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Proposed EQS for Water Framework Directive Annex VIII substances: linuron

Science Report – HOEP670085/SR18

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The UK Technical Advisory Group (UKTAG) supporting the implementation of the Water Framework Directive (2000/60/EC) is a partnership of UK environmental and conservation agencies. It also includes partners from the Republic of Ireland. This report is the result of research commissioned and funded on behalf of UKTAG by the Environment Agency.

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Steve Killeen

Head of Science

Use of this report

The development of UK-wide classification methods and environmental standards that aim to meet the requirements of the Water Framework Directive (WFD) is being sponsored by the UK Technical Advisory Group (UKTAG) for WFD on behalf of its members and partners.

This technical document has been developed through a project managed by the Environment Agency and has involved members and partners of UKTAG. It provides background information to support the ongoing development of the standards and classification methods.

While this report is considered to represent the best available scientific information and expert opinion available at the time of its completion, it does not necessarily represent the final or policy positions of UKTAG or any of its partner agencies.

Executive summary

The UK Technical Advisory Group (UKTAG) has commissioned a programme of work to derive Environmental Quality Standards (EQSs) for substances falling under Annex VIII of the Water Framework Directive (WFD). This report proposes predicted no-effect concentrations (PNECs) for linuron using the methodology described in Annex V of the Directive. There are existing EQSs for linuron, but the method used to derive these is not considered to comply with the requirements of Annex V and so is unsuitable for deriving Annex VIII EQSs.

The PNECs described in this report are based on a technical assessment of the available ecotoxicity data for linuron, along with any data that relate impacts under field conditions to exposure concentrations. The data have been subjected to rigorous quality assessment such that decisions are based only on scientifically sound data. Following consultation with an independent peer review group, critical data have been identified and assessment factors selected in accordance with the guidance given in Annex V of the WFD.

Where possible, PNECs have been derived for freshwater and saltwater environments, and for long-term/continuous exposure and short-term/transient exposure. If they were to be adopted as EQSs, the long-term PNEC would normally be expressed as an annual average concentration and the short-term PNEC as a 95th percentile concentration.

The feasibility of implementing these PNECs as EQSs has not been considered at this stage. However, this would be an essential step before a regulatory EQS can be recommended.

Properties and fate in water

Linuron is a selective, systemic herbicide absorbed principally through the roots, but also through the foliage, with translocation in the xylem. It inhibits photosynthesis – mainly in photosystem II between quencher and plastoquinone – by blocking electron transport, which leads to the production of a range of powerful oxidants causing rapid destruction of plant cells.

Linuron degrades moderately rapidly in water and, based on a log Koc of 2.83, it will mostly remain in the water column, rather than be adsorbed to sediment. Bioconcentration in aquatic organisms is low.

Availability of data

Long-term laboratory data are available for seven different freshwater taxonomic groups (algae, annelids, crustaceans, fish, macrophytes, molluscs and platyhelminths). Freshwater short-term toxicity data are available for nine taxonomic groups (algae, annelids, bryophytes, crustaceans, fish, insects, macrophytes, molluscs and platyhelminths). Freshwater algae and macrophytes are the most sensitive taxa of those tested with both technical linuron and various linuron formulations. For marine organisms, single species short-term toxicity data are available for six different taxonomic groups (bacteria, algae, crustaceans, fish, macrophytes and molluscs). However, long-term toxicity data are available only for macrophytes. Laboratory data are supplemented by freshwater mesocosm data, which confirm the high sensitivity of algae and macrophytes to linuron.

Limited evidence suggests that linuron is a potential vertebrate (fish and mammals) endocrine disruptor by acting as an androgen receptor antagonist.

Derivation of PNECs

Long-term PNEC for freshwaters

The lowest reliable long-term (lt) laboratory test result is an EC10 of 1.0 µg active ingredient (a.i.) l^{-1} for the macrophyte *Ceratophyllum demersum*. Long-term data are also available for algae, invertebrates and fish. Vascular plants such as *C.demersum* are expected to be sensitive to linuron, so it is unlikely that substantially more sensitive species will be found. In addition, exposure of a wide variety of organisms in several well-performed microcosm and mesocosm studies produced a lowest no observed effect concentration (NOEC) of 0.5 µg l^{-1} after continuous exposure. Therefore, application of an assessment factor of 2 to the *C.demersum* EC10 is recommended resulting in a $PNEC_{freshwater\_lt}$ of 0.5 µg l^{-1}.

This value is lower than the existing EQS of 2.0 µg l^{-1}, which was derived by applying a safety factor of 100 to the lowest acute effects concentration (i.e. a 48-hour EC50 to first instar *Daphnia magna* of 210 µg l^{-1}).

Short-term PNEC for freshwaters

Reliable short-term (st) toxicity data are available for algae, invertebrates and fish. The lowest short-term value is a 24-hour EC50 of 9.0 µg a.i. l^{-1} for inhibition of photosystem II in the macrophyte *Elodea nuttallii*. Since there is a considerable short-term toxicity database for freshwater organisms which shows that macrophytes such as *Elodea* are likely to be among the most sensitive to linuron, an assessment factor of 10 rather than 100 should be applied resulting in a $PNEC_{freshwater\_st}$ of 0.9 µg l^{-1}.

This value is lower than the existing EQS of 20 µg l^{-1}, which was derived by applying a safety factor of 10 to the lowest acute effects concentration (i.e. a 48-hour EC50 to first instar *Daphnia magna* of 210 µg l^{-1}).

Long-term PNEC for saltwaters

Long-term single species toxicity data for saltwater organisms are available only for two macrophyte species. The absence of long-term data for algae, invertebrates and fish means that it is not possible to generate a $PNEC_{saltwater\_lt}$ based on the saltwater data alone. Therefore, it is proposed that the combined freshwater and saltwater dataset is used for the PNEC generation.

The lowest long-term laboratory test result is therefore a 5-week EC10 of 1.0 µg a.i. l^{-1} for inhibition of photosystem II in the macrophyte *Ceratophyllum demersum*. The argument used in the EU Technical Guidance Document (TGD) for an additional assessment factor of 10 in saltwater systems to account for greater biodiversity should not be applied to linuron because of its mode of toxic action and the likelihood that photosystem II photosynthesisers such as *Ceratophyllum* are the most sensitive organisms. Therefore maintaining the freshwater assessment factor of 2 for calculating a $PNEC_{salwater\_lt}$ is recommended, resulting in a $PNEC_{saltwater\_lt}$ of 0.5 µg l^{-1}.

This value is slightly lower than the interim guideline of 2.0 µg l^{-1} in the existing EQS report, which was 'read across' from the freshwater long-term value.

Short-term PNEC for saltwaters

Single species acute toxicity data for marine organisms are available for bacteria, algae, crustaceans, fish and molluscs, with algae being the most sensitive group. The highly specific mode of action of linuron strongly suggests that a maximum allowable concentration in saltwater should be similar to one set on the basis of more extensive data for freshwater plants. Therefore the use of the 24-h growth EC50 of 9.0 µg a.i. l^{-1} for the macrophyte *E. nuttallii* and application of an assessment factor of 10 is recommended, resulting in a $PNEC_{saltwater\_st}$ of 0.9 µg l^{-1}.

There is no current short-term EQS for saltwaters.

PNECs for sediment

The log Kow of linuron lies between 2.76 and 3.0, which is on the borderline for requiring the derivation of PNECs for the protection of benthic organisms under the TGD. However, there is no evidence from microcosm and mesocosm studies to suggest that sediment-dwelling organisms (invertebrates and decomposers) are directly affected at concentrations near those that affect photosystem II in plants. Therefore derivation of a sediment PNEC is not recommended.

PNECs for secondary poisoning

Bioconcentration data – as bioconcentration factor (BCF) values – for linuron for aquatic organisms are low, with values for fish ranging from 38 to 49. Hence the TGD BCF trigger of 100 in whole fish is not exceeded and the derivation of PNECs for secondary poisoning of predators is not required.

Summary of proposed PNECs

Receiving medium/exposure scenario	**Proposed PNEC ($\mu g\ l^{-1}$)**	**Existing EQS ($\mu g\ l^{-1}$)**
Freshwater/long-term	0.5	2.0 (AA)
Freshwater/short-term	0.9	20 (MAC)
Saltwater/long-term	0.5	2.0 (AA)
Saltwater/short-term	0.9	–
Sediment	Not required	–
Secondary poisoning	Not required	–

AA = annual average
MAC = maximum allowable concentration

Analysis

The data quality requirements are that, at a third of the EQS, total error of measurement should not exceed 50 per cent. Using this criterion, it is evident that current analytical methodologies (non-standard) employing gas chromatography/mass spectrometry (GC-MS) and capable of achieving detection limits as low as 5 ng l^{-1} should offer adequate performance to analyse for linuron.

Implementation issues

These PNECS are suitable for use as EQSs because they are not subject to excessive uncertainty and analytical capability should be adequate for compliance assessment purposes.

Contents

1 Introduction

The UK Technical Advisory Group (UKTAG) supporting the implementation of the Water Framework Directive (2000/60/EC)[1] is a partnership of UK environmental and conservation agencies. It also includes partners from the Republic of Ireland. UKTAG has commissioned a programme of work to derive Environmental Quality Standards (EQSs) for substances falling under Annex VIII of the Water Framework Directive (WFD). This report proposes predicted no-effect concentrations (PNECs) for linuron using the methodology described in Annex V of the Directive. There are existing EQSs for linuron, but the method used to derive these is not considered to comply with the requirements of Annex V and so is unsuitable for deriving Annex VIII EQSs.

The PNECs described in this report are based on a technical assessment of the available ecotoxicity data for linuron, along with any data that relate impacts under field conditions to exposure concentrations. The data have been subjected to rigorous quality assessment such that decisions are based only on scientifically sound data.[2] Following consultation with an independent peer review group, critical data have been identified and assessment factors selected in accordance with the guidance given in Annex V of the WFD. The feasibility of implementing these PNECs as EQSs has not been considered at this stage. However, this would be an essential step before a regulatory EQS can be recommended.

This report provides a data sheet for linuron.

1.1 Properties and fate in water

Linuron is a selective, systemic herbicide absorbed principally through the roots, but also through the foliage, with translocation in the xylem. It inhibits photosynthesis – mainly in photosystem II between quencher and plastoquinone – by blocking electron transport, which leads to the production of a range of powerful oxidants causing rapid destruction of plant cells.

Linuron degrades moderately rapidly in water and, based on a log Koc of 2.83, it will mostly remain in the water column, rather than be adsorbed to sediment. Bioconcentration in aquatic organisms is low.

[1] *Official Journal of the European Communities*, **L327**, 1–72 (22/12/2000). Can be downloaded from http://www.eu.int/comm/environment/water/water-framework/index_en.html

[2] Data quality assessment sheets are provided in Annex 1 of this report.

2 Results and observations

2.1 Identity of substance

Table 2.1 gives the chemical name and Chemical Abstracts Service (CAS) number for the species of interest.

Table 2.1 Species covered by this report

Name	CAS Number
Linuron	330-55-2

2.2 PNECs proposed for derivation of quality standards

Table 2.2 lists proposed PNECs obtained using the methodology described in the Technical Guidance Document (TGD) issued by the European Chemicals Bureau (ECB) on risk assessment of chemical substances (ECB 2003).

Section 2.6 summarises the effects data identified from the literature for linuron. The use of these data to derive the values given in Table 2.2 is explained in Section 3.

Table 2.2 Proposed overall PNECs as basis for quality standard setting

PNEC	TGD deterministic approach (AFs)	TGD probabilistic approach (SSDs)	Existing EQS
Freshwater short-term	0.9 µg l^{-1}	–	20 µg l^{-1} (MAC)
Freshwater long-term	0.5 µg l^{-1}	Insufficient data	2.0 µg l^{-1} (AA)
Saltwater short-term	0.9 µg l^{-1}	–	–
Saltwater long-term	0.5 µg l^{-1}	Insufficient data	2.0 µg l^{-1} (AA)
Sediment	Not required	–	–
Secondary poisoning	Not required	–	–

AA = annual average
AF = assessment factor
MAC = maximum allowable concentration
SSD = species sensitivity distribution

2.3 Hazard classification

Table 2.3 gives the R-phrases (Risk-phrases) and labelling for the species of interest.

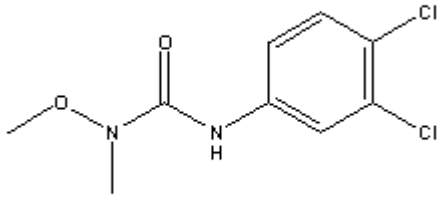
Table 2.3 Hazard classification

R-phrases and labelling	Reference
R22, 40, 48/22, 50/53 S2, 36/37, 60, 61	IUCLID (2000)

2.4 Physical and chemical properties

Table 2.4 summarises the physical and chemical properties of the species of interest.

Table 2.4 Physical and chemical properties of linuron

Property	Value	Reference
CAS number	330-55-2	ECB (2005)
Substance name	3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea	ECB (2005)
Molecular formula	$C_9H_{10}Cl_2N_2O_2$	ECB (2005)
Molecular structure		Chemfinder (2005)
Molecular weight	249.1	HSDB (2005)
Colour/form	White crystalline solid, fine flakes, coarse powder, or colourless crystals	HSDB (2005), IPCS (2004)
Odour	Odourless	HSDB (2005)
Melting point (°C)	93–94°C	HSDB (2005)
Boiling point (°C)	180–190°C	Chemfinder (2005)
Vapour pressure	0.002 Pa at 24°C	IPCS (2004)
Density/specific gravity	1.49 at 20°C	HSDB (2005)
Henry's Law constant	2.0×10^{-4} Pa m^3 mol^{-1}	EC (2002)
Solubility in water	75 mg l^{-1} at 25°C	HSDB (2005)
	52.7 mg l^{-1} at pH 5, 20°C 63.8 mg l^{-1} at pH 7, 20°C 74.5 mg l^{-1} at pH 9, 20°C	EC (2002)
Solubility in organic solvents	395 g l^{-1} acetone at 20°C 2.3 g l^{-1} hexane at 20°C 170 g l^{-1} methanol at 20°C	EC (2002)

2.5 Environmental fate and partitioning

Table 2.5 summarises the information obtained from the literature on the environmental fate and partitioning of linuron.

Table 2.5 Environmental fate and partitioning of linuron

Property	Value	Reference
Abiotic fate	The rate constant for the vapour phase reaction with photochemically produced hydroxyl radicals has been estimated using a structure estimation method as 1.0×10^{-11} cm^3 $molecule^{-1}$ sec^{-1} at 25°C. This corresponds to an atmospheric half-life of approximately 12 hours at an atmospheric concentration of 5×10^5 hydroxyl radicals cm^{-3}.	Meylan and Howard (1993)
Speciation	Not applicable	
Hydrolytic stability	Hydrolysis is not expected to occur in water except at high alkaline conditions (pH >9). DT50 = 1,220 days at pH 5 DT50 = 1,460 days at pH 7 DT50 = 1,080 days at pH 9	HSDB (2005) EC (2002)
Photostability	Linuron is completely, although slowly, degraded by direct photolysis in sterile, aqueous solution ultimately resulting in the formation of inorganic compounds (CO_2). Under realistic use conditions, it is to be expected that photolytic degradation is considerably accelerated by the sensitisers always present in natural bodies of surface water. An aqueous sample exposed to sunlight outdoors in Fargo, North Dakota, resulted in a 43% loss of linuron in 24 days.	EC 2002 Tanaka *et al.* (1986)
Volatilisation	Volatilisation from water surfaces is not expected to be an important environmental fate process, based upon the estimated Henry's Law constant of 2.0 × 10-4 Pa m^3 mol^{-1}.	EC (2002)
Distribution in water/sediment systems (active substance)	Absorption–desorption kinetic studies of linuron in freshwater sediment slurries showed that both processes occur rapidly, approaching 75% equilibrium values within 3–6 minutes, and that the processes are reversible.	Wauchope and Myers (1985)
Metabolites	The major degradation products are: • desmethoxy linuron (loss of a methoxy group); • desmethoxy monolinuron (loss of methoxy group and a chlorine); • norlinuron (loss of methoxy and a methyl group).	Caux *et al.* (1998)

Property	Value	Reference
Degradation in soil	Loss of linuron is principally due to biodegradation, which is mainly co-metabolic. The degradation rate also correlates with soil respiration, organic carbon content, adsorptivity and clay content. At higher soil moistures and temperatures, linuron degrades faster. In most soils, at normal application rates, linuron degrades within 3–4 months. The half-lives of 4 parts per million (ppm) linuron incubated at 25°C in 18 mineral soils ranged from 22 to 86 days.	HSDB (2005) Walker and Zimdahl 1981
Biodegradation in water	Biodegradation of linuron in surface waters appears to be particularly site-specific. Calculated half-lives range from 2–11.8 days in stagnant ditches; 16–42 days in 1 m^3 outdoor experimental enclosures and 10 weeks in Lake Balaton, Hungary. Degradation is more rapid at higher temperatures and at high or low pH when compared with intermediate pH, and follows first order reaction kinetics with an average Q10 of 2.77.	HSDB (2005) Cserháti *et al.* (1976)
Octanol-water coefficient (log Kow)	2.76 3.0	Caux *et al.* (1998) Worthing and Hance (1991)
Log Koc	2.83	Caux *et al.* (1998)
Bioaccumulation BCF values	Bioconcentration factor (BCF) values of 38 and 49 for bluegill sunfish (*Lepomis macrochirus*) suggest that bioconcentration in aquatic organisms is low.	DuPont 1984, cited in Caux *et al.* (1998)

Crum *et al.* (1998) studied the fate of linuron in experimental ditches. Eight ditches were sprayed with an emulsifiable formulation (active ingredient linuron at 450 g l^{-1}) three times at monthly intervals, while two served as controls. As a simulation of spray drift, four doses (nominal: 0.5, 5, 15 and 50 µg a.i. l^{-1}) were applied in duplicate. After each herbicide application, the ditches were kept stagnant for a week, followed by a flow period of three weeks with fresh water. No clear stratification of linuron was found in the water compartment. During the stagnant period, half-lives for the disappearance of linuron in the water compartment ranged from 7.2 to 11.8 days. The rate of disappearance was slower in the ditches treated with the highest dose and during colder treatment periods. A maximum of 6 per cent of the linuron dose was found in the sediment and 1 per cent in the macrophyte compartment. Approximately 20 per cent of the fraction in the sediment compartment was present in the pore water.

The degradation rate of linuron under anaerobic conditions in an aquatic environment was studied using sterilised and unsterilised water, and sediment samples from two different ponds (DuPont 1986). Radio-labelled linuron at 5 mg l^{-1} was added to each of the different systems for 52 weeks. The shortest half-life, 7 days, was observed in the unsterilised water/sediment system

with the highest organic matter content and pH 6.5. The half-life increased to 22 days at pH 7.8. The half-life was 42 weeks in the sterilised alkaline water/sediment system compared with 3.5 weeks in the sterilised acidic water/sediment system.

These data suggest that degradation of linuron is moderately rapid and that it will mostly remain in the water column rather than be adsorbed to sediment.

2.6 Effects data

A summary of the mode of action of this substance can be found in Section 2.6.5.

Data collation followed a tiered approach.

First, critical freshwater and saltwater data were compiled from existing EQS documents. Further data published after derivation of the current UK EQS were then retrieved from the US Environmental Protection Agency (US EPA) ECOTOX database.[3]

In addition, data were sought from:

- ScienceDirect®;[4]
- Hazardous Substances Data Bank (HSDB®) database of the US National Library of Medicine;[5]
- US EPA Integrated Risk Information System (IRIS) database;[6]
- US EPA Reregistration Eligibility Report (RED) for linuron (US EPA 1995) – referred to in this report as US EPA RED 1995;
- European Commission Plant Products Protection (PPP) review report on linuron (EC 2002);
- Draft Assessment Report (DAR) prepared under Council Directive 91/414/EEC (EU 1996) – referred to in this report as EU DAR 1996;
- Advisory Committee on Pesticides evaluation report on linuron (ACP 1995).

2.6.1 Toxicity to freshwater organisms

Freshwater toxicity data on linuron are available for various taxonomic groups including algae, invertebrates and fish, as required for the application of the approach specified in the EU Technical Guidance Document (ECB 2003). Long-term data were available for seven taxonomic groups including algae, annelids, crustaceans, fish, macrophytes, molluscs and platyhelminths. Freshwater short-term toxicity data were available for nine taxonomic groups including algae, annelids, bryophytes, crustaceans, fish, insects, macrophytes, molluscs and platyhelminths.

Table 2.6 summarises the available data on the effects of different forms of linuron to freshwater organisms in long-term and short-term toxicity studies.

[3] http://www.epa.gov/ecotox/
[4] http://www.sciencedirect.com
[5] http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB
[6] http://www.epa.gov/iris/index.html

Table 2.6 Summary of available data for linuron

Type of data	Form of substance	Taxonomic groups for which information is available
Long-term	Technical grade linuron	Algae, macrophytes, crustaceans, platyhelminths, molluscs, annelids and fish
	48.7–49.3% w/w wettable product formulation	Algae and crustaceans
	450 g l^{-1} formulation	Algae and macrophytes
	Not stated	Algae and fish
Short-term	Technical grade linuron	Algae, macrophytes, bryophytes, crustaceans, insects, platyhelminths, molluscs, annelids and fish
	47.5–50.0% w/w wettable product formulation	Algae, crustaceans and fish
	197 g l^{-1} formulation	Crustaceans and fish
	Not stated	Algae, crustaceans and fish

Overall, the available short-term and long-term toxicity test data indicate that algae and macrophytes are the most sensitive taxa of those tested for both technical linuron and the various linuron formulations. The greater toxicity of technical linuron and the various formulations to algae and macrophytes compared with other taxonomic groups (where comparative data are available) is consistent with the use of the substance as a herbicide.

Table 2.7 indicates that there are no marked differences in the toxicity of technical grade linuron and linuron in formulations for given taxonomic groups (e.g. see studies with *Elodea* and *Scenedesmus*). However, there will be variability associated with the toxicity data set for different forms of linuron. Therefore, a relatively large difference between the toxicity data for different forms of linuron would be needed to conclude that one form of linuron was consistently more toxic than others. In view of the absence of clear differences between the toxicity of different forms of linuron, all the available data were used to derive the PNECs.

Table 2.7 Comparative toxicity of different forms of linuron to four species

Data type	Form of substance	Freshwater toxicity data*			
		Algae (*S. subspicatus*)	**Macrophyte (*Elodea* sp.)**	**Invertebrate (*D.magna*)**	**Fish (*O.mykiss*)**
Long-term	Technical grade linuron	72-hour NOEC = 5.6 µg a.i. l^{-1}	28-day NOEC = <20 µg a.i. l^{-1}	21-day NOEC = 180 µg a.i. l^{-1}	21-day NOEC = <42 µg a.i. l^{-1}
	Formulations	72-hour NOEC = 4.0 µg a.i. l^{-1}	21-day NOEC = 5.0 µg a.i. l^{-1}	21-day NOEC = 160 µg a.i. l^{-1}	ND
Short-term	Technical grade linuron	72-hour EC50 = 16 µg a.i. l^{-1}	ND	48-hour EC50 = 120–750 µg a.i. l^{-1}	96-hour LC50 = 3,150–3,300 µg a.i. l^{-1}
	Formulations	72-hour EC50 = 50 µg a.i. l^{-1}	ND	48-hour EC50 = 210 µg a.i. l^{-1}	96-hour LC50 = 3,360–6,700 µg a.i. l^{-1}

* From Tables 2.8 and 2.9.
ND = no data
NOEC = no observed effect concentration

Diagrammatic representations of the available freshwater data (cumulative distribution functions) for linuron are presented in Figures 2.1 and 2.2. These diagrams include all data regardless of quality and provide an overview of the spread of the available data, but they are not species sensitivity distributions and have not been used to derive linuron PNECs. The lowest critical freshwater data for linuron are presented in Tables 2.8 and 2.9.

Figure 2.1 Cumulative distribution function of freshwater long-term data (µg a.i. l^{-1}) for linuron

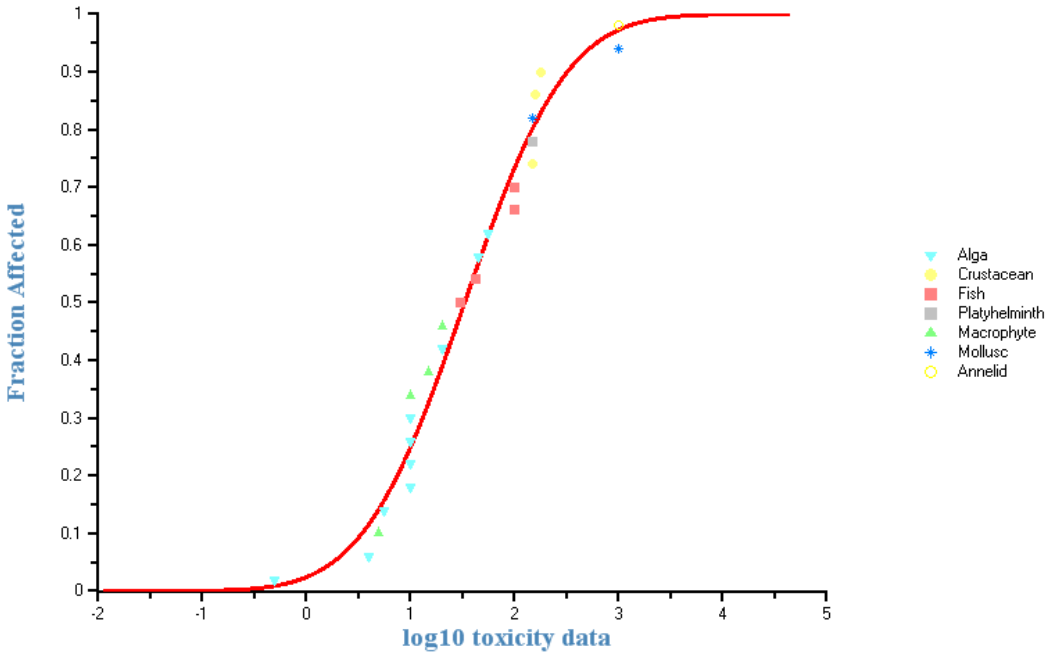


Figure 2.2 Cumulative distribution function of freshwater short-term data (µg a.i. l^{-1}) for linuron

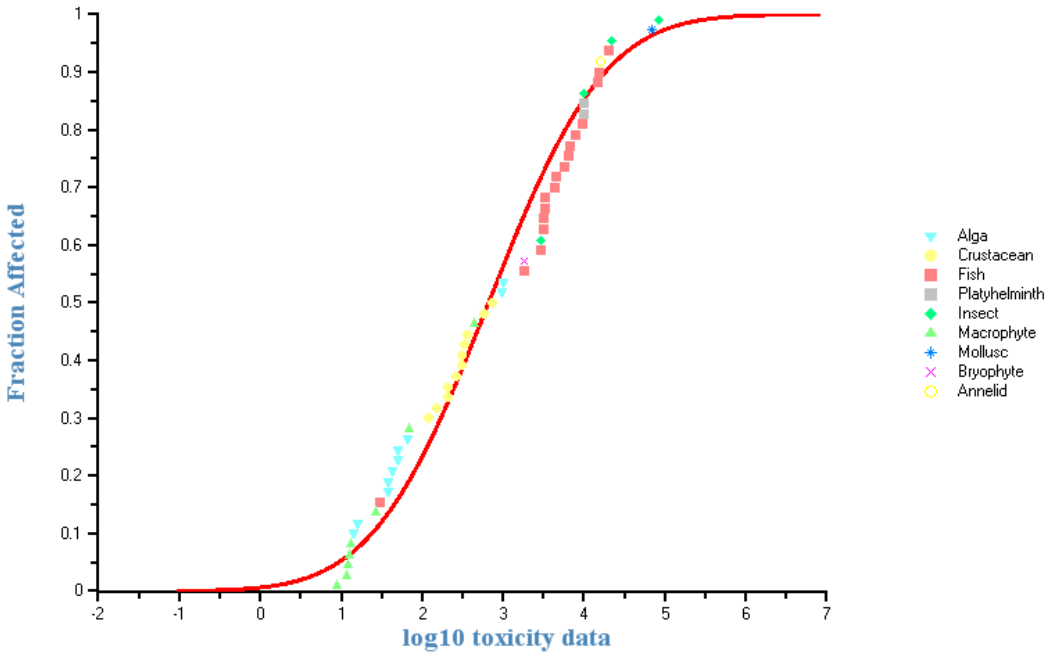


Table 2.8 Most sensitive long-term aquatic toxicity data for freshwater organisms exposed to linuron

Form of the chemical	Scientific name	Common name	Taxonomic group	Effect	End-point	Test duration	Conc. (µg a.i. l^{-1})	Exposure[1]	Toxicant analysis[2]	Comments	Reliability (Klimisch Code*)	Reference
94.8% purity	*Scenedesmus subspicatus*	Green alga	ALG	Growth/cell deformation	NOEC	72 hours	5.6	s	n	-	4	Fischer 1989a[#]
Technical grade purity not stated	*Selenastrum capricornutum*	Green alga	ALG	Growth	NOEC	120 hours	10	s	n	-	4	Cited in ACP 1995
Technical grade purity not stated	*Chlorella vulgaris*	Green alga	ALG	Growth rate/ photosynthesis	NOEC	96 hours	10	s	n	-	2	Knauf and Schulze 1972
97% purity	*Chlorella vulgaris*	Green alga	ALG	Growth	NOEC	21 days	20	s	y	Microcosm test	1	Slijkerman *et al.* 2005
98% purity	*Nostoc muscorum*	Cyano-bacterium	ALG	Chlorophyll a	LOEC	7 days	45	s	n	-	2	Abou-Waly and Shabana 1993
Technical grade purity not stated	*Lemna minor*	Lesser duckweed	MAC	Growth	NOEC	7 days	10	s	n	25°C	2	Hulsen *et al.* 2002
97% purity	*Elodea* sp.	Waterweed	MAC	Biomass	NOEC	28 days	<20	s	y	Microcosm test	1	Slijkerman *et al.* 2005
98.4% purity	*Daphnia magna*	Water flea	CRU	Reproduction	MATC	21 days	>130 <240	–	–	-	4	Baer 1991 Cited in US EPA RED 1995
94.8% purity	*Daphnia magna*	Water flea	CRU	Reproduction	NOEC	21 days	180	ss	y		3	Fischer 1987[#]
99.96% purity	*Oncorhynchus mykiss*	Rainbow trout	FIS	Cytopathology of liver and kidney	LOEC	5 weeks	30	f	y	9.4±0.5°C	1	Oulmi *et al.* 1995
98.4% purity	*Oncorhynchus mykiss*	Rainbow trout	FIS	Early life cycle	NOEC	21 days	< 42	–	–	-	4	Cited in US EPA RED 1995
94.8% purity	*Oncorhynchus mykiss*	Rainbow trout	FIS	Behaviour	NOEC	21 days	100	–	y	-	4	Fischer 1989f#
Technical grade purity not stated	*Cyprinus carpio*	Common carp	FIS	Behaviour	NOEC	28 days	100	s	n	-	2	Knauf and Schulze 1972
48.7% w/w WP	*Scenedesmus subspicatus*	Green alga	ALG	Growth	NOEC	72 hours	4.0	s	n	-	4	Fischer 1989c[#]
49.3% w/w WP	*Daphnia magna*	Water flea	CRU	Reproduction	NOEC	21 days	160	ss	y	-	4	Fischer 1989g[#]
450 g l^{-1} SC	*Spirogyra* sp.	Filamentous alga	ALG	Photosystem II inhibition	EC10	5 weeks	11.1	s	y	Microcosm test	2	Snel *et al.* 1998

Form of the chemical	Scientific name	Common name	Taxonomic group	Effect	End-point	Test duration	Conc. (µg a.i. l^{-1})	Exposure[1]	Toxicant analysis[2]	Comments	Reliability (Klimisch Code*)	Reference
450 g l^{-1} SC	*Ceratophyllum demersum*	Common hornwort	MAC	Photosystem II inhibition	EC10	5 weeks	1.0	s	y	Microcosm test	2	Snel *et al.* 1998
450 g l^{-1} SC	*Elodea nuttallii*	Nuttall's waterweed	MAC	Photosystem II inhibition	EC10	8 weeks	1.3	s	y	Microcosm test	2	Snel *et al.* 1998
450 g l^{-1} SC	*Elodea nuttallii*	Nuttall's waterweed	MAC	Biomass	NOEC	21 days	5.0	ss	y	Microcosm test	1	van den Brink *et al.* 1997
450 g l^{-1} SC	*Myriophyllum spicatum*	Eurasian water milfoil	MAC	Biomass	NOEC	4 weeks	15	ss	y	Mesocosm test	1	van Geest *et al.* 1999
Not stated	*Scenedesmus acutus*	Green alga	ALG	Growth	EC10	72 hours	1.2	s	y	-	2	Snel *et al.* 1998
Not stated	*Pseudokirchneriella subcapitata and Chlorella vulgaris*	Green alga	ALG	Growth	NOEC LOEC	96 hours	10 100	s	n	-	_	Garten 1990 Reported in US EPA ECOTOX database
Not stated	*Oncorhynchus mykiss*	Rainbow trout	FIS	Haematocrit	LOEC	28 days	30	f	y	-	4	Brüggeman *et al.* 1995

* See Annex 1.
[#] Cited in EU DAR 1996.
[1] Exposure: s = static; ss = semi-static; f = flow-through.
[2] Toxicant analysis: y = measured; n = not measured.
ALG = algae; CRU = crustaceans; FIS = fish; MAC = macrophytes
LOEC = lowest observed effect concentration
MATC = maximum allowable toxicant concentration
NOEC = no observed effect concentration
EC10 = concentration effective against 10% of the organisms tested
SC = suspension concentrate
WP = wettable product

Table 2.9 Most sensitive short-term aquatic toxicity data for freshwater organisms exposed to linuron

Form of the chemical	Scientific name	Common name	Taxonomic group	Effect	End-point	Test duration (hours)	Conc. (µg a.i. l^{-1})	Exposure[1]	Toxicant analysis[2]	Comments	Reliability (Klimisch Code*)	Reference
94.8% purity	*Scenedesmus subspicatus*	Green alga	ALG	Growth/cell deformation	EC50	72	16	s	n	-	4	Fischer 1989a[#]
Technical grade purity not stated	*Pseudokirchneriella subcapitata*	Green alga	ALG	Growth	EC50	120	43	s	n	-	4	Cited by UK rapporteur in EU DAR 1996
80% purity	*Chlorella vulgaris*	Green alga	ALG	Growth	EC50	120	50	s	n	-	2	Stephenson and Kane 1984
100% purity	*Pseudokirchneriella subcapitata*	Green alga	ALG	Population growth	EC50	120	67	s	-	-	4	Douglas and Handley 1988 Cited in US EPA RED 1995
Technical grade purity not stated	*Chlorella* sp.	Green alga	ALG	Photosynthesis inhibition	EC50	24	960	s	n	-	2	Knauf and Schulze 1972
80% purity	*Lemna minor*	Lesser duckweed	MAC	Growth	EC50	120	70	s	n	-	2	Stephenson and Kane 1984
Technical grade purity not stated	*Lemna* sp.	Duckweed	MAC		EC50	24	450	s	n	-	2	Knauf and Schulze 1972
Technical grade purity not stated	*Rhiziella* sp.	Moss	BRY		EC50	24	1,800	s	n	-	2	Knauf and Schulze 1972
94.4% purity	*Daphnia magna*	Water flea	CRU	Immobility	EC50	48	120	s	-	-	-	Hall 1985 Cited in US EPA RED 1995
94.4% purity	*Daphnia magna*	Water flea	CRU	Immobility	EC50	48	120	s	n	-	4	Cited in ACP 1995
95.1% purity	*Daphnia magna*	Water flea	CRU	Immobility	EC50	48	270	s	n	17°C	2	Mayer and Ellersieck 1986
80% purity	*Daphnia magna*	Water flea	CRU	Immobility	EC50	24	310	s	n	19.5±1°C	2	Stephenson and Kane 1984
80% purity	*Diaptomus gracilis*	Copepod	CRU	Immobility	EC50	24	330	s	n	19.5±1°C	2	Stephenson and Kane 1984
80% purity	*Daphnia* spp.	Water flea	CRU	Immobility	EC50	24	360	s	n	19.5±1°C	2	Stephenson and Kane 1984
94.4% purity	*Daphnia magna*	Water flea	CRU	Immobility	EC50	48	750	s	n	-	4	Knauf 1980a[#]

Form of the chemical	Scientific name	Common name	Taxonomic group	Effect	End-point	Test duration (hours)	Conc. (µg a.i. l^{-1})	Exposure[1]	Toxicant analysis[2]	Comments	Reliability (Klimisch Code*)	Reference
Technical grade purity not stated	*Lebistes reticulatus*	Guppy	FIS	Lethality	LC0	24	1,500	s	n	-	2	Knauf and Schulze 1972
95.1% purity	*Ictalurus punctatus*	Channel catfish	FIS	Lethality	LC50	96	2,900	s	n	22°C	2	Mayer and Ellersieck 1986
Technical grade purity not stated	*Oncorhynchus mykiss*	Rainbow trout	FIS	Lethality	LC50	96	3,150	s	n	-	4	Knauf 1980b#
96.2% purity	*Oncorhynchus mykiss*	Rainbow trout	FIS	Lethality	LC50	96	3,300	s	y	-	4	Cited in ACP 1995
Technical grade purity not stated	*Ictalurus nebulosus*	Catfish	FIS	Lethality	LC50	96	4,900	s	n	-	4	Knauf 1980e#
96.2% purity	*Lepomis macrochirus*	Bluegill sunfish	FIS	Lethality	LC50	96	9,600	s	y	-	4	Cited in ACP 1995
48.7% w/w WP	*Scenedesmus subspicatus*	Green alga	ALG	Growth	EC50	72	50	s	n	-	4	Fischer 1989c#
50% w/w WP	*Daphnia magna*	Water flea	CRU	Immobility	EC50	48	210	s	n	17°C	2	Mayer and Ellersieck 1986
48.7% w/w WP	*Daphnia magna*	Water flea	CRU	Immobility	EC50	48	210	s	y	-	4	Fischer 1989b#
50% w/w WP	*Ictalurus punctatus*	Channel catfish	FIS	Lethality	LC50	96	1,800	s	n	22°C	2	Mayer and Ellersieck 1986
48.7% w/w WP	*Oncorhynchus mykiss*	Rainbow trout	FIS	Lethality	LC50	96	6,700	s	y	-	4	Fischer 1989d#
47.5% w/w WP	*Cyprinus carpio*	Common carp	FIS	Lethality	LC50	96	15,500	s	y	-	4	Fischer 1989e#
197g l^{-1} EC	*Daphnia magna*	Water flea	CRU	Immobility	EC50	48	590	s	n	-	4	Fischer 1981#
197g l^{-1} EC	*Oncorhynchus mykiss*	Rainbow trout	FIS	Lethality	LC50	96	3,360	s	n	-	4	Knauf 1980c#
197g l^{-1} EC	*Idus melanotus*	Golden orfe	FIS	Lethality	LC50	96	4,380	s	n	-	4	Knauf 1980d#
Not stated	*Scenedesmus acutus*	Green alga	ALG	Growth	EC50	72	6	s	y	-	2	Snel *et al.* 1998
Not stated	*Navicula pelliculosa*	Diatom	ALG	Population abundance	EC50	-	14	s	-	-	-	Reported in US EPA ECOTOX database
Not stated	*Anabaena flos-aquae*	Blue-green alga	ALG	Population abundance	EC50	120	38	s	-	-	-	Reported in US EPA ECOTOX database

Form of the chemical	Scientific name	Common name	Taxonomic group	Effect	End-point	Test duration (hours)	Conc. (µg a.i. l^{-1})	Exposure[1]	Toxicant analysis[2]	Comments	Reliability (Klimisch Code*)	Reference
Not stated	*Elodea nuttallii*	Nuttall's waterweed	MAC	Photosystem II inhibition	EC50	24	9	s	y	-	2	Snel *et al.* 1998
Not stated	*Myriophyllum spicatum*	Eurasian water milfoil	MAC	Photosystem II inhibition	EC50	24	11.8	s	y	-	2	Snel *et al.* 1998
Not stated	*Chara globularis*	Fragile stonewort	MAC	Photosystem II inhibition	EC50	24	12.1	s	y	-	2	Snel *et al.* 1998
Not stated	*Potamogeton crispus*	Curly pondweed	MAC	Photosystem II inhibition	EC50	24	12.9	s	y	-	2	Snel *et al.* 1998
Not stated	*Ranunculus circinatus*	Fan-leaved water crowfoot	MAC	Photosystem II inhibition	EC50	24	13.2	s	y	-	2	Snel *et al.* 1998
Not stated	*Lemna gibba*	Inflated duckweed	MAC	Population abundance	EC50	14	27	s	-	-	-	Reported in US EPA ECOTOX database
Not stated	*Daphnia magna*	Water flea	CRU	Immobility	EC50	96	7,000	s	n	-	4	Hernando *et al.* 2003

* See Annex 1.
# Cited in EU DAR 1996.
[1] Exposure: s = static.
[2] Toxicant analysis: y = measured; n = not measured.
ALG = algae; BRY = bryophytes; CRU = crustaceans; FIS = fish; MAC = macrophytes
EC50 = concentration effective against 50% of the organisms tested
LCx = concentration effective against X% of the organisms tested
EC = emulsifiable concentrate
WP = wettable product

2.6.2 Toxicity to saltwater organisms

Single species linuron short-term toxicity data for saltwater organisms are available for six different taxonomic groups (bacteria, algae, crustaceans, fish, macrophytes and molluscs). Long-term toxicity data are available only for macrophytes. Long-term and short-term toxicity data for marine species are summarised in Tables 2.10 and 2.11 respectively.

A diagrammatic representation of all the available saltwater data (cumulative distribution function) for linuron is presented in Figure 2.3. This diagram includes all data regardless of quality and provides an overview of the spread of the available data. This diagram is not a species sensitivity distribution and has not been used to set the linuron PNECs.

Figure 2.3 Cumulative distribution function of saltwater long-term and short-term data (µg a.i. l^{-1}) for linuron (combined data presented due to limited data available)

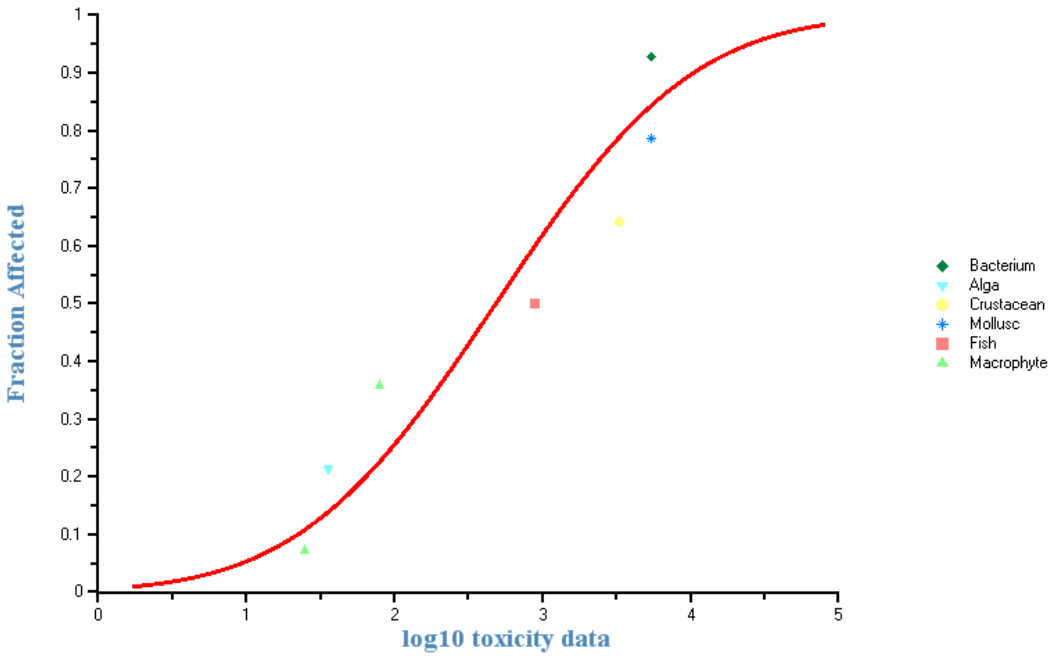


Table 2.10 Most sensitive long-term aquatic toxicity data for saltwater organisms exposed to linuron†

Form of the chemical	Scientific name	Common name	Taxonomic group	Effect	End-point	Test duration (weeks)	Conc. (µg a.i. l^{-1})	Exposure[1]	Toxicant analysis[2]	Comments	Reliability (Klimisch Code*)	Reference
Technical grade purity not stated	*Myriophyllum spicatum*	Eurasian water milfoil	MAC	Photosynthesis	NOEC	4	5	s	y	Salinity 9‰	1	Kemp *et al.* 1985
Technical grade purity not stated	*Potamogeton perfoliatus*	Clasping leaf pondweed	MAC	Photosynthesis and biomass	NOEC	4–5	5	s	y	Salinity 9‰	1	Kemp *et al.* 1985
Technical grade purity not stated	*Myriophyllum spicatum*	Eurasian water milfoil	MAC	Biomass	NOEC	5	100	s	y	Salinity 9‰	1	Kemp *et al.* 1985

† Note that these species are not strictly marine, although they will tolerate brackish water up to a salinity of 10 parts per thousand. * See Annex 1.
[1] Exposure: s = static. [2] Toxicant analysis: y = measured.
MAC = macrophytes
NOEC = no observed effect concentration

Table 2.11 Most sensitive short-term aquatic toxicity data for saltwater organisms exposed to linuron

Form of the chemical	Scientific name	Common name	Taxonomic group	Effect	End-point	Test duration	Conc. (µg a.i. l^{-1})	Exposure[1]	Toxicant analysis[2]	Comments	Reliability (Klimisch Code*)	Reference
98.4% pure	*Mysidopsis bahia*	Mysid shrimp	CRU	Lethality	LC50	96 hours	3,300	s	-	-	4	Cited in US EPA RED 1995
98.4% pure	*Crassostrea virginica*	Eastern oyster	MOL	Lethality	LC50	48 hours	5,400	s	-	-	4	Ward and Boeri 1991a Cited in US EPA RED 1995
98.4% pure	*Cyprinodon variegatus*	Sheepshead minnow	FIS	Lethality	LC50	96 hours	890	-	-	-	4	Ward and Boeri 1991b Cited in US EPA RED 1995
Not stated	*Skeletonema costatum*	Diatom	ALG	Population abundance	EC50	120 hours	36	s	-	-	4	Reported in US EPA ECOTOX database
Not stated	*Vibrio fischeri*	Luminescent bacterium	BAC	Bioluminescence	EC50	30 minutes	5,500	s	n	-	4	Hernando *et al.* 2003

* See Annex 1.
[1] Exposure: s = static. [2] Toxicant analysis: n = not measured.
ALG = algae; BAC = bacteria; CRU = crustaceans; FIS = fish; MOL = molluscs
EC50 = concentration effective against 50% of the organisms tested
LC50 = concentration effective against 50% of the organisms tested

2.6.3 Toxicity to sediment-dwelling organisms

Data on direct toxicity to sediment-dwelling organisms were not found, except in the microcosm and mesocosm studies described in Section 2.6.6.

2.6.4 Endocrine-disrupting effects

Linuron binds to mammalian and fish androgen receptors (Wilson *et al.* 2004), but Kashian and Dodson (2002) found no endocrine-related effects on *Daphnia magna* sex ratios, moult frequency or other endocrine-mediated endpoints at concentrations up to 100 µg l^{-1}.

The limited evidence, therefore, suggests that linuron is a potential vertebrate endocrine disruptor by acting as an androgen receptor antagonist.

2.6.5 Mode of action of linuron

Linuron is a selective, systemic herbicide absorbed principally through the roots, but also through the foliage, with translocation in the xylem. It inhibits photosynthesis – mainly in photosystem II between quencher and plastoquinone – by blocking electron transport. This leads to the production of a range of powerful oxidants causing rapid destruction of the plant cell.

The basis of its different toxicity to different species selectivity is a combination of differential rates of absorption and transport together with different rates of detoxification. Detoxification reactions in the plant include N-demethylation, N-demethoxylation, ring hydroxylation, which can lead to the formation of conjugates with carbohydrates and peptides, and hydrolysis to the corresponding aniline (Lewis and Gardiner 1996).

2.6.6 Mesocosm and field studies

Freshwater mesocosm and field studies

Several freshwater microcosm and mesocosm studies have been performed with linuron using single, continuous or pulsed exposures.

Single application

In a study by Stephenson and Kane (1984), an artificial pond 10 m × 5 m × 1 m deep had nine 1 m^3 polythene cylinders sunk at random 5 cm into the sediment to create individual enclosures. One day after the cylinders had been placed in the pond, linuron (dissolved in acetone) was mixed into the water of three replicate enclosures to give a nominal concentration of 1,000 µg l^{-1}. Three enclosures were used as controls and the remaining three enclosures were dosed with methyl-parathion in a parallel experiment. The effects on macrophytes, zooplankton and macro-invertebrate populations were monitored over a 49-day period.

Linuron was not detected in water samples taken from the enclosures immediately before treatment. From day 1 onwards, the rate of loss of linuron was described adequately by an exponential function and calculated half-lives ranged from 16 to 40

days. Levels of dissolved oxygen declined from 13 to 4 mg l^{-1} after linuron treatment, whereas there was an increase in dissolved oxygen to day 23 in the control and then a decrease. In the open pond, there was no change in oxygen level. This difference was attributed to a lack of photosynthesis caused by linuron. The pH in linuron enclosures showed a similar pattern to the open pond. No macrophytes or filamentous algae were visible to the naked eye on day 0 in any enclosure. By day 28, considerable vegetation was present in the control enclosures, but there was still no angiosperms or algae present in the linuron enclosures on day 49. Populations of *Daphnia* spp. and *Diaptomus* declined rapidly following application of linuron and *Daphnia* did not repopulate the treated enclosures. Occasional individuals of *Diaptomus* were present in treated enclosures from day 1 onwards. Similarly, *Cyclops strenuous* and *C.agilis* populations declined to low numbers in the treatment enclosures on days 2–5. No nauplii were present by day 2 and, although recovery started after day 23, populations were still low by day 33. Overall, there were fewer families of arthropods represented in linuron enclosures compared with controls (eight versus14). Compared with the open pond, oligochaetes and chironomids were not affected. There were reduced numbers of molluscs.

Slijkerman *et al.* (2005) exposed phytoplankton and zooplankton – plus introduced macrophytes (*Elodea* sp.) and immobilised algal bioassays (*Chlorella vulgaris*) – to nominal concentrations of 20, 60 or 180 µg l^{-1} linuron in freshwater microcosms. The 3,000-litre microcosms were dosed once after a three-week acclimation period and then monitored for 28 days. Measured concentrations at the start of the study were lower than nominal concentrations and declined further during the study. Between days 0 and 28, concentrations declined from 14 to 11 µg l^{-1} in the 20 µg l^{-1} treatment, from 43 to 28 µg l^{-1} in the 60 µg l^{-1} treatment, and from 115 to 74 µg l^{-1} in the 180 µg l^{-1} treatment. All concentrations of linuron reduced dissolved oxygen and pH concentrations, and increased NH_4 and NO_3 in a dose-dependent manner. Differences in chlorophyll *a* concentrations were apparent, but were not consistently related to linuron exposure. Dose-dependent effects were also evident on the growth of *Elodea* placed in the microcosms, which were more sensitive than the immobilised algal bioassay. Effects on phytoplankton community structure occurred at all doses, with cyanobacteria (e.g. *Chroococcus*) dominating in the controls and flagellates (e.g. *Chlamydomonas*) dominating in the dosed microcosms, with no recovery by the end of the study. There were no clear effects on zooplankton.

Continuous exposure

Van den Brink *et al.* (1997) dosed duplicate freshwater microcosms with 0, 0.5, 5, 15, 50 or 150 µg l^{-1} linuron, and maintained this exposure concentration for a period of four weeks by adding linuron twice a week to compensate for any losses. Water samples were taken at several points during the study for linuron analysis. The microcosms measured 1.1 m long, 1.1 m wide, 0.7 m high, and contained 600 litres of well water over a 10-cm layer of lake sediment. The macrophyte *Elodea nuttallii* and several macroinvertebrate populations were introduced to the microcosms during the three-month acclimation period before addition of linuron. The nutrients phosphorus (0.05 mg l^{-1}) and nitrogen (0.3 mg l^{-1}) were also applied during this period.

Results for 11 weeks from the start of linuron application are reported for:

- phytoplankton (species composition and chlorophyll *a* concentration);
- periphyton (species composition, chlorophyll *a* concentration on glass slides or on *E. nuttallii*, and cell biovolume of *Cocconeis*);
- neuston (organisms in the water surface layer: species composition and chlorophyll a concentration);
- *E. nuttallii* dry weight.

Mean linuron concentrations in the microcosms were within 10 per cent of the nominal concentration for the microcosms dosed at 0.5 µg l^{-1}, and within 5 per cent for the remaining doses. The lowest no observed effect concentration (NOEC) in this study was 0.5 µg l^{-1} for *E. nuttallii* growth and photosynthesis, algal (*Cocconeis* and *Chroomonas*) abundance, and effects on oxygen and pH levels. The alga *Chlamydomonas* increased in abundance at the two highest doses, which resulted in higher chlorophyll *a* concentrations in the microcosms. Laboratory bioassays on pre-exposed *Chlamydomonas* suggest that this alga can adapt to high linuron concentrations.

Cuppen *et al.* (1997) report results from community metabolism and invertebrate endpoints measured in the study described by van den Brink *et al.* (1997). Although there were clear effects on community metabolism, as shown by the effects on pH and dissolved oxygen mentioned above, there was no effect at any concentration on the decomposition of particulate organic matter in litter bags. There was also no evidence of direct effects on invertebrates. However, indirect effects were observed as a result of changes in the abundance and composition of primary producers. The reduction in abundance of cryptophytes and diatoms and the increased abundance of *Chlamydomonas* led to a decline in rotifer abundance and an increase in copepod abundance. The elimination of *E. nuttallii* in the 150 µg l^{-1} treatments and a 50 per cent biomass reduction at 50 µg l^{-1} reduced both snail *Physella acuta* and isopod *Asellus aquaticus* abundance at the end of the study.

Pulsed exposure

Kersting and van Wijngaarden (1999) exposed organisms in large mesocosms to repeated pulses of linuron at four-week intervals. The mesocosms were designed to simulate agricultural ditches and measured 40 m long, 3.4 m wide at the water surface and 0.5 m deep, with a water capacity of 60 m^3 and a 25-cm layer of sandy loam lake sediment at the bottom. They were filled with well water, which was allowed to circulate between mesocosms for more than two years before dosing with linuron. Duplicate mesocosms were dosed with 0, 0.5, 5, 15 or 50 µg l^{-1} linuron and the water in them was kept static for seven days before a three-week period during which the mesocosms were flushed with undosed water. This was repeated so that each mesocosm was dosed on three separate occasions.

During each seven-day static period, measured linuron concentrations decreased to 50–70 per cent of nominal concentrations. The lowest NOEC reported in this study was 0.5 µg l^{-1} for effects on dissolved oxygen and pH levels. The NOEC for effects on gross primary production was 5 µg l^{-1}, with EC10 values rising with each successive exposure from 6 µg l^{-1} in May to 8 µg l^{-1} in June and 13 µg l^{-1} in July, suggesting system acclimation to linuron exposure.

Van Geest *et al.* (1999) reported on the diversity and abundance of organisms sampled in the same study as Kersting and Wijngaarden (1999). There were no significant effects of linuron on:

- macrophyte species number;
- mean area of the ponds that was covered;
- above-sediment biomass – although biomass was lower in mesocosms dosed with linuron at 50 µg l^{-1} from the second treatment on.

In situ bioassays with the macrophyte *Myriophyllum spicatum* also showed effects on dry weight in the 50 µg l^{-1} treatment. Although there were a small number of differences in the abundance of periphyton and phytoplankton species at different sampling dates, in most cases these were not consistently related to linuron exposure. Univariate analyses of 52 phytoplankton species showed consistent differences between dosed

and control mesocosms only for *Chlamydomonas*, with a NOEC of 5 µg l^{-1}. There were no effects on planktonic chlorophyll *a* levels, but there were slightly higher periphyton chlorophyll *a* concentrations at doses of 15 and 50 µg l^{-1}. There were few significant effects of linuron overall on the invertebrate community, with univariate analyses of 53 different species showing that only the rotifer *Keratella quadrata* had lower abundance than controls earlier in the study and higher abundance later in the study. The number of macroinvertebrate taxa declined slightly with increasing linuron concentrations, and tubificids and the dragonfly *Anax imperator* were more abundant in the 50 µg l^{-1} treatment.

Summary

Several well-performed microcosm and mesocosm studies with linuron have been reported, generating consistent results. The study reported by van den Brink *et al.* (1997) and Cuppen *et al.* (1997) provides the soundest basis for establishing a long-term quality standard for linuron because exposure concentrations were maintained for four weeks and a wide range of primary and secondary producers were studied. Effects occurred on some endpoints at 5 µg l^{-1}, but no effects occurred at 0.5 µg l^{-1}.

Saltwater mesocosm and field studies

No data from mesocosm or field studies using saltwater organisms were located.

3 Calculation of PNECs as a basis for the derivation of quality standards

3.1 Derivation of PNECs by the TGD deterministic approach (AF method)

3.1.1 PNECs for freshwaters

PNEC accounting for an annual average concentration

For the freshwater environment, data are available for the 'base set' of toxicity tests (i.e. tests with algae, crustaceans and fish) and, therefore, the EU Technical Guidance Document assessment factor (AF) method can be applied. Long-term (lt) data were available for seven taxonomic groups (algae, annelids, crustaceans, fish, macrophytes, molluscs and platyhelminths), with algae and macrophytes being markedly more sensitive than the other taxa. Table 2.8 summarises the most sensitive long-term freshwater toxicity data found for linuron. In the EU DAR (1996), only summary information on the tests was given and it was not possible to assign reliability codes based on the information presented.

The lowest long-term result for algae is a 72-hour growth inhibition EC10 of 1.2 µg a.i. l^{-1} for *Scenedesmus acutus* (Snel *et al.* 1998). The 95 per cent confidence limits around the EC10 were 0.7 to 2.0 µg a.i. l^{-1}, indicating that the estimate is precise. The study can be considered valid since the methodology used was described and there was analytical confirmation of the exposure concentrations. The sensitivity of algae is supported by results from other commercially confidential studies and the enclosure study of Slijkerman *et al.* (2005) (see Section 2.6.6).

The lowest long-term result for macrophytes is also reported by Snel *et al.* (1998), who estimated EC10 values for inhibition of photosystem II of 1.0 µg a.i. l^{-1} for *Ceratophyllum demersum* after five weeks of exposure and 1.3 µg a.i. l^{-1} for *Elodea nuttallii* after eight weeks of exposure. The sensitivity of macrophytes is supported by effects reported from other laboratory studies and the microcosm studies performed by van den Brink *et al.* (1997), van Geest *et al.* (1999) and Slijkerman *et al.* (2005) (see Section 2.6.6).

The lowest long-term result for invertebrates is a 21-day reproduction maximum allowable toxicant concentration (MATC) of 130–240 µg a.i. l^{-1} for *D. magna* (Baer 1991), which is supported by similar 21-day reproduction NOECs of 160 µg a.i. l^{-1} (Fischer 1989g) and 180 µg a.i. l^{-1} (Fischer 1987). However, it is not possible to assess the reliability of any of these studies because information is available only in compendium reports.

The lowest long-term result for fish is a lowest observed effect concentration (LOEC) of 30 µg a.i. l^{-1} in rainbow trout based upon haematological endpoints (Brüggeman *et al.* 1995) or cytopathological endpoints (Oulmi *et al.* 1995). These results are supported by a study cited by the US EPA (US EPA 1995), in which effects on rainbow trout early life

stages were found at the lowest test concentration of 42 µg a.i. l^{-1}. However, it is not possible to assess the reliability of this study because information is available only in a compendium report. The report by Brüggeman *et al.* (1995) contained very little methodological detail.

The lowest reliable long-term laboratory test result is, therefore, an EC10 of 1 µg a.i. l^{-1} for the macrophyte *Ceratophyllum demersum*. Long-term data are also available for algae, invertebrates and fish. Vascular plants such as *C.demersum* are expected to be sensitive to linuron, so it is unlikely that substantially more sensitive species will be found. In addition, exposure of a wide variety of organisms in several well-performed microcosm and mesocosm studies produced a lowest NOEC of 0.5 µg l^{-1} after continuous exposure (van den Brink *et al.* 1997). Therefore, application of an assessment factor of 2 to the *C. demersum* EC10 is recommended resulting in the following value:

$PNEC_{freshwater\_lt}$ = 1.0 µg l^{-1}/AF (2) = 0.5 µg l^{-1} linuron

PNEC accounting for a maximum allowable concentration

Freshwater short-term (st) toxicity data were available for nine taxonomic groups, i.e. algae, annelids, bryophytes, crustaceans, fish, insects, macrophytes, molluscs and platyhelminths. Table 2.9 summarises the most sensitive short-term freshwater toxicity data found for linuron.

As expected, these data show that linuron has the greatest effects on algae and macrophytes, with Snel *et al.* (1998) reporting a 24-hour growth EC50 of 23.6 µg a.i. l^{-1} and a 72-hour EC50 of 6.0 µg a.i. l^{-1} for the alga *Scenedesmus acutus*, and a 24-hour EC50 of 9 µg a.i. l^{-1} for inhibition of photosystem II in *Elodea nuttallii*. The study can be considered valid since the methodology used was described and there was analytical confirmation of the exposure concentrations. Data are available for invertebrates and fish, which are considerably less sensitive than plants with toxicity values ranging from 120 to 15,500 µg l^{-1} (see Table 2.9). The short-term algae and macrophyte data are within the same order of magnitude of sensitivity as the long-term data, suggesting that brief exposure to linuron may have a similar severity of effect as prolonged exposure.

Based on the guidance given in the TGD on effects assessment for intermittent releases (Section 3.3.2 of Part II of the TGD document (EU 2003) and the fact that there is a considerable short-term toxicity database for freshwater organisms, an assessment factor of 10 rather than 100 should be applied to the *E. nuttallii* 24-hour EC50. This results in the following value:

$PNEC_{freshwater\_st}$ = 9.0 µg l^{-1}/AF (10) = 0.9 µg l^{-1} linuron

3.1.2 PNECs for saltwaters

Very few data could be found on the effects of linuron to saltwater species (see Tables 2.10 and 2.11). However, data are available for the macrophytes *Myriophyllum spicatum* and *Potamogeton perfoliatus* exposed in saline medium. These data suggest that prolonged (4–5 week) exposure to linuron produces effects at similar concentrations to those found in freshwater macrophytes.

Since there are no obvious differences in the sensitivity of freshwater or saltwater species of the same taxonomic group, the TGD approach of using freshwater data within the marine effect assessment can be used where appropriate. Therefore, the freshwater data have been used in conjunction with the saltwater dataset.

PNEC accounting for an annual average concentration

Long-term single species toxicity data for saltwater organisms are only available for two macrophyte species, *Myriophyllum spicatum* and *Potamogeton perfoliatus* (Kemp *et al.* 1985) (Table 2.10). Plants were grown in 50-litre laboratory microcosms containing filtered estuarine water and sediments for a period of five weeks and then treated with linuron at concentrations ranging from 5 to 1,000 µg l^{-1}. Initial biomass was estimated directly by obtaining a blotted wet weight prior to planting and then developing a regression of wet to dry weight on a subsample of 35–40 plants. At the end of the experiment, all plants were harvested from each microcosm and shoots dried to a constant weight. After five-weeks' exposure to linuron, there was a clear trend of decreasing *P.perfoliatus* biomass with treatment at concentrations ≥50 µg l^{-1} . There was a small but insignificant increase in biomass for *M. spicatum* at 5 µg l^{-1}. A trend similar to that for *P.perfoliatus* occurred for M. *spicatum,* with mean biomass decreasing as treatments increased from 50 to 1,000 µg l^{-1}; however, significant reductions were seen only at concentrations ≥500 µg l^{-1}. The study can be considered valid since the approach adopted was well-described and there was analytical confirmation of the exposure concentrations.

Overall, the absence of long-term data for both invertebrates and fish means that it is not appropriate to generate a $PNEC_{saltwater\_lt}$ based on the saltwater data alone. However, since the long-term data for saltwater macrophytes are similar to that for corresponding freshwater species and, given the specific mode of action of linuron, it is proposed that the combined freshwater and saltwater dataset for linuron be used for PNEC generation.

The lowest long-term laboratory test result is therefore a 5-week EC10 of 1.0 µg a.i. l^{-1} for inhibition of photosystem II in the macrophyte *Ceratophyllum demersum* (Snel *et al.* 1998). The argument used in the TGD for an additional assessment factor of 10 in saltwater systems to account for greater biodiversity should not apply to linuron because of its mode of toxic action and the likelihood that PSII photosynthesisers are the most sensitive organisms. Therefore, maintaining the freshwater assessment factor of 2 for calculating a $PNEC_{salwater\_lt}$ is recommended resulting in the following value:

$PNEC_{salwater\_lt}$ = 1.0 µg l^{-1}/AF (2) = 0.5 µg l^{-1} linuron

PNEC accounting for a maximum allowable concentration

Single species short-term toxicity data for marine organisms are available for bacteria, algae, crustaceans, fish and molluscs, with algae being the most sensitive group (as shown by a 120-hour EC50 of 36 µg a.i. l^{-1} for effects of linuron on the population abundance of the diatom *Skeletonema costatum*). The available data for bacteria, invertebrates and fish show markedly lower toxicity with values, being in the range 890–5,500 µg a.i. l^{-1}. However, all the data are of questionable quality (see Table 2.11).

Once again, the highly specific mode of action of linuron strongly suggests that a maximum allowable concentration (MAC) in saltwater should be similar to one set on the basis of more extensive data for freshwater plants. Therefore, the use of the 24-hour growth EC50 of 9.0 µg a.i. l^{-1} for the macrophyte *E.nuttallii* (Snel *et al.* 1998) and application of an assessment factor of 10 is recommended resulting in the following value:

$PNEC_{saltwater\_st}$ = 9.0 µg l^{-1}/AF (10) = 0.9 µg l^{-1} linuron

3.2 Derivation of PNECs by the TGD probabilistic approach (SSD method)

The minimum number of long-term toxicity data (at least 10 NOECs from eight taxonomic groups) is not available. Therefore, the species sensitivity distribution (SSD) approach cannot be used for PNEC derivation.

3.3 Derivation of existing EQSs

In freshwaters, the annual average was derived in the UK EQS report (Lewis and Gardiner 1996) by applying a safety factor of 100 to the lowest acute effects concentration (i.e. a 48-hour EC50 to first instar *Daphnia magna* of 210 µg l^{-1}), resulting in an EQS of 2 µg l^{-1} linuron. The freshwater MAC for linuron was based on the same datum by applying a safety factor of 10, resulting in an EQS of 20 µg l^{-1} linuron expressed as a MAC.

Although it was recognised that the proposed MAC value might cause algistatic effects on some species of algae, the standard was designed as a protection against short-term high concentrations. The view was taken that, as the effects demonstrated on algae at these concentrations are algistatic rather than algicidal, populations should not be adversely affected.

It was considered that there were too few data for marine organisms to be able to set reliable EQSs for the protection of aquatic life in estuaries and coastal waters. A guideline of 2 µg l^{-1} linuron, based on the EQS derived for the protection of freshwater organisms was proposed as an interim measure. Because of the lack of data and an assumption that linuron concentrations are likely to be lower in saltwater than in freshwater systems, no EQS expressed as a MAC was proposed.

3.4 Derivation of PNECs for sediment

Linuron has been shown to remain largely in the water phase in natural sediment/water systems, with little partitioning of linuron to sediment. Consequently the risk to sediment-dwelling species through this route of exposure should be low. Although sediment may eventually become contaminated with linuron degradation products, the greatest risk to sediment-dwelling species is likely to be through exposure to linuron in the aqueous phase.

The log Kow of linuron of 2.76–3.0 (see Section 2.5) is on the borderline for requiring the derivation of PNECs for the protection of benthic organisms under the TGD. However, there is no evidence from microcosm and mesocosm studies to suggest that sediment-dwelling organisms (invertebrates and decomposers) are affected directly at concentrations near those that affect photosystem II in plants. Therefore, derivation of a sediment PNEC is not recommended.

3.5 Derivation of PNECs for secondary poisoning of predators

3.5.1 Mammalian and avian toxicity data

Several reviews have been published regarding linuron (ACP 1995, IUCLID 2000, EC 2002). The most recent reports by the European Commission[7] and IUCLID were assumed to contain the most sound and scientifically accurate mammalian data. These were therefore the primary sources used. However, the ACP review was also consulted. Additional literature searches were performed to locate any lower effect data reported since 2002, but none were located.

Due to the lack of relevant data in the IUCLID and EC reviews, the ACP review was assumed to contain the most sound and scientifically accurate data for avian toxicity. However, similarly to the mammalian data, a comprehensive literature search was also performed to locate any lower effect data reported since 1995.

In a study in rats, linuron was metabolised by demethoxylation followed by hydroxylation of the benzene ring. Major urinary metabolites were urea derivatives and no evidence of unchanged linuron was reported. Only trace amounts of 3,4-dichloroaniline were found (Gosselin *et al.* 1984)

The acute oral LD50s of linuron vary between (Maier-Bode and Hartel 1981, RSC 1991):

- 400–1,500 mg kg^{-1} body weight (bw) for rats;
- 2,400 mg kg^{-1} body weight for the mouse;
- 2,400 mg kg^{-1} body weight for the rabbit;
- 800 mg kg^{-1} body weight for the dog.

Gaines and Linder (1986) reported acute oral LD50s for adult male, adult female and weanling male rats of 1,150, 1,500 and 2,100 mg kg^{-1} body weight respectively.

Table 3.1 summarises the data available for linuron.

Table 3.1 Most sensitive mammalian and bird oral toxicity data relevant for the assessment of secondary poisoning

Study and result	Details
Sub-chronic toxicity to mammals	
Cited in: EC 2002 Lewis and Gardner 1996 **Sub-chronic NOAEL = 0.9 mg/kg bw/day**	Male and female Beagle dogs (four per sex per group) received linuron orally via their diet for 13 weeks at doses of 0, 25, 125 or 625 mg/kg diet. The NOAEL was based on haemolytic effects, increased methaemoglobin, increased Heinz bodies, slightly increased liver and spleen weights, and haemosiderin deposition that occurred at the top two doses. It is unclear in both reviews as to the identity of the original paper.

[7] Carried out when linuron added to the list of substances covered by the Plant Protection Products (PPP) Directive.

Study and result	Details
Cited in Lewis and Gardner 1996 **Sub-chronic NOAEL = 2 mg/kg bw/day**	Male and female Wistar rats (ten per sex per group) received linuron orally via their diet for 13 weeks at doses of 0, 25, 200 or 1,600 mg/kg diet. The NOAEL was based on decreased body weight gain, decreased food consumption, decreased red blood count (RBC) count, increased methaemoglobin, increased Heinz bodies and increased haemosiderin deposition in the spleen that occurred at the top two doses. It is unclear in the review as to the identity of the original paper.
Chronic toxicity to mammals	
Cited in: EC 2002 Lewis and Gardner 1996 **Chronic NOAEL = 0.9 mg/kg bw/day**	Male and female Beagle dogs (six per sex per group) received linuron orally via their diets for 52 weeks at doses of 0, 25, 125 or 625 mg/kg diet. The NOAEL was based on haemolytic effects, methaemoglobin formation, increased liver and spleen weights, and haemosiderin deposition in the liver, spleen and kidneys that occurred at the top two doses. It is unclear in both reviews as to the identity of the original paper.
Cited in Lewis and Gardner 1996 **Chronic LOEL = 1.8 mg/kg bw/day**	Male and female CD rats (80 per sex per group) received linuron orally via their diet for two years at doses of 0, 50, 125 or 625 mg/kg diet (approximately 0, 1.8, 4.5 and 25 mg/kg bw/day). The LOAEL was based on: • haemolytic effects; • reduced body weight gain; • increased testes weight; • increased haemosiderin deposition in the liver, spleen, bone marrow and lymph nodes; • increased incidences of Leydig cell adenomas, uterine adenocarinomas and ovarian tumours. It is unclear in the review as to the identity of the original paper.
Cited in: EC 2002 Lewis and Gardner 1996 **Chronic NOAEL = 1.6 mg/kg bw/day (females)**	Male and female Wistar rats (80 per sex per group) received linuron orally via their diet for two years at doses of 0, 25, 200 or 1600 mg/kg diet. The NOAEL was based on: • increased mortality, decreased body weight, haemolytic effects, haemosiderin deposition in the spleen and kidneys, and increased incidences of benign testicular interstitial tumours, uterine adenocarcinomas and ovarian tumours that occurred at the top two doses; • reduced incidences of pituitary tumours that occurred at all three doses in males and at the top two doses in females. Thus a female NOAEL can be set, but not a male NOAEL. However, the EC review (2002) states a NOAEL of 1.3 mg/kg bw/day, which is the equivalent male concentration of the 25 mg/kg diet dose. It is unclear in both reviews as to the identity of the original paper.

Study and result	Details
Effects on reproduction of mammals	
Cited in EC 2002 **Reproductive NOAEL = 0.8–1.0 mg/kg bw/day**	Experimental animals (species, strain and sex unspecified) received linuron orally (vehicle unspecified) for an unspecified duration at unspecified doses. The NOAEL was based on unspecified reproductive effects that occurred at unspecified doses that were maternally toxic. No further details were provided and it is unclear in the review as to the identity of the original paper.
Cited in Lewis and Gardner 1996 **Reproductive adult NOAEL = 0.8–1.0 mg/kg bw/day** **Reproductive offspring NOAEL = 6.4–8.0 mg/kg bw/day**	Male and female rats (30 per sex per group) received linuron orally via their diet at doses of 0, 12.5, 100 or 625 mg/kg diet during three generations. Parental rats were pre-treated for 72 days and then bred within treatment groups to produce F1 litters. After weaning, F1 animals were fed their respective treatment group's diet until all rats had been treated for 105 days and these animals were then bred to produce F2 litters. The adult NOAEL was based on decreased body weight that occurred at the top two doses, while the offspring NOAEL was based on litter viability and pathological changes that occurred at the top dose. Other effects included: • decreased general health status of F1 animals at the top dose; • corneal opacity in F1 animals at the top dose • decreased fertility in F1 males at the top dose • decreased offspring born alive and offspring viability in F1 and F2 animals at the top dose • decreased testes weight in F1 males at the top dose; • increased incidences of small testes and small and/or deformed epididymides in F1 males at the top dose. It is unclear in the review as to the identity of the original paper.
Embryotoxicity and teratogenicity	
Cited in EC 2002 **Developmental NOAEL = 10 mg/kg bw/day**	Rabbits (strain and sex unspecified) received linuron orally (vehicle unspecified) for an unspecified duration at unspecified doses. The NOAEL was based on unspecified foetotoxicity that occurred at unspecified doses that were maternally toxic. No further details were provided and it is unclear in the review as to the identity of the original paper.
Cited in Lewis and Gardner 1996 **Maternal and foetal NOAEL = 20 mg/kg bw/day**	Female Wistar rats (20 per group) received linuron orally (vehicle unspecified) during gestation days 7–16 at doses of 0, 20, 60 or 180 mg/kg bw/day. The NOAEL was based on: • decreased food consumption and body weight gain, and increased kidney and spleen weights that occurred in dams; • decreased number of live pups; • decreased body weight, delayed ossification and an increased incidence of sternal abnormalities that

Study and result	Details
	occurred in foetuses. No evidence of teratogenicity was observed, except at the top dose, at which severe maternal toxicity was also observed. This study was conducted to Good Laboratory Practice (GLP). It is unclear in the review as to the identity of the original paper.
Cited in Lewis and Gardner 1996 **Foetal NOAEL = 25 mg/kg bw/day**	Female Himalayan rabbits (15 per group) received linuron orally (vehicle unspecified) during gestation days 6 to 18 at doses of 0, 10, 25 or 62.5 mg/kg bw/day. A maternal NOAEL of 10 mg/kg bw/day was set, which was based on: • decreased food consumption and body weight gain, pregnancy abortions, mortality and liver histopathology that occurred at the top dose; • decreased food consumption at the 25 mg/kg bw/day dose. The foetal NOAEL was based on increased intrauterine deaths that occurred at the top dose. No evidence of teratogenicity was observed, except at the top dose, at which severe maternal toxicity was also observed. This study was conducted to GLP. It is unclear in the review as to the identity of the original paper.
Endocrine disruption Several studies report evidence for weak anti-androgenic activity (i.e. linuron is a weak androgen receptor antagonist), but no specific NOAELs were stated (EC 2002, Lewis and Gardiner 1996).	
Sub-chronic toxicity to birds	
Cited in Lewis and Gardner 1996 **Sub-chronic NOEC = 312.5 mg/kg diet**	Ten-day old bobwhite quails (*Colinus virginianus*; sex unspecified) received linuron via their diet at doses of 0, 312.5, 625, 1,250, 2,500 or 5,000 mg linuron (94.7% purity)/kg diet for five days. The NOEC was based on mortality, decreased food consumption, decreased body weight gain and decreased spontaneous activity that occurred at higher doses. It is unclear in the review as to the identity of the original paper.
OPP (2000). Reported in US EPA ECOTOX database **Sub-chronic LD50 = 1700 mg/kg diet**	Ten-day old bobwhite quails (*Colinus virginianus*; sex unspecified) received linuron orally via their diet at unspecified doses for eight days. The LD50 represents that dose at which 50% mortality occurs. No further details were provided.
OPP (2000). Reported in US EPA ECOTOX database **Sub-chronic LD50 = 940 mg/kg bw**	Seventeen-week old bobwhite quails (*Colinus virginianus*; sex unspecified) received linuron orally via capsules at unspecified doses for 21 days. The LD50 was based on mortality. The LD50 represents that dose at which 50% mortality occurs. No further details were provided.
No studies were available on the potential effects of linuron on avian reproduction, development or potential carcinogenicity.	

LOAEL = lowest observed adverse effect level
NOAEL = no observed adverse effect level
NOEC= no observed effect concentration

3.5.2 PNECs for secondary poisoning of predators

Lewis and Gardiner (1996) describe a bioaccumulation study on bluegill sunfish using exposure concentrations of 0.1 and 0.95 mg l^{-1} submitted by a manufacturer. They report peak whole fish bioconcentration factors (BCFs) of 49 and 38 at the low and high concentrations respectively. Corresponding peak BCFs in the muscle were 34 and 19 and in the viscera they were 200 and 170. In the remaining carcass, they were 39 and 29. There was rapid accumulation of linuron, particularly in the viscera, and equally rapid depuration from all tissues with >92 per cent loss after 14 days.

The TGD BCF trigger of 100 in whole fish is not exceeded by linuron, so there is no need to derive PNECs for secondary poisoning.

4 Analysis and monitoring

Several analytical methodologies have been developed to measure linuron concentrations in water, soil, air, plants and animal tissue. Unlike other phenylureas, linuron does not decompose into isocyanates at high temperatures, thus allowing analysis by gas chromatography (GC).

For soil samples, the limit of detection ranges from 0.01 to 0.03 mg kg^{-1} (Caux *et al.* 1998). An alternative to GC analysis is high performance liquid chromatography (HPLC) with ultraviolet (UV), electrochemical, or mass spectrometric detection. At equal injection volumes, HPLC is less sensitive than GC. But analysis of water samples, HPLC methods are superior since GC methods require the conversion of phenylureas into thermally stable derivatives and are unable to separate linuron, diuron, monolinuron, buturon and monuron.

A Standing Committee of Analysts 'Blue Book' method for determining phenylurea herbicides in river and drinking water was published in 1994 (SCA 1994). Using thermospray liquid chromatography/mass spectrometry (LC-MS), limits of detection of 5 ng l^{-1} have been achieved for linuron.

An analytical method based on HPLC with photodiode array detection has been developed for the determination of linuron in aqueous soil extracts containing different amounts of organic matter (0.7–11.7 per cent) (Sanchez *et al.* 1996). The detection limit of linuron in the aqueous soil extracts was 0.01 µg ml^{-1} (0.2 ng) and the relative standard deviation for repeatability based on peak area measurement ranged between 0.7 and 3.1 per cent. Recoveries from spiked samples ranged from 106.3 to 116.1 per cent. The method uses a C-18 reversed-phase column, a mobile phase of methanol/water (65:35 v/v) at a flow rate of 1 ml min^{-1} and UV detection at 210 nm. The method was applied to determine the adsorption of linuron by soils

Liquid chromatography with diode array UV detection (LC-DAD UV) and positive ion electrospray mass spectrometry (LC-ESI-MS) were used for the separation, identification and quantification of 10 sulfonyl- and phenylurea herbicides. Both procedures – solid-phase extraction (SPE) LC-DAD UV and LC-ESI-MS – were applied to spiked river waters and were compared in terms of selectivity and sensitivity. The detection limits obtained in river water samples were below 50 ng l^{-1} for both procedures (Carabias-Martinez *et al.* 2004).

A multi-residue method to screen ultra traces of neutral pesticides from water samples in compliance with European Union regulations has been reported using SPE followed by HPLC coupled to atmospheric pressure electrospray ionisation mass spectrometry (ESI-MS) (Nogueira *et al.* 2004). Calibration conditions of the method in the selected ion monitoring (SIM) mode showed excellent linear response for the 12 pesticides studied (carbaryl, carbofuran, methomyl, oxamyl, pirimicarb, chlortoluron, diuron, isoproturon, linuron, atrazine, propazine and simazine) in the range from 1 to 50 µg l^{-1}. Instrumental limits of detection of 0.10 µg l^{-1} were measured for linuron. The SPE assays using octadecilsilica cartridges are relatively simple and highly reproducible, requiring low volume water samples (50 ml)

For water, proposed PNECs derived for linuron range from 0.3 to 0.6 µg l^{-1}. The data quality requirements are that, at a third of the EQS, total error of measurement should not exceed 50 per cent. Using this criterion, it is evident that current analytical methodologies (non-standard) employing thermospray LC-MS, capable of achieving detection limits as low as 5 ng l^{-1}, should offer adequate performance to analyse for linuron.

5 Conclusions

5.1 Availability of data

Long-term laboratory data are available for seven different freshwater taxonomic groups including algae, annelids, crustaceans, fish, macrophytes, molluscs and platyhelminths. Freshwater short-term toxicity data are available for nine taxonomic groups including algae, annelids, bryophytes, crustaceans, fish, insects, macrophytes, molluscs and platyhelminths. Freshwater algae and macrophytes are the most sensitive taxa of those tested with both technical linuron and various linuron formulations. For marine organisms, single species short-term toxicity data are available for six different taxonomic groups (bacteria, algae, crustaceans, fish, macrophytes and molluscs). However, long-term toxicity data are available only for macrophytes. Laboratory data are supplemented by freshwater mesocosm data, which confirm the high sensitivity of algae and macrophytes to linuron.

Limited evidence suggests that linuron is a potential vertebrate (fish and mammals) endocrine disruptor by acting as an androgen receptor antagonist.

5.2 Derivation of PNECs

The proposed PNECS are described below and summarised in Table 5.1.

5.2.1 Long-term PNEC for freshwaters

The lowest reliable long-term laboratory test result is an EC10 of 1.0 µg active ingredient (a.i.) l^{-1} for the macrophyte *Ceratophyllum demersum*. Long-term data are also available for algae, invertebrates and fish. Vascular plants such as *C.demersum* are expected to be sensitive to linuron, so it is unlikely that substantially more sensitive species will be found. In addition, exposure of a wide variety of organisms in several well-performed microcosm and mesocosm studies produced a lowest NOEC of 0.5 µg l^{-1} after continuous exposure. Therefore, application of an assessment factor of 2 to the *C.demersum* EC10 is recommended resulting in a $PNEC_{freshwater\_lt}$ of 0.5 µg l^{-1}.

This value is lower than the existing EQS of 2.0 µg l^{-1}, which was derived by applying a safety factor of 100 to the lowest acute effects concentration (i.e. a 48-hour EC50 to first instar *Daphnia magna* of 210 µg l^{-1}).

5.2.2 Short-term PNEC for freshwaters

Reliable short-term toxicity data are available for algae, invertebrates and fish. The lowest short-term value is a 24-hour EC50 of 9.0 µg a.i. l^{-1} for inhibition of photosystem II in the macrophyte *Elodea nuttallii*. Since there is a considerable short-term toxicity database for freshwater organisms which shows that macrophytes such as *Elodea* are likely to be among the most sensitive to linuron, an assessment factor of 10 rather than 100 should be applied resulting in a $PNEC_{freshwater\_st}$ of 0.9 µg l^{-1}.

This value is lower than the existing EQS of 20 µg l^{-1}, which was derived by applying a safety factor of 10 to the lowest acute effects concentration (i.e. a 48-hour EC50 to first instar *Daphnia magna* of 210 µg l^{-1}).

5.2.3 Long-term PNEC for saltwaters

Long-term single species toxicity data for saltwater organisms are available only for two macrophyte species. The absence of long-term data for algae, invertebrates and fish means that it is not possible to generate a $PNEC_{saltwater\_lt}$ based on the saltwater data alone. Therefore, it is proposed that the combined freshwater and saltwater dataset is used for the PNEC generation.

The lowest long-term laboratory test result is therefore a 5-week EC10 of 1.0 µg a.i. l^{-1} for inhibition of photosystem II in the macrophyte *Ceratophyllum demersum*. The argument used in the EU Technical Guidance Document for an additional assessment factor of 10 in saltwater systems to account for greater biodiversity should not be applied to linuron because of its mode of toxic action and the likelihood that photosystem II photosynthesisers such as *Ceratophyllum* are the most sensitive organisms. Therefore maintaining the freshwater assessment factor of 2 for calculating a $PNEC_{salwater\_lt}$ is recommended, resulting in a $PNEC_{saltwater\_lt}$ of 0.5 µg l^{-1}.

This value is slightly lower than the interim guideline of 2.0 µg l^{-1} in the existing EQS report, which was 'read across' from the freshwater long-term value.

5.2.4 Short-term PNEC for saltwaters

Single species acute toxicity data for marine organisms are available for bacteria, algae, crustaceans, fish and molluscs, with algae being the most sensitive group. The highly specific mode of action of linuron strongly suggests that a maximum allowable concentration in saltwater should be similar to one set on the basis of more extensive data for freshwater plants. Therefore the use of the 24-h growth EC50 of 9.0 µg a.i. l^{-1} for the macrophyte *E.nuttallii* and application of an assessment factor of 10 is recommended, resulting in a $PNEC_{saltwater\_st}$ of 0.9 µg l^{-1}.

There is no current short-term EQS for saltwaters.

5.2.5 PNEC for sediments

The log Kow of linuron lies between 2.76 and 3.0, which is on the borderline for requiring the derivation of PNECs for the protection of benthic organisms under the TGD. However, there is no evidence from microcosm and mesocosm studies to suggest that sediment-dwelling organisms (invertebrates and decomposers) are directly affected at concentrations near those that affect photosystem II in plants. Therefore derivation of a sediment PNEC is not recommended.

5.2.6 PNEC for secondary poisoning

Bioconcentration data (as BCF values) for linuron for aquatic organisms are low, with values for fish ranging from 38 to 49. Hence the TGD BCF trigger of 100 in whole fish is not exceeded and the derivation of PNECs for secondary poisoning of predators is not required.

Table 5.1 Summary of proposed PNECs

Receiving medium/exposure scenario	Proposed PNEC (µg l^{-1})	Existing EQS (µg l^{-1})
Freshwater/long-term	0.5	2.0 (AA)
Freshwater/short-term	0.9	20 (MAC)
Saltwater/long-term	0.5	2.0 (AA)
Saltwater/short-term	0.9	–
Sediment	Not required	–
Secondary poisoning	Not required	–

AA = annual average
MAC = maximum allowable concentration

5.3 Analysis

The data quality requirements are that, at a third of the EQS, total error of measurement should not exceed 50 per cent. Using this criterion, it is evident that current analytical methodologies (non-standard) employing GC-MS and capable of achieving detection limits as low as 5 ng l^{-1} should offer adequate performance to analyse for linuron.

5.4 Implementation issues

These PNECS are suitable for use as EQSs because they are not subject to excessive uncertainty and analytical capability should be adequate for compliance assessment purposes.

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List of abbreviations

AA	annual average
AF	assessment factor
a.i.	active ingredient
BCF	bioconcentration factor
bw	body weight
CAS	Chemical Abstracts Service
DAR	Draft Assessment Report
EC50	concentration effective against 50% of the organisms tested
EQS	Environmental Quality Standard
GC	gas chromatography
GLP	Good Laboratory Practice (OECD)
HPLC	high performance liquid chromatography
LC50	concentration lethal to 50% of the organisms tested
LC-MS	liquid chromatography/mass spectrometry
LOAEL	lowest observed adverse effect level
LOEC	lowest observed effect concentration
lt	long term
MAC	maximum allowable concentration
MATC	maximum allowable toxicant concentration
NA	not applicable
ND	no data
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
OECD	Organization for Economic Co-operation and Development
PNEC	predicted no-effect concentration
ppm	parts per million
SPE	solid-phase extraction
SSD	species sensitivity distribution
st	short term
TGD	Technical Guidance Document
UKTAG	UK Technical Advisory Group
UV	ultraviolet
US EPA	US Environmental Protection Agency
WFD	Water Framework Directive

ANNEX 1 Data quality assessment sheets

Identified and ordered by alphabetical order of references.

Data relevant for PNEC derivation were quality assessed in accordance with the so-called Klimisch Criteria (Table A1).

Table A1 Klimisch Criteria*

Code	Category	Description
1	Reliable without restrictions	Refers to studies/data carried out or generated according to internationally accepted testing-guidelines (preferably GLP**) or in which the test parameters documented are based on a specific (national) testing guideline (preferably GLP), or in which all parameters described are closely related/comparable to a guideline method.
2	Reliable with restrictions	Studies or data (mostly not performed according to GLP) in which the test parameters documented do not comply totally with the specific testing guideline, but are sufficient to accept the data or in which investigations are described that cannot be subsumed under a testing guideline, but which are nevertheless well-documented and scientifically acceptable.
3	Not reliable	Studies/data in which there are interferences between the measuring system and the test substance, or in which organisms/test systems were used that are not relevant in relation to exposure, or which were carried out or generated according to a method which is not acceptable, the documentation of which is not sufficient for an assessment and which is not convincing for an expert assessment.
4	Not assignable	Studies or data which do not give sufficient experimental details and which are only listed in short abstracts or secondary literature.

* Klimisch H.-J, Andreae M and Tillmann U (1997) A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*, **25**, 1–5.
** OECD Principles of Good Laboratory Practice (GLP). See: http://www.oecd.org/department/0,2688,en_2649_34381_1_1_1_1_1,00.html

Reference	(cited by) ACP 1995

Information on the test species	
Test species used	*Oncorhynchus mykiss*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	juvenile

Information on the test design	
Methodology used	Not stated
Form of the test substance	Technical grade linuron (96.2% purity) solubilised with DMF
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	Yes (mean measured concentrations 53–97% of nominal)
Measurement of water quality parameters	Yes (temperature = 11.4–12.9°C; dissolved oxygen = 7.2–9.6 mg l^{-1}; pH 7.3–8.0)
Test validity criteria satisfied	Fish loading factor 3.6 g l^{-1}
Water quality criteria satisfied	Yes
Study conducted to GLP	Not stated
Comments	Response curve was nearly asymptotic. Undissolved material was seen in the 10.4 and 17.4 mg l^{-1} tanks

Reliability of study	**Not assignable**
Relevance of study	**Relevant**
Klimisch Code	**4**

Reference	(cited by) ACP 1995

Information on the test species	
Test species used	*Lepomis macrochirus*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	juvenile

Information on the test design	
Methodology used	Not stated
Form of the test substance	Technical grade linuron (96.2% purity) solubilised with DMF
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	Yes (mean measured concentrations 93–109% of nominal)
Measurement of water quality parameters	Yes (temperature = 21.0–21.2°C; dissolved oxygen = 5.7–8.7 mg l^{-1}; pH 7.0–8.2)
Test validity criteria satisfied	Fish loading factor 4.2 g l^{-1}
Water quality criteria satisfied	Yes
Study conducted to GLP	Not stated
Comments	Undissolved material was seen in the two highest concentrations 12.6 and 18.9 mg l^{-1}

Reliability of study	**Not assignable**
Relevance of study	**Relevant**
Klimisch Code	**4**

Reference	(cited by) ACP 1995

Information on the test species	
Test species used	*Daphnia magna*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	neonates

Information on the test design	
Methodology used	Not stated
Form of the test substance	Technical grade linuron (94.4% purity) solubilised with DMF
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	No
Measurement of water quality parameters	Yes (temperature = 19.9°C; dissolved oxygen = 7.2–8.5 mg l^{-1}; pH 7.3–7.8)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Yes
Study conducted to GLP	Not stated
Comments	-

Reliability of study	**Not assignable**
Relevance of study	**Relevant**
Klimisch Code	**4**

Reference	(cited by) ACP 1995

Information on the test species	
Test species used	*Selenastrum capricornutum*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	neonates

Information on the test design	
Methodology used	Not stated
Form of the test substance	Technical grade linuron (purity not stated) solubilised with Tween80/acetone
Source of the test substance	Not stated
Type and source of the exposure medium	Artificial growth medium; temperature = 24 ± 1°C; pH 7.6–10.4, light intensity ~ 7,000 lux
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	No
Measurement of water quality parameters	Not applicable
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not applicable
Study conducted to GLP	Not stated
Comments	-

Reliability of study	**Not assignable**
Relevance of study	**Relevant**
Klimisch Code	**4**

Reference	Abou-Waly and Shabana 1993

Information on the test species	
Test species used	*Nostoc muscorum*
Source of the test organisms	Isolated from Nile River water
Holding conditions prior to test	Cultured in sterile modified Watanabe medium
Life stage of the test species used	Exponential growth

Information on the test design	
Methodology used	Algae were exposed to linuron for seven days and then washed, placed in uncontaminated media and their growth monitored for a further 23 days.
Form of the test substance	Linuron (98% purity)
Source of the test substance	Ciba-Geigy, Switzerland
Type and source of the exposure medium	Sterile modified Watanabe medium
Test concentrations used	0.045, 0.375, 0.75, 3 and 4 mg l^{-1}.
Number of replicates per concentration	3
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	No
Measurement of water quality parameters	No
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Comments	-

Reliability of study	**Reliable with restrictions (standard exposure not fully reported)**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	Brüggemann *et al.* 1995

Information on the test species	
Test species used	*Oncorhynchus mykiss*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	28-day flow-through test and examination of haematology and histopathology.
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Spring water
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Flow-through
Measurement of exposure concentrations	Yes
Measurement of water quality parameters	Yes (temperature = 10±1°C; pH 7.7–7.8; oxygen saturation = 90%)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Comments	-

Reliability of study	**Not assignable**
Relevance of study	**Relevant**
Klimisch Code	**4**

Reference	Cuppen *et al.* 1997

Information on the test species	
Test species used	Macrophyte dominated freshwater microcosms
Source of the test organisms	Natural lake sediment plus introduced macrophytes (*Elodea nuttallii*) and populations of macroinvertebrates
Holding conditions prior to test	Three-month acclimation period during which water was circulated between microcosms.
Life stage of the test species used	Various

Information on the test design	
Methodology used	Freshwater microcosms (1.1 m long, 1.1 m wide, 0.7 m high, with water volume of 600 litres) were dosed twice a week to maintain stable concentrations of linuron for four weeks.
Form of the test substance	As the product Afalon
Source of the test substance	Not stated
Type and source of the exposure medium	Well water and natural lake sediment
Test concentrations used	0.5, 5, 15, 50 and 150 µg l^{-1}
Number of replicates per concentration	2
Number of organisms per replicate	Natural populations
Nature of test system (static, semi-static or flow-through, duration, feeding)	Semi-static
Measurement of exposure concentrations	Yes (Mean measured concentrations were within 10% of nominal at lowest dose and within 5% at all other doses.)
Measurement of water quality parameters	Yes
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Study conducted to GLP	Not stated
Comments	-

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	Fischer 1981 Unpublished, cited in EU DAR 1996

Information on the test species	
Test species used	*Daphnia magna*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Study conducted in accordance with US EPA guidelines.
Form of the test substance	Formulation - emulsifiable concentrate 198 g l^{-1} linuron
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	No
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	No
Comments	-

Reliability of study	**Not assignable**
Relevance of study	**Relevant**
Klimisch Code	**4**

Reference	Fischer 1987 Unpublished, cited in EU DAR 1996

Information on the test species	
Test species used	*Daphnia magna*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Neonates < 24-hours-old

Information on the test design	
Methodology used	Study conducted in accordance with US EPA guidelines.
Form of the test substance	Technical grade linuron 94.8% purity
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	0, 0.032, 0.56, 0.1, 0.18 and 0.32 mg l^{-1} nominal
Number of replicates per concentration	20
Number of organisms per replicate	1 or 5 (total 44)
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static renewal three times per week
Measurement of exposure concentrations	Yes
Measurement of water quality parameters	Yes (temperature = 19.6–20.8°C; dissolved oxygen = 6.2–9.5 mg l^{-1}; hardness = 41.5–49.9 mg l^{-1} $CaCO_3$; pH 7.1–7.9)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Yes
Comments	Mean measured concentrations were 100–107% of nominal for new solutions and 100–109% for old solutions. However, individual replicates ranged from 70–180% and 48–190% of nominal for old and new respectively. This may explain why no clear dose-response relationship was observed.

Reliability of study	**Unreliable**
Relevance of study	**Relevant**
Klimisch Code	**3**

Reference	Fischer 1989a Unpublished, cited in EU DAR 1996

Information on the test species	
Test species used	*Scenedesmus subspicatus* chodat
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Study conducted in accordance with OECD Guideline 201
Form of the test substance	Technical linuron 94.8% purity
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	1.8, 3.2, 5.6, 10, 18, 32, 56, 100, 180 and 320 µg l^{-1} plus blank and solvent controls
Number of replicates per concentration	3
Number of organisms per replicate	Initial 10^4 cells ml^{-1}
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	No
Measurement of water quality parameters	Not applicable
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not applicable
Study conducted to GLP	Yes
Comments	-

Reliability of study	**Not assignable**
Relevance of study	**Relevant**
Klimisch Code	**4**

Reference	Fischer 1989b Unpublished, cited in EU DAR 1996

Information on the test species	
Test species used	*Daphnia magna*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Study conducted in accordance with OECD Guideline 202
Form of the test substance	Formulation – wettable powder 48.7% w/w
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	Yes (actual 99.0–101.6% of nominal)
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Yes
Comments	-

Reliability of study	**Not assignable**
Relevance of study	**Relevant**
Klimisch Code	**4**

Reference	Fischer 1989c Unpublished, cited in EU DAR 1996

Information on the test species	
Test species used	*Scenedesmus subspicatus* CHODAT
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Study conducted in accordance with OECD Guideline 201
Form of the test substance	Formulation – wettable powder 48.7% w/w
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	No
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Yes
Comments	-

Reliability of study	**Not assignable**
Relevance of study	**Relevant**
Klimisch Code	**4**

Reference	Fischer 1989d Unpublished, cited in EU DAR 1996

Information on the test species	
Test species used	*Salmo gairdneri (Oncorhynchus mykiss)*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Study conducted in accordance with OECD Guideline 203
Form of the test substance	Formulation – wettable powder 48.7% w/w
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	Yes (actual 99.7–101.2% of nominal)
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Yes
Comments	-

Reliability of study	**Not assignable**
Relevance of study	**Relevant**
Klimisch Code	**4**

Reference	Fischer 1989e Unpublished, cited in EU DAR 1996

Information on the test species	
Test species used	*Cyprinus carpo*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Study conducted in accordance with OECD Guideline 203
Form of the test substance	Formulation – wettable powder 47.5% w/w
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	Yes (actual 96.9–97% of nominal)
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Yes
Comments	-

Reliability of study	**Not assignable**
Relevance of study	**Relevant**
Klimisch Code	**4**

Reference	Fischer 1989f Unpublished, cited in EU DAR 1996

Information on the test species	
Test species used	*Salmo gairdneri (Oncorhynchus mykiss)*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Mean weight 1.58–2.03 g

Information on the test design	
Methodology used	Study conducted in accordance with OECD Guideline 204
Form of the test substance	Technical grade linuron 94.8% purity
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	0.01, 0.05,0.1,0.5 and 1.0 mg l^{-1} nominal 0.01, ND, 0.12,0.64, and 1.34 mg l^{-1} measured
Number of replicates per concentration	1
Number of organisms per replicate	10
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	Yes
Measurement of water quality parameters	Yes (temperature = 13.2–14.5°C; dissolved oxygen = 9.0–11.3 mg l^{-1}; hardness = 335–350 mg l^{-1} $CaCO_3$; pH 7.8–8.5)
Test validity criteria satisfied	Fish loading 0.04 g $l^{-1}day^{-1}$ at the start of testing
Water quality criteria satisfied	Not stated
Study conducted to GLP	Yes
Comments	Fish exposed to ≥0.5 mg l^{-1} (nominal) were reported to suffer slowed reactions, narcotic condition, swimming head up or at the surface and reduced food intake. Symptoms appeared day 5 at 1.0 mg l^{-1} and day 20 at 0.5 mg l^{-1}. No significant effects on growth seen. This study did not cover different fish growth stages or reproductive effects and is not a true chronic study by today's standards.

Reliability of study	**Not assignable**
Relevance of study	**Relevant**
Klimisch Code	**4**

Reference	Fischer 1989g Unpublished, cited in EU DAR 1996

Information on the test species	
Test species used	*Daphnia magna*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Neonates < 24-hours-old

Information on the test design	
Methodology used	Study conducted in accordance with US EPA guidelines.
Form of the test substance	Formulation – wettable powder 49.3% w/w linuron
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	0, 0.0032, 0.01, 0.032, 0.1, 0.32, and 1.0 mg l^{-1} nominal
Number of replicates per concentration	4
Number of organisms per replicate	10
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static renewal three times per week
Measurement of exposure concentrations	Yes
Measurement of water quality parameters	Yes (temperature = 19.7–20.3°C; dissolved oxygen = 4.6–9.7 mg l^{-1}; hardness = 244–263 mg l^{-1} $CaCO_3$; pH 7.5–8.2)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	No – dissolved oxygen fell below acceptable levels.
Study conducted to GLP	Yes
Comments	Mean measured concentrations were 97.1–128% of nominal for new solutions and 89.4–106.3% for old solutions.

Reliability of study	**Not assignable**
Relevance of study	**Relevant**
Klimisch Code	**4**

Reference	Hernando *et al.* 2003

Information on the test species	
Test species used	(1) *Vibrio fischeri* (2) *Daphnia magna*
Source of the test organisms	(1) *Vibrio fischeri*: Biotox test (Turku, Finland) (2) *Daphnia magna*: Daphtoxkit (Creasol, Belgium)
Holding conditions prior to test	Freeze-dried bacteria or daphnid cysts
Life stage of the test species used	Bacteria or 24-hour daphnids

Information on the test design	
Methodology used	(1) *V. fischeri*: ISO 11348 (2) *D. magna*: OECD Guideline 202
Form of the test substance	'Highest purity available'
Source of the test substance	Chem-Service
Type and source of the exposure medium	(1) *V. fischeri*: distilled water (2) *D. magna*: culture medium (non-chlorinated water/spring water (1:1) to give 170 mg $CaCO_3$ l^{-1}; temperature 21±1°C
Test concentrations used	Not stated
Number of replicates per concentration	6
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	No
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Comments	-

Reliability of study	**Not assignable**
Relevance of study	**Relevant**
Klimisch Code	**4**

Reference	Hulsen *et al.* 2002

Information on the test species	
Test species used	*Lemna minor*
Source of the test organisms	Pond in Ghent University botanical garden.
Holding conditions prior to test	Stock culture grown at 25°C in pH 6.5 medium.
Life stage of the test species used	Fronds

Information on the test design	
Methodology used	7-day exposure and assessment of total number of fronds, doubling time and total frond area.
Form of the test substance	Analytical grade linuron
Source of the test substance	Chem-Service
Type and source of the exposure medium	*Lemna* mineral medium
Test concentrations used	Eight concentrations ranging from 0.0025 to 0.32 mg l^{-1}.
Number of replicates per concentration	6
Number of organisms per replicate	5
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	No
Measurement of water quality parameters	No
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Comments	-

Reliability of study	**Reliable with restrictions (no chemical analysis)**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	Kemp *et al.* 1985

Information on the test species	
Test species used	*Potamogeton perfoliatus* L. *Myriophyllum spicatum* L.
Source of the test organisms	Choptank River estuary, a tributary of Chesapeake Bay
Holding conditions prior to test	Microcosms planted and allowed to acclimatise for seven weeks prior to dosing. Temperature = 21.5±1.5°C; salinity = 9±1.1‰; dissolved oxygen = >3–4 mg l^{-1}
Life stage of the test species used	Size at start 6–14 cm stem length; 2–6 cm rhizome length

Information on the test design	
Methodology used	Plants were established in 50-litres aquaria and effects of linuron on respiration and growth were measured over a four-week period.
Form of the test substance	Technical grade (purity not stated) dissolved in 100 ml methanol
Source of the test substance	E.I. du Pont de Nemours Co
Type and source of the exposure medium	Filtered estuarine water (salinity ~9‰) and sediment obtained from brackish portion of Choptank River estuary
Test concentrations used	0, 0.005, 0.05, 0.10, 0.5 and 1.0 mg l^{-1} nominal + solvent control
Number of replicates per concentration	2
Number of organisms per replicate	10
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	Yes. Concentrations analysed in water, sediment and plants at termination of experiment
Measurement of water quality parameters	Yes (temperature, salinity, dissolved oxygen)
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Study conducted to GLP	Not stated
Comments	At the end of the experiment 9–22% of linuron remained in the water column; approximately 1.5% was in sediment.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	Kersting and van Wijngaarden 1999

Information on the test species	
Test species used	Naturally colonising organisms plus introduced *Elodea nuttalli*.
Source of the test organisms	Sediment from a non-polluted lake and *Elodea* from *Elodea*-rich mesocosms.
Holding conditions prior to test	Water circulated between mesocosms for >2 years before study.
Life stage of the test species used	Various

Information on the test design	
Methodology used	Freshwater mesocosms (40 m long, 3.4 m wide, 0.5 m deep, with water volume of 600 m^3) were dosed three times with the same dose at four week intervals. After each treatment, the mesocosms were kept static for seven days and then flushed with undosed water for three weeks before the next dose.
Form of the test substance	Afalon
Source of the test substance	Hoechst Holland
Type and source of the exposure medium	Natural well water overlying 25 cm of sandy loam sediment from a non-polluted lake
Test concentrations used	0, 0.5, 5, 15 and 50 µg l^{-1}.
Number of replicates per concentration	2
Number of organisms per replicate	Natural populations
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static during exposure (seven days) and then flushed through before next dose (water residence time = 8 days)
Measurement of exposure concentrations	Yes (Measured concentrations were close to nominal at the start of each seven-day exposure period and declined to 50–70% of nominal by the end of this period.)
Measurement of water quality parameters	Yes (Dissolved oxygen and pH were measured to examine ecosystem metabolism.)
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Study conducted to GLP	Not stated
Comments	-

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	Knauf 1980a Unpublished, cited in EU DAR 1996

Information on the test species	
Test species used	*Daphnia magna*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Neonates 24-hours-old

Information on the test design	
Methodology used	Conducted to US EPA guidelines.
Form of the test substance	Technical linuron (94.4% purity) dissolved in dimethylformamide (DMF)
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	12 nominal concentrations ranging from 0.18 to 100 mg l^{-1} plus water and solvent controls
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not sated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	No
Measurement of water quality parameters	Yes (temperature = 22±1°C; dissolved oxygen = 7.9–9 mg l^{-1}; hardness = 44 mg l^{-1} $CaCO_3$; pH 7.6–7.8)
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	No
Comments	At 0.56 mg l^{-1}, daphnids became 'irritated' and movements were uncoordinated; therefore NOEC stated to be 0.32 mg l^{-1}.

Reliability of study	**Not assignable**
Relevance of study	**Relevant**
Klimisch Code	**4**

Reference	Knauf 1980b Unpublished, cited in EU DAR 1996

Information on the test species	
Test species used	*Salmo gairdneri (Oncorhynchus mykiss)*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Conducted to US EPA guidelines.
Form of the test substance	Technical grade linuron (purity not stated)
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not sated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	No
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	No
Comments	This data point was considered the most reliable acute value for fish by the UK rapporteur to EU DAR.

Reliability of study	**Not assignable**
Relevance of study	**Relevant**
Klimisch Code	**4**

Reference	Knauf 1980c Unpublished, cited in EU DAR 1996

Information on the test species	
Test species used	*Salmo gairdneri (Oncorhynchus mykiss)*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Conducted to BBA* guidelines
Form of the test substance	Formulation – emulsifiable concentrate 197 g l^{-1} linuron
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not sated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	No
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	No
Comments	-

Reliability of study	**Not assignable**
Relevance of study	**Relevant**
Klimisch Code	**4**

* Biologische Bundesanstalt (German Federal Biological Research Centre).

Reference	Knauf 1980d Unpublished, cited in EU DAR 1996

Information on the test species	
Test species used	*Idus melanotus*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Conducted to BBA guidelines
Form of the test substance	Formulation – emulsifiable concentrate 197g l^{-1} linuron
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not sated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	No
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	No
Comments	-

Reliability of study	**Not assignable**
Relevance of study	**Relevant**
Klimisch Code	**4**

Reference	Knauf 1980e Unpublished, cited in EU DAR 1996

Information on the test species	
Test species used	*Ictalurus nebulosus*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Juvenile

Information on the test design	
Methodology used	Conducted to US EPA guidelines.
Form of the test substance	Technical grade linuron (purity not stated), solubilised with acetone
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not sated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	No
Measurement of water quality parameters	Yes (temperature = 21–22°C; pH 6.8–8.0)
Test validity criteria satisfied	Fish loading 0.1 g l^{-1}
Water quality criteria satisfied	Dissolved oxygen low at 72 and 96 hours
Study conducted to GLP	No
Comments	-

Reliability of study	**Not assignable**
Relevance of study	**Relevant**
Klimisch Code	**4**

Reference	Knauf and Shulze 1972

Information on the test species	
Test species used	*Chlorella vulgaris* *Lemna* sp. *Rhiziella* sp. *Cyprinus carpio* *Lebistes reticulatus*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	Not stated
Form of the test substance	Technical grade (purity not stated)
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated except 96-hour test with *C.vulgaris* 0.01, 0.05, 0.2 and 1 mg l^{-1}.
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	No
Measurement of water quality parameters	Not stated
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	No
Comments	Few details of the test methodology are given and the tests were conducted prior to GLP. The UK rapporteur to EU DAR noted that the notifier considered that the studies were conducted to acceptable prevailing standards and that the result is valid.

Reliability of study	**Reliable with restriction (few methodology details and no chemical analysis)**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	Mayer and Ellersieck 1986

Information on the test species	
Test species used	(1) *Daphnia magna* (2) *Chironomus plumosus* (3) *Ictalurus punctatus*
Source of the test organisms	Not stated
Holding conditions prior to test	Not stated
Life stage of the test species used	(1) 1st instar (2) 3rd instar (3) 0.4 g (technical) and 0.6 g (wettable powder)

Information on the test design	
Methodology used	ASTM 1980 or Committee on Methods for Toxicity Tests with Aquatic Organisms 1975
Form of the test substance	Technical grade linuron (95.1%) and wettable powder (50% active ingredient)
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	Not stated
Measurement of water quality parameters	(1) Temperature = 17°C; pH 7; hardness = 43 mg l^{-1} $CaCO_3$ (2) Temperature = 22°C; pH 7; hardness = 43 mg l^{-1} $CaCO_3$ (3) Temperature = 22°C; pH 7; hardness = 40 mg l^{-1} $CaCO_3$
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Study conducted to GLP	Not stated
Comments	-

Reliability of study	**Reliable with restriction (standard procedures not fully reported, no chemical analysis)**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	Oulmi *et al.* 1995

Information on the test species	
Test species used	*Oncorhynchus mykiss*
Source of the test organisms	Reared from eggs at field station of Bavarian Federal Agency for Water Research, Germany.
Holding conditions prior to test	Well water
Life stage of the test species used	Weight = 14.1±2.3 g Length = 10±1.8 cm

Information on the test design	
Methodology used	Exposed for five weeks and then examined cytopathologically.
Form of the test substance	Linuron: 99.96% purity.
Source of the test substance	Ehrenstorfer GmbH, Augsburg, Germany.
Type and source of the exposure medium	Well water. Hardness = 379 mg l^{-1} $CaCO_3$; conductivity = 744 µS; pH 7.66 ± 0.1; dissolved oxygen = 10.5±0.5 mg l^{-1}; temperature = 9.4±0.5^{o}C.
Test concentrations used	Nominal = 0, 30, 120 and 240 µg l^{-1}. Measured = 0, 30.8±6.2, 125.8±17.3 and 235.8±10.4 µg l^{-1}.
Number of replicates per concentration	2
Number of organisms per replicate	10
Nature of test system (static, semi-static or flow-through, duration, feeding)	Flow-through
Measurement of exposure concentrations	Yes
Measurement of water quality parameters	Yes
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Study conducted to GLP	Not stated
Comments	-

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	Slijkerman *et al.* 2005

Information on the test species	
Test species used	Naturally colonising organisms plus introduced *Elodea* and immobilised algal bioassays (*Chlorella vulgaris*).
Source of the test organisms	Water and sediment from Lake Markermeer in the Netherlands.
Holding conditions prior to test	Water was mixed within each microcosm for ~3 weeks before dosing.
Life stage of the test species used	Various

Information on the test design	
Methodology used	Microcosms (circular glass fibre tanks 1.95 m high, with internal diameter of 2.25 m (top) and 1.7 m (bottom) and 3,000-litre capacity) dosed once and zooplankton and phytoplankton then monitored for 27 days. The growth of *Elodea* and immobilised algae in calcium-alginate beads placed in the microcosms was also measured over 27 and 21 days respectively.
Form of the test substance	Linuron (97% active ingredient)
Source of the test substance	Luxan, Elst, the Netherlands
Type and source of the exposure medium	Lake Markermeer water (average P = 0.22 mg l^{-1}; average N =1.91 mg l^{-1}) and sediment (3% organic carbon, 50% particles <63 µm).
Test concentrations used	20, 60 and 180 µg l^{-1}.
Number of replicates per concentration	2
Number of organisms per replicate	Various natural populations, plus two bunches of five stems of *Elodea* and three sets of 30 algal (*C. vulgaris*) beads per microcosm.
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	Yes (between days 0 and 28, concentrations declined from 14 to 11 µg l^{-1} in the 20 µg l^{-1} treatment, from 43 to 28 µg l^{-1} in the 60 µg l^{-1} treatment, and from 115 to 74 µg l^{-1} in the 180 µg l^{-1} treatment.)
Measurement of water quality parameters	Yes (effects of linuron apparent on dissolved oxygen and pH at all concentrations)
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Study conducted to GLP	Not stated
Comments	-

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	Snel *et al.* 1998

Information on the test species	
Test species used	*Elodea nuttallii* *Myriophyllum spicatum* *Potamogeton crispus* *Ranunculus circinatus* *Ceratophyllum demersum* *Chara globularis* *Scenedesmus acutus*
Source of the test organisms	Macrophytes collected from experimental ditches in the Netherlands. Algae (*Scenedesmus*) were grown axenically and then immobilised in alginate beads.
Holding conditions prior to test	Incubation for 24 hours in nutrient-supplemented well water
Life stage of the test species used	Young macrophyte shoots (5 cm) and immobilised algae.

Information on the test design	
Methodology used	PSII electron flow was measured after 24-hour exposure of macrophytes and after four-week exposure in microcosms (see van den Brink *et al.* 1997 for details). PSII efficiency and cell number were measured for *S.acutus* during 72-hour exposures.
Form of the test substance	Not stated for laboratory tests; as Afalon for microcosm tests.
Source of the test substance	Not stated
Type and source of the exposure medium	Nutrient-supplemented well water.
Test concentrations used	Laboratory tests: 0, 0.1, 1, 10, 25, 100 and 150 µg l^{-1}. Microcosms: 0, 0.5, 5, 15, 50 and 150 µg l^{-1}.
Number of replicates per concentration	Laboratory tests: 3 Microcosms: duplicates
Number of organisms per replicate	Laboratory tests: 1 Microcosms: 4g of *E.nuttallii* shoots.
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static.
Measurement of exposure concentrations	Yes (but results for laboratory tests not stated)
Measurement of water quality parameters	Laboratory tests: no; microcosm tests: yes.
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Comments	-

Reliability of study	**Reliable with restrictions**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	Stephenson and Kane 1984

Information on the test species	
Test species used	(1) *Lemna minor* (L) (2) *Chlorella vulgaris* (Beij) (3) *Daphnia magna* (Straus) (4) *Daphnia* spp (pulex and longispina) (5) *Diaptomus gracilis* (Sars)
Source of the test organisms	(1) and (2) not stated (3) laboratory culture (4) and (5) field collected
Holding conditions prior to test	Not stated
Life stage of the test species used	(3) <24-hours-old and adults

Information on the test design	
Methodology used	The laboratory test procedures are described but not in detail.
Form of the test substance	Technical grade linuron, 80% pure
Source of the test substance	Not stated
Type and source of the exposure medium	(1) Hutners medium (2) Culture media (3) (4) and (5) dechlorinated tap water ~ 250 mg l^{-1} as $CaCO_3$
Test concentrations used	(1) logarithmically spaced series (2) (3) (4) and (5) not stated
Number of replicates per concentration	(1) 4 (2) 3 (3) (4) and (5) not stated
Number of organisms per replicate	(1) 6 fronds (2) initial conc 5 x 10^3 cells ml^{-1} (3) 20 (4) 8 (5) 20
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	No
Measurement of water quality parameters	(1) N/A (2) N/A (3) (4) and (5) temperature = 19.5±1°C
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Comments	The EC50 value for *L.minor* is given as 7 µg l^{-1}. However, Lewis and Gardiner (1996) report the EC50 as 70 µg l^{-1} following communications with the authors. EC50 *D. magna* 590 µg l^{-1} <24-hours-old and 310 µg l^{-1} adults.

Reliability of study	**Reliable with restrictions (standard exposures not fully reported, no chemical analysis)**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	Stephenson and Kane 1984

Information on the test species	
Test species used	Macrophytes; zooplankton; macroinvertebrates
Source of the test organisms	Natural populations
Holding conditions prior to test	Not stated
Life stage of the test species used	Various

Information on the test design	
Methodology used	Polythene enclosures (1 m^3) were placed, 5 cm into the sediment, within artificial ponds 1 m deep. In addition, three sampling stations were established in the pond. The ponds were dosed once. Zooplankton were sampled prior to dosing and on days 0, 1, 2, 4, 7, 12, 23 and 33. Macroinvertebrates sampled on days -6, -2 and day 49, Macrophytes on days 0, 28 and 49. Changes in toxicity of water within enclosures were followed by inhibition growth bioassays using *L. minor*.
Form of the test substance	Technical grade linuron, 80% pure
Source of the test substance	Not stated
Type and source of the exposure medium	Not stated
Test concentrations used	1 mg l^{-1} plus control
Number of replicates per concentration	3
Number of organisms per replicate	Natural populations
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	Yes
Measurement of water quality parameters	Yes (temperature, dissolved oxygen and pH, alkalinity; decline in dissolved oxygen up to day 23 and then progressive recovery thereafter)
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Study conducted to GLP	Not stated
Comments	-

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	van den Brink *et al.* 1997

Information on the test species	
Test species used	Macrophyte dominated freshwater microcosms
Source of the test organisms	Natural lake sediment plus introduced macrophytes (*Elodea nuttallii*) and populations of macroinvertebrates
Holding conditions prior to test	Three-month acclimation period during which water was circulated between microcosms.
Life stage of the test species used	Various

Information on the test design	
Methodology used	Freshwater microcosms (1.1 m long, 1.1 m wide, 0.7 m high, with water volume of 600 litres were dosed twice a week to maintain stable concentrations of linuron for four weeks.
Form of the test substance	As the product Afalon
Source of the test substance	Not stated
Type and source of the exposure medium	Well water and natural lake sediment
Test concentrations used	0.5, 5, 15, 50 and 150 µg l^{-1}
Number of replicates per concentration	2
Number of organisms per replicate	Natural populations
Nature of test system (static, semi-static or flow-through, duration, feeding)	Semi-static
Measurement of exposure concentrations	Yes. Mean measured concentrations were within 10% of nominal at lowest dose and within 5% at all other doses.
Measurement of water quality parameters	Yes (reported in associated paper by Cuppen *et al.* 1997)
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Study conducted to GLP	Not stated
Comments	-

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	Van Geest *et al.* 1999

Information on the test species	
Test species used	Naturally colonising organisms plus introduced *Elodea nuttalli*.
Source of the test organisms	Sediment from a non-polluted lake and *Elodea* from *Elodea*-rich mesocosms.
Holding conditions prior to test	Water circulated between mesocosms for >2 years before study.
Life stage of the test species used	Various

Information on the test design	
Methodology used	Freshwater mesocosms (40 m long, 3.4 m wide, 0.5 m deep, with water volume of 600 m^3) were dosed three times with the same dose at four week intervals. After each treatment, the mesocosms were kept static for seven days and then flushed with undosed water for three weeks before the next dose. *In situ* bioassays were also run with *Myriophyllum spicatum*.
Form of the test substance	Afalon
Source of the test substance	Hoechst Holland
Type and source of the exposure medium	Natural well water overlying 25 cm of sandy loam sediment from a non-polluted lake
Test concentrations used	0, 0.5, 5, 15 and 50 µg l^{-1}
Number of replicates per concentration	2
Number of organisms per replicate	Natural populations
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static during exposure (seven days) and then flushed through before next dose (water residence time = 8 days)
Measurement of exposure concentrations	Yes (Measured concentrations were close to nominal at the start of each seven-day exposure period and declined to 50–70% of nominal by the end of this period.)
Measurement of water quality parameters	Yes (Dissolved oxygen and pH were measured to examine ecosystem metabolism.)
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Study conducted to GLP	Not stated
Comments	-

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**1**

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