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Maximum Permissible Concentrations and Negligible Concentrations for phthalates (dibutylphthalate and di(2-ethyl-hexyl) phthalate), *with special emphasis on endocrine disruptive properties*

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ABSTRACT

In the current report maximal permissible concentrations (MPCs) and negligible concentrations (NCs) are derived for di-*n*-butylphthalate (DBP) and di(2-ethylhexyl)phthalate (DEHP).
Phthalates are often mentioned as suspected endocrine disrupters. Data are collected with endpoints related to the endocrine or reproductive system for *in vitro* as well as in *in vivo* tests. Especially the two-generation reproduction studies appear sensitive in detecting endocrine disruptive effects. None of the tests with uterine weight (often in combination with vaginal cornification) showed positive results
The most sensitive endpoints in the *in vivo* studies are converted to concentrations in the organic carbon exerting adverse effects. It is concluded based on the available data that the MPCs derived based upon the classically used endpoints (survival, growth and reproduction), give sufficient protection against adverse endocrine disruptive effects.

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CONTENTS

SAMENVATTING

In dit rapport worden maximaal toelaatbare risiconiveaus (MTRs) en verwaarloosbare risiconiveaus (VRs) gepresenteerd voor di-*n*-butylftalaat (DBP) en di(2-ethylhexyl) ftalaat (DEHP).
Ftalaat esters worden veelvuldig gebruikt als weekmakers. In sommige plastics bestaat de helft van het totale gewicht uit ftalaten. Een kwart van de geproduceerde ftalaten is DEHP. Ftalaat esters zijn niet persistent; ze hebben verschillende afbraakroutes. Door de aanzienlijke directe en indirecte emissies, kunnen de in het milieu aangetroffen concentraties verklaard worden. Ophoping in de voedselketen is niet te verwachten vanwege biotransformatie van de ftalaten.

De MTRs zijn afgeleid op basis van ecotoxicologische en milieuchemische gegevens. Voor DBP zijn chronische toxiciteits data voor het aquatisch ecosysteem beschikbaar voor algen, crustaceën en vissen. De chronische toxiciteit van DBP voor zoetwater organismen ligt in de orde van mg/l. De meest gevoelige NOEC is voor de vis *Oncorhynchus mykiss*. Er zijn veel acute toxiciteit data voor aquatische organismen. De verschillende taxonomische groepen (protozoa, algen, crustaceën, insekten en vissen) zijn vergelijkbaar in hun gevoeligheid. Er zijn geen verschillen in de gevoeligheid van zoet- en zoutwater organismen. Voor DBP zijn geen bruikbare bodem- of sediment toxiciteit testen gevonden.
Voor DEHP zijn in de meerderheid van de acute en chronische toxiciteitstudies (voor algen, crustaceën, vissen, mollusken en amphibiën) geen effecten gevonden in de hoogst geteste concentratie. Deze hoogst geteste concentratie overstijgt veelal de wateroplosbaarheid. Voor oligochaeten en microbiële processen in bodem is eveneens geen toxiciteit gevonden van DEHP in de hoogst geteste concentratie. Een studie in sediment met als eindpunt het uit het ei komen van de kikker *Rana arvalis*, geeft een NOEC van 10 mg/kg versgewicht.
De op basis van bovenstaande gegevens afgeleide MTRs en VRs zijn vermeld in Tabel A.

Tabel A. Overzicht van de MTRs en VRs die zijn afgeleid voor water en standaard bodem en/of sediment (10% organisch koolstof).

Stof	MTR_{water} (µg/l)		VR_{water} (µg/l)	$MTR_{bodem/sediment}$ (mg/kg versgewicht)		$VR_{bodem/sediment}$ (mg/kg versgewicht)
DBP	10	*EPA/10*	0.1	0.7	*EP*	0.007
DEHP	0.19	*EP*	0.002	1.0	*EPA/10*	0.01

EPA/10: Afgeleid met behulp van een assessment factor van 10. EP: Deze waarde is het resultaat van harmonizatie via de 'equilibrium partitioning methode'

Ftalaten zijn vaak genoemd als verdachte endocrien verstorende stoffen. Een doel van onderhavig rapport is te beslissen of en hoe effecten op het endocriene en/of reproduktieve systeem geïncorporeerd moeten worden in MTRs voor ftalaten. Daarvoor zijn gegevens verzameld van *in vitro* en *in vivo* testen met ftalaten, met eindpunten die verband houden met het endocriene of reproductieve systeem. Voor verschillende ftalaten (DIDP, DMP, DOP, DIHP, DINP, zie de voetnoot bij Tabel 4.1. voor de gebruikte afkortingen) zijn geen effecten gerapporteerd bij de hoogst geteste concentraties in de *in vitro* systemen. Voor andere verbindingen (DBP, DEP, DHP,

DEHP, DIBP en MEHP) zijn wel endocrien vestorende effecten gevonden. De relatieve potentie afgezet tegen het natuurlijk hormoon 17β-oestradiol is altijd laag (10^{-4}-10^{-8}). Sommige auteurs melden een verschil in potentie tussen de verschillende ftalaten, anderen vinden geen duidelijke potentie verschillen.
De verdenking van endocrien verstorende potentie moet bevestigd worden in gegevens verkregen in *in vivo* testen. Redenen hiervoor zijn verschillen in metabolisme, biobeschikbaarheid en toxicokinetiek tussen *in vitro* en *in vivo* test systemen, en de afwezigheid van intercellulaire interactie en mechanismen gerelateerd aan endocriene homeostase in *in vitro* systemen. In de *in vivo* testen zijn meestal de muis of de rat gebruikt als proefdier. Endocrien verstorende effecten zijn gevonden in deze testen voor DBP, DEHP, BBP, DEP en DHP, maar niet voor de andere geteste ftalaten (DOP, DIHP, DINP, DIDP). Vooral de twee-generatie reproductie studies blijken gevoelig om endocrien verstorende effecten op te pikken. Geen van de testen met als eindpunt uterien gewicht (vaak gemeten in combinatie met vaginale cornificatie) liet een positief resultaat zien. Het onderscheid tussen ftalaten die al dan niet endocrien verstorend zijn, blijkt hetzelfde te zijn voor *in vitro* en *in vivo* testen. Het is aangenomen dat de gevoeligheid van de zoogdieren die getest zijn niet verschilt van in het wild levende zoogdieren. De meest gevoelige eindpunten gemeten in de *in vivo* testen zijn omgerekend naar concentraties in het organisch koolstof die gepaard gaan met ongewenste effecten.
De conclusie is dat de MTRs die zijn afgeleid (Tabel A) voldoende bescherming bieden tegen endocrien verstorende effecten, en dat er geen noodzaak is voor deze ftalaten om extra gegevens in de MTR-afleiding te incorporeren.

Gemeten concentraties van DBP zijn zelden hoger dat de MTR voor water of sediment. Gemeten concentraties voor DEHP in water zijn 3 tot 20 maal hoger dan de MTR, en ook de concentraties die gerapporteerd zijn in sediment zijn hoger dan de afgeleide MTR.

SUMMARY

This report presents maximum permissible concentrations (MPCs) and Negligible Concentrations (NCs) for di-*n*-butylphthalate (DBP) and di(2-ethylhexyl)phthalate (DEHP).
Phthalate esters (esters of 1,2-benzene dicarboxylic acid) are widely used as plasticisers. In some plastics, phthalates comprise up to 50% of the total weight. A quarter of the plasticisers produced is DEHP. Phthalate esters have several degradation pathways and therefore are not considered to be intrinsically highly persistent chemicals. In view of the considerable direct and expected indirect emission however, fluxes may be high explaining the concentrations found in the environment. Bioaccumulation in the food chain is limited because of biotransformation.

The MPCs are derived using data on (eco)toxicology and environmental chemistry, and represent the potential risk of the substances to the ecosystem.
For DBP, chronic toxicity data for aquatic organisms are found for algae, crustaceans and fishes. The chronic toxicity of DBP on freshwater organisms is in the order of mg/l (0.1-6.1 mg/l). The most sensitive NOEC is observed for Oncorhynchus mykiss. Acute toxicity data for aquatic organisms are abundant, and the various taxonomic groups (protozoa, algae, crustaceans, insects and fish) are comparable in their sensitivity. No differences are found in the sensitivity of freshwater and saltwater organisms. No useful soil or sediment tests were found for DBP.
For DEHP, in the majority of acute and chronic studies (on algae, crustaceans, fish, mollusks and amphibians) no effects are found at the highest tested concentration. This highest tested concentration often exceeds the water solubility.
For *oligochaeta* and microbial processes in soil no toxicity of DEHP is found at the highest tested concentration. A study in sediment on hatching success from the moorfrog *Rana arvalis* reports a NOEC of 10 mg/kg fresh weight.
The MPCs and NCs derived are given in Table A.

Table A. Overview of the maximum permissible concentrations and NCs that are derived for water and standard soil/sediment (10% organic matter).

Compound	MPC_{water} (µg/l)		NC_{water} (µg/l)	$MPC_{soil/sediment}$ (mg/kg fresh weight)		$NC_{soil/sediment}$ (mg/kg fresh weight)
Dibutylphthalate	10	*EPA/10*	0.1	0.7	*EP*	0.007
Di-n-ethylhexyl-phthalate	0.19	*EP*	0.002	1.0	*EPA/10*	0.01

EPA/10: Preliminary effects assessment is used with an assessment factor of 10. EP: this value is the result of harmonization via the equilibrium partitioning method

Phthalates are often mentioned as suspected endocrine disrupters. An objective of the present report is to develop a viewpoint concerning if and how effects on the endocrine/reproductive system should be incorporated in MPCs for phthalates.
Data are collected for *in vitro* as well as in *in vivo* tests performed with phthalates, with endpoints related to the endocrine or reproductive system.
For several of the phthalate compounds (DIDP, DMP, DOP, DIHP, DINP, see note to Table 4.1. for the abbreviations used) no effects were found at the highest tested concentration in any of the *in vitro* tests. For other compounds (DBP, DEP, DHP,

BBP, DEHP, DIBP and MEHP) endocrine disruptive effects are reported. The relative potency related to the potency of the natural estrogen, 17ß-estradiol, is always low (10^{-4}-10^{-8}). Some authors report differences in potency between the phthalates tested, others do not find clear potency differences.
The suspicion of endocrine disruptive potency must be confirmed in *in vivo* data. Reasons therefore are differences in metabolism, bioavailability and toxicokinetics between *in vitro* and *in vivo* test systems, and the absence of intercellular interaction and mechanisms related to endocrine homeostasis in *in vitro* systems. Mostly mouse and rat are used as test organisms. Endocrine disruptive effects are found in *in vivo* tests for DBP, DEHP, BBP, DEP and DHP, but not for the other phthalates tested (DOP, DIHP, DINP, DIDP). Especially the two-generation reproduction studies appear sensitive in detecting endocrine disruptive effects. None of the tests with uterine weight (often in combination with vaginal cornification) showed positive results. The distinction between phthalates which can or cannot act as endocrine disrupters appears to be the same from *in vitro* and *in vivo* tests.
It is assumed that the sensitivity of the mammals that are tested in the *in vivo* tests does not differ from mammalian wildlife. The most sensitive endpoints in the *in vivo* studies are converted to concentrations in the organic carbon exerting adverse effects. It is concluded base on the available data that the MPCs derived (Table A), will give sufficient protection against endocrine disruptive effects. There is no need for these phthalates to include extra data in the derivation of MPCs.

Measured concentrations of DBP are seldom above the MPCs for water and sediment. However, reported concentrations in water for DEHP are 3 to 20 times higher than the MPC, and for sediments reported concentrations are also higher that the MPC for sediment.

1. INTRODUCTION

1.1. The project 'Setting Integrated Environmental Quality Standards'

This report is produced in the framework of the project 'Setting Integrated Environmental Quality Standards'. The aim of the project is to derive concentration limits for substances in the environment for the different compartments, air, water, sediment and soil. It is based on the risk philosophy of the Ministry of Housing, Spatial Planning and the Environment which is laid down in the policy document "Premises for Risk Management" (VROM, 1989b). The concentration limits are referred to as Environmental Quality Standards (EQSs) in Dutch Environmental Policy.
The EQSs set by the Ministry of VROM are based on risk limits, the Maximum Permissible Concentrations (MPC) and Negligible Concentrations (NCs).

This report presents MPCs and NCs for di-*n*-butylphthalate (DBP) and di(2-ethylhexyl)phthalate (DEHP). The MPCs are derived using data on (eco)toxicology and environmental chemistry, and they represent the potential risk of the substances to the ecosystem. The NCs are derived by applying a factor of 100 towards the MPC. The derivation of the MPC is performed at the National Institute of Public Health and the Environment (RIVM), under the authority of the Ministry of Housing, Spatial Planning and the Environment (Ministry of VROM). A commission of experts from a variety of governmental research institutes, non-governmental organisations and industry is involved in the supervision of the derivation of risk limits. The process of deriving integrated EQSs is shown schematically in Figure 1.1.
Both DBP and DEHP are currently undergoing a risk assessment in the framework of the EU-existing chemicals programme (according to council regulation 793/93).

The results obtained until now in the project 'Setting Integrated Environmental Quality Standards' are laid down in several reports. In the report 'Desire for Levels' (Van de Meent *et al.*, 1990) a methodology was proposed for deriving MPCs for several compounds such as heavy metals, chlorophenols, pesticides and polycyclic aromatic hydrocarbons (PAHs). Based on this method, EQSs for water, sediment and soil were set by the Minister of VROM (VROM, 1991). In subsequent reports, MPCs an NCs were proposed for nine trace metals (Van de Plassche and De Bruijn, 1992), several volatile compounds (Van de Plassche and Bockting, 1993) and substances with a potential for secondary poisoning (Van de Plassche, 1994). The MPCs and NCs for PAHs as derived in Van de Meent *et al.* (1990), have been updated by Kalf *et al.* (1995; 1997). MPCs and NCs for metals and pesticides have been updated by Crommentuijn *et al.* (1997ab). Reuther *et al.* (1998) derived MPCs and NCs for aniline derivatives. Risk limits for boron, silver, titanium, tellurium, uranium and organosilicon compounds are derived in Van de Plassche *et al.* (1999), and recently MPCs have been proposed for PCBs (Van Wezel *et al.*, 1999). The MPCs and NCs derived until 1997 are summarised by De Bruijn *et al.* (1999).

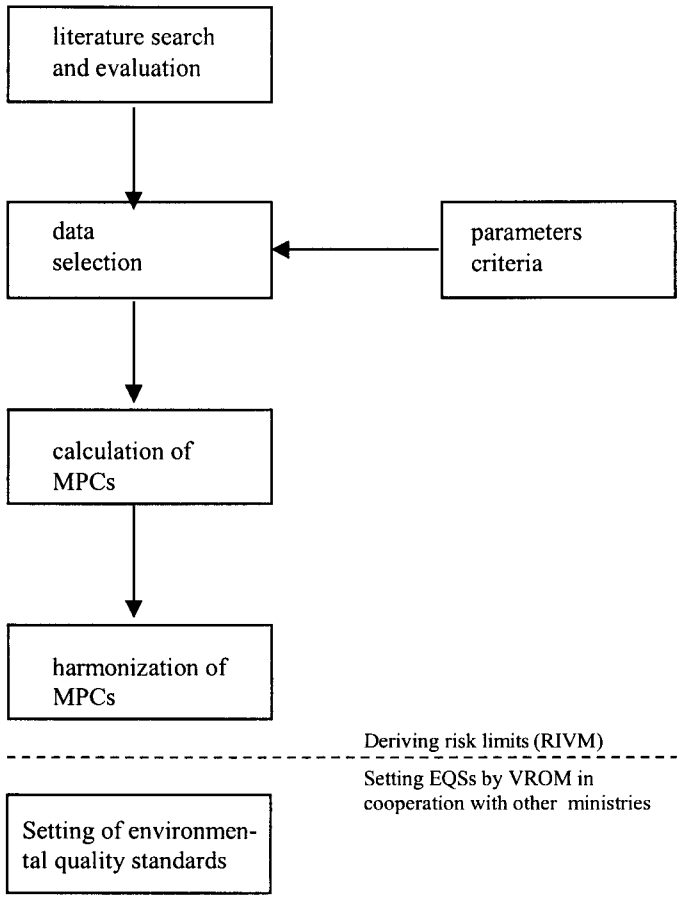

Figure 1.1. The process of deriving Integrated Environmental Quality Standards

1.2. Phthalates

Phthalate esters (esters of 1,2-benzene dicarboxylic acid) are widely used as plasticisers to increase the flexibility and workability of high-molecular-weight polymers. Their low melting point and high boiling point make them also very useful as heat transfer fluids and carriers. In some plastics, phthalates comprise up to 50% of the total weight. Phthalates can be found in ink, paint, adhesives, vinyl flooring and some food products (e.g. baby milk formula and cheese) (MAFF, 1995, 1996ab). The world-wide production of phthalates approximates 2.7 million metric tons a year (Bauer & Herrmann, 1997). A quarter of the plasticisers produced is DEHP. The Dutch registration of emission to air for total phthalates emission by industry gives a figure of 40022 kg/year for the Netherlands in the year 1995. The direct emission is decreasing; in 1997 the emission is estimated to be 14200 kg/year. However, also indirect emission by leaching, volatilisation and migration during the use of products and during disposal is of great importance for the phthalates. In domestic waste, DEHP and DBP can be measured in concentrations up to 1500 and 18 mg/kg d.w. respectively (Bauer & Herrmann, 1997). As phthalates are not chemically bound to the polymeric matrix, they may relatively easy enter into the environment.

The molecular structure of DBP and DEHP is given in Figure 1.2. Some physical-chemical properties are given in Table 1.1.

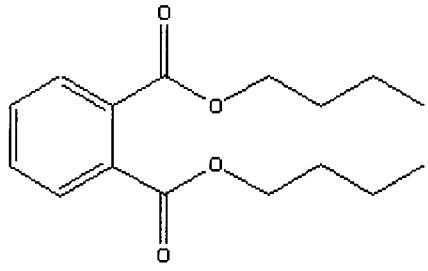

Figure 1.2.a. Di-*n*-butyl-phthalate (DBP), with an alkyl chain length of 4 carbon atoms

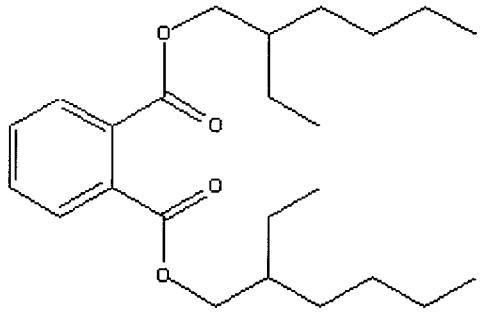

Figure 1.2.b. Di(2-ethylhexyl)phthalate (DEHP), with an alkyl chain length of 8 carbon atoms

According to Staples *et al.* (1997a) The best estimates for the water solubility's of DBP and DEHP are 11.2 and 0.003 mg/l, respectively. Experimental determination of the water solubility of a compound such as DEHP meets many difficulties. DEHP, and to a lesser extent DBP, is bipolar in nature. It may form micelles or other types of aggregates. Experimentally determined water solubility for DEHP, as given in the literature, span several orders of magnitude: from 0.6 µg/l in seawater obtained using a generator column to 0.34 mg/l using a shake flask/centrifugation method. Calculations based on structure activity relationships (SPARC, EPIWIN) give in general lower estimates for the water solubility than experimental results, i.e. respectively 4.9 and 1.5 mg/l for DBP and 1.1 and 2.6 µg/l for DEHP (Staples *et al.*, 1997a).

In the atmosphere, phthalates are easily photodegraded via free-radical attack (half-lives of ±1 day). Phthalates can also be degraded by bacteria and actinomycetes, under both aerobic as well as anaerobic conditions (≥60% in 28 days in standard aerobic biodegradation tests) (Staples *et al.*, 1997a). In unacclimated test systems consisting of fresh or marine waters, primary degradation half-lives are between 2 and 30 days for DEHP and between 0.2 and 10 days for DBP. In unacclimated test systems for soil, results for degradation half-lives for DBP vary between 0.6 and 100 days and for DEHP between 20 and 400 days (Staples *et al.*, 1997a). It is concluded

Table 1.1.: Physical-chemical properties of dibutylphthalate and di(2-ethylhexyl)phthalate

Compound	CAS-No	Formula	MW (g/mol)	Water solubility[a] (mg/l)	log Kow[a]	Henry's constant ($Pa \cdot m^3 \cdot mol^{-1}$)[a]	Vapour Pressure (mm Hg)[a]	Melting point (°C)[a]
Di-n-butylphthalate (DBP)	84-74-2	$C_{16}H_{22}O_4$	278.34	11.2	4.45	0.089	$2.7 \cdot 10^{-5}$	-35
Di-(2-ethylhexyl)phthalate (DEHP)	117-81-7	$C_{24}H_{38}O_4$	390.56	$3 \cdot 10^{-3}$	7.50	1.73	$1.0 \cdot 10^{-7}$	-47

[a]Staples *et al.*, 1997a; recommended data based on review of the literature

that phthalate esters have several degradation pathways and therefore are not considered to be intrinsically highly persistent chemicals. In view of the considerable direct and expected indirect emission however, fluxes may be high explaining the concentrations found in the environment (see Chapter 5).
Published data for partitioning between organic carbon and water (K_{oc}) are reviewed by Staples *et al.* (1997a). For DBP, K_{oc} values vary over two orders of magnitude, and for DEHP differences of approximately a factor of 50 are found between different sources (Table 1.2.). The differences can at least partly be attributed to difficulties in discriminating between truly dissolved chemicals and chemicals which formed complexes with organic colloids.
Bioaccumulation in the food chain is limited because of biotransformation (Staples *et al.*, 1997a). For this reason, the extra risk related to accumulation in the food chain was not explicitly considered in the derivation of the MPC. The normal procedure in the project 'Setting Integrated Environmental Quality Objectives' is to consider this risk for compounds with a relatively high log K_{ow} (Van de Plassche, 1994; Van Wezel et al., 1999). Elimination rate constants are higher than predicted based on the K_{ow}, which is commonly used to predict elimination rate constants (De Wolf *et al.*, 1992). Bioconcentration factors for fish show no relationship with K_{ow}. Bioconcentration to other aquatic organisms (e.g. algae, molluscs, etc.) seems to be higher than to fish, pointing to lower metabolic capacities of these organisms.

Table 1.2.: Properties related to environmental fate processes of dibutylphthalate and di(2-ethylhexyl)phthalate

Compound	K_{oc} (L/kg)[a] soil/sediment	K_{oc} (L/kg)[a] suspended solids	BCF for fish[a] (ml/g wet)	BCF for other organisms[a] (ml/g wet)
Di-n-butylphthalate (DBP)	$1.4 \cdot 10^3$-$15 \cdot 10^3$	$1.2 \cdot 10^3$-$16 \cdot 10^4$	3.6-172	78-5500
Di-(2-ethylhexyl)phthalate (DEHP)	$87 \cdot 10^3$-$51 \cdot 10^4$	$22 \cdot 10^3$-$1.0 \cdot 10^6$	2-6510 mean:280±230	30-5400

[a]Staples *et al.*, 1997a

1.3. Aim of this report

MPCs are, among other things, used for setting emission reduction objectives. Currently in the Netherlands, a 'desirable level' (in Dutch: *streefwaarde*) of 0.1 mg/kg d.w. standard soil/sediment for the sum of phthalates is in use (IWINS, 1997). However, this value is not derived on basis of risk limits and is not reliably underpinned by data on the ecotoxicology and the environmental chemistry of phthalates. There is a need for more information on the risks of phthalates and the goal of this report is to meet this demand.
For the compounds di-n-butylphthalate (DBP) and di(2-ethylhexyl)phthalate (DEHP), an MPC is derived following the methods that are commonly used in the project 'Setting integrated environmental quality standards' (CSR, 1996). These phthalate compounds are most relevant as they occur in the highest concentrations in the environment (Belfroid, 1998). Much research results are expected to be published concerned with phthalates in the coming years. Therefore it was not felt appropriate to derive MPCs for a whole series of phthalates.

Phthalates are often mentioned as suspected endocrine disrupters, i.e. (anti-)estrogens or -androgens (e.g. Colborn *et al.*, 1993; Gillesby & Zacharewski, 1998; Sohoni & Sumpter, 1998). Especially information from *in vitro* testing give rise to this suspicion

(e.g. Jobling *et al.*, 1995; see for more information chapter 4). Given the ongoing scientific and political debate on this subject, it is important to develop a position concerning if and how these effects on the endocrine/reproductive system should be incorporated in the setting environmental quality standards for phthalates. This is a second objective of the current report. In this report effects related to endocrine disruption as observed in *in vitro* as well as in *in vivo* assays are collected for phthalates, and are discussed. Results from *in vitro* assays can give a first indication that the compound is able to act as a xeno-estrogen, these results may or may not be confirmed in *in vivo* studies with different species. The endocrine disruptive potency of a chemical may differ per species, as the receptor as well as metabolic capacities and pathways differ between species. It is discussed how data from *in vitro* and *in vivo* test with endpoints specifically related to endocrine disruption can be used and compared with the MPCs derived.

2. METHODOLOGY

2.1. General scheme

The maximum permissible concentrations and negligible concentrations are derived as described in CSR (1996) and the methods generally applied in the reports produced within the framework of the project 'Setting Integrated Environmental Quality Standards' (Van de Meent *et al.*, 1990; Van de Plassche and De Bruijn, 1992; Van de Plassche and Bockting, 1993; Van de Plassche, 1994, 1999; Kalf *et al.*, 1995; Crommentuijn *et al.*, 1997ab; Reuther *et al.*, 1998).
In short, data on chronic and acute toxicity for aquatic and terrestrial species of a compound are searched for, evaluated and selected or rejected. The maximum permissible concentration (MPC) is derived using either the refined assessment method as described by Aldenberg and Slob (1993), or assessment factors modified to the EPA method. The MPCs are harmonised according to the equilibrium partition theory. In this way it is prevented that a concentration on an MPC-level in one compartment leads to an exceeding of the MPC in another compartment.

2.2. Data collection

Main literature sources for the required information on DBP and DEHP are Staples *et al.* (1997ab) that review the aquatic toxicity and the environmental fate of phthalate esters, and the documents that are in preparation in the EU-existing chemicals programme for both compounds. Additional literature is searched for via the databases Biosis and Toxline, via the library of the National Institute for Public Health and the Environment, and via reptrospective search based upon public literature and reviews. Data are collected until the beginning of 1999.

2.3. Data selection

A toxicity study is considered reliable if the design of the experiment is in agreement with international accepted guidelines, such as the guidelines described by the OECD (1984a-e, 1992a-b). To judge studies which have not been performed according to these guidelines, criteria are developed within the framework of the project 'Setting Integrated Environmental Quality Standards' (CSR, 1996).
Effects on growth, reproduction or survival are used in the derivation of MPCs, as they are directly related to population dynamics. In order to protect the soil ecosystem, also microbial processes are taken into account. Toxicity data from soil or sediment studies are normalised to organic matter, they are recalculated into toxic concentrations in a so-called standard soil with 10% organic matter.
For each species and each compound, the most sensitive toxicity test is selected. If for a single species several toxicity values are found for the same effect parameter, the geometric mean is calculated.

Table 2.1.: Assessment factors used in the preliminary effect assessment

Available information	Assessment factor
Aquatic ecosystems	
lowest acute L(E)C_{50}-values or QSAR estimate for acute toxicity	1000
lowest acute L(E)C_{50}-value or QSAR estimate for acute toxicity for minimal algae/crustaceae/pisces	100
lowest NOEC-value or QSAR estimate for chronic toxicity	10*
lowest NOEC-value or QSAR estimate for minimal algae/crustaceae/pisces	10
Terrestrial ecosystems	
lowest acute L(E)C_{50}-values or QSAR estimate for acute toxicity	1000
lowest acute L(E)C_{50}-value or QSAR estimate for acute toxicity for three representatives of microbial processes, earthworms or arthropods and plants	100
lowest NOEC-value or QSAR estimate for chronic toxicity	10*
lowest NOEC-value or QSAR estimate for three representatives of microbial processes, earthworms or arthropods and plants	10

*value is subsequently compared to the calculated value based on the lowest L(E)C50, the lower is selected

2.4. Extrapolation towards an MPC

2.4.1. Preliminary effect assessment

If chronic or acute toxicity data are available for less than four taxonomic groups, assessment factors are used. Assessment factors are developed by the Environmental Protection Agency and modified by van der Meent *et al.* (1990). The magnitude of the assessment factor depends on the number and the kind of the available toxicity data (Table 2.1.).

2.4.2. Refined effect assessment

The aim of environmental quality standards as derived in the project 'Setting Integrated Environmental Quality Standards' is to protect all species in the ecosystem. For statistical considerations the MPC is set equal to the concentration at which 95% of the species is protected, assuming thereby to protect the whole ecosystem (VROM, 1989; Van Leeuwen *et al.*, 1992). A detailed description of the statistical background of the refined effect assessment method is given in the literature (Kooijman, 1987; Van Straalen and Denneman, 1989; Aldenberg and Slob, 1993).
It is assumed that sensitivities of species in an ecosystem can be described by a log-logistic frequency distribution. It can be tested with the Kolmogorov-Smirnov D*sqrt(n) test if a log-logistic distribution adequately describes the underlying data. This distribution is defined by α (the mean of the log-logistic distribution) and by β, which determines the width of the distribution:

$$\beta = \sigma \frac{\sqrt{3}}{\pi}$$

where σ stands for the sample standard deviation.
The concentration at which 95% of the species is protected can be derived by:

$$MPC = \alpha - \beta \cdot ln \frac{1 - 0.05}{0.05}$$

2.5. Harmonisation between the compartments

The partition coefficient between organic carbon in the sediment and water (K_{oc}) is used to derive an MPC for soil or sediment when no data on terrestrial or sediment-dwelling organisms are available. In addition, it is used to harmonise the MPCs between the different compartments. Harmonisation prevents that reaching an MPC in one compartment leads to an exceeding of the MPC in a second compartment.

By applying this equilibrium partitioning concept (DiToro *et al.*, 1991), it is assumed that there is an equilibrium between the concentration in organic carbon and water. This equilibrium is described by the K_{oc}. It is furthermore assumed that toxicity is related to the pore water concentrations, and that aquatic organisms are comparable in their sensitivity to the compound to the organisms living in soil or sediment.

2.6. Effects related to endocrine disruption

Concerning the suspected endocrine disruptive properties, data are collected for *in vitro* as well as in *in vivo* tests performed with phthalates (not only DBP and DEHP), with endpoints related to the endocrine or reproductive system. The *in vivo* studies are in the majority performed with mammals. The results of the different study types are compared. The most sensitive endpoints in the *in vivo* studies are converted to concentrations in the organic carbon exerting adverse effects. These concentrations are compared with the MPC derived for sediment, in order to check the need for adjusting the MPC for endocrine disruptive effects.

3. DERIVATION OF MPCs AND NCs FOR DBP AND DEHP

In this chapter, MPCs and NCs are derived following the methods generally applied within the project 'Setting integrated environmental quality objectives'. Tests with endpoints related to effects upon the endocrine or reproductive system, other than the toxicity tests related to reproduction that are normally taken into account in the derivation of MPCs, are reviewed and discussed in chapter 4 of this report.

3.1. Results from toxicity tests

3.1.1. Dibutylphthalate

Data on the toxicity of DBP can be found in appendices 1.2. (chronic toxicity to freshwater organisms), 1.4. (acute toxicity to freshwater organisms), 2.2. (chronic toxicity to saltwater organisms), and 2.4. (acute toxicity to saltwater organisms). The data that are used as input for the derivation of the MPC are listed in Table 3.1. Chronic toxicity data for freshwater organisms are found for algae, crustaceans and fishes. Differences between the taxonomic groups are less than a factor of 30. The chronic toxicity of DBP on freshwater organisms is in the order of mg/l (0.1-6.1 mg/l). The most sensitive NOEC is observed for Oncorhynchus mykiss, and is taken from a study by Rhodes *et al.* (1995). Exposure duration in this study is 60 d, and both reproduction and growth are studied as an end-point. One study was found for chronic toxicity to saltwater organisms. The studied species is an algae, and its sensitivity to DBP in the same order of magnitude as observed for the freshwater species (Table 3.1.).
Acute toxicity data for freshwater organisms are abundant, and the various taxonomic groups (protozoa, algae, crustaceans, insects and fish) are comparable in their sensitivity. Acute toxicity for freshwater organisms is found at concentrations 100 to 10 times below the water solubility of DBP. EC50 values with growth as an endpoint are found for protozoa and algae, LC50 data are found for crustaceans and fish. Different studies for the same taxonomic group show comparable results; the highest difference between studies was a factor of 22 for the algae. Data on the acute toxicity of DBP to saltwater organisms (bacteria, algae, crustaceans and fish) are in the same range as data for freshwater organisms (Figure 3.1.).

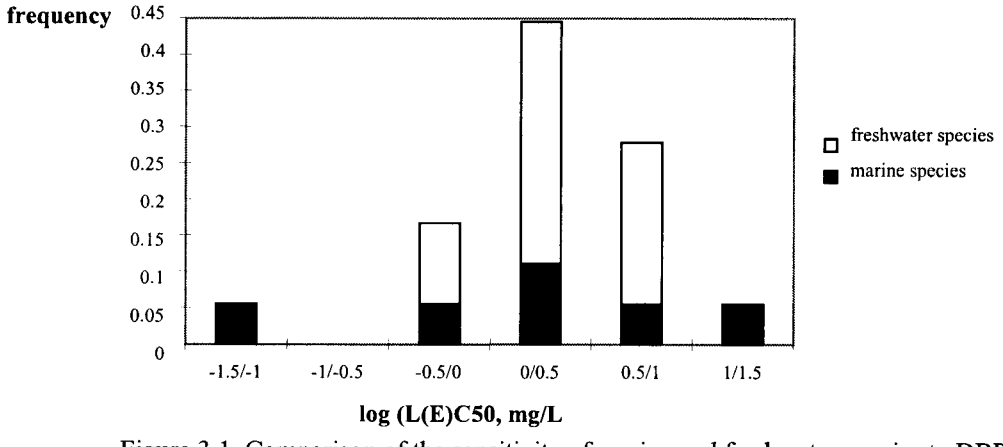

Figure 3.1. Comparison of the sensitivity of marine and freshwater species to DBP; acute toxicity.

Table 3.1. Data that are used as input for the derivation of the maximum permissible concentrations for DBP

Chronic toxicity of DBP to aquatic organisms		
Taxonomic group	**Species**	**NOEC, mg/l**
Algae	*Chlorella emersoni*	2.8
Algae	*Pseudokirchnerellia subspicata*	0.77[a]
Algae	*Scenedesmus subspicatus*	6.1
Algae (saltwater)	*Dunaliella parva*	0.28
Crustacea	*Daphnia magna*	0.88[c]
Pisces	*Oncorhynchus mykiss*	0.1
Pisces	*Pimephales promelas*	0.56
Acute toxicity of DBP to aquatic organisms		
Taxonomic group	**Species**	**L(E)C50, mg/l**
Bacteria (saltwater)	*Vibrio fisheri*	11-23
Protozoa	*Tetrahymena pyriformis*	7.0
Algea	*Pseudokirchnerellia subspicata*	0.40
Algea	*Scenedesmus subspicatus*	4.2[a]
Algea (saltwater)	*Gymnodinium breve*	0.05[f]
Crustacea	*Daphnia magna*	3.9[b]
Crustacea	*Gammarus pseudolimnaeus*	2.1
Crustacea (saltwater)	*Artemia salina*	8.0
Crustacea (saltwater)	*Mysidopsis bahia*	0.50
Crustacea (saltwater)	*Nitroca spinipes*	1.7
Insecta	*Chironimus plumosus*	2.5[b]
Insecta	*Paratanytarsus parthenogenica*	6.3
Pisces	*Brachydanio rerio*	2.2
Pisces	*Lepomis macrochirus*	1.5[d]
Pisces	*Oncorhynchus mykiss*	2.3[d]
Pisces	*Perca flavescens*	0.35
Pisces	*Pimephales promelas*	1.2[e]
Pisces (saltwater)	*Ictalurus punctatus*	1.2[a]

[a]Geometric mean of two values; [b]Geometric mean of three values; [c]Geometric mean of four values; [d]Geometric mean of five values; [e]Geometric mean of eight values; [f]Geometric mean of four values, obtained in two replicate tests with over an order of magnitude difference in the results.

As no differences are observed in the sensitivity of freshwater and saltwater organisms for both chronic and acute toxicity, it is concluded that all data can be combined for the derivation of the MPC.

No clear differences are observed between the results of studies in which the actual concentration of the compound is measured, or in which the nominal concentration is used. Such a difference was more or less expected because of difficulties concerning the analysis of the compounds. Contamination with phthalates during experimentation is easily obtained (Hendriks, 1998), especially for DBP and DEHP (van der Velde, pers. comm.). This may lead to an overestimation of the exposure concentration in tests where the actual concentration is measured. However, this is not reflected in the data.

Concerning soil or sediment tests, no useful tests were found for DBP (see appendix 4.2.).

3.1.2. Di(2-ethylhexyl)phthalate

Data on the toxicity of DEHP can be found in appendices 1.3. (chronic toxicity to freshwater organisms), 1.5. (acute toxicity to freshwater organisms), 2.3. (chronic toxicity to saltwater organisms), 2.5. (acute toxicity to saltwater organisms), 4.3. (chronic toxicity to sediment organisms), 4.4. (acute toxicity to soil organisms) and 4.5. (toxicity to soil microbial processes).

Chronic toxicity studies with DEHP have been reported for freshwater organisms from four taxonomic groups (algae, crustaceans, fish and amphibians), and for saltwater organisms from two taxonomic groups (crustaceans and molluscs). In the majority of studies no effects are found at the highest tested concentration. This highest tested concentration often exceeds the value of 3 µg/l that is recommended as the best estimate of water solubility by Staples *et al.* (1997a). In those studies where adverse effects are observed and NOECs could be specified, the NOEC is always above the water solubility (see appendix 1.3.).
Acute toxicity studies with DEHP have been performed with four taxonomic groups of freshwater organisms (algae, crustaceans, insects and fish) and with one taxonomic group for the saltwater organisms (fish). Again in practically all studies no toxicity is observed at the highest test concentration. In the few cases where LC50s are reported, these values are far above the recommended value for the water solubility of 3 µg/l. Although these results are not useful for risk assessment they are listed in the appendices to emphasise that DEHP does not exhibit any short-term toxicity to aquatic organisms at concentrations below the aqueous solubility. It is suggested in the literature that exposure concentrations in toxicity tests can easily exceed water solubility because of micelle formation (e.g. Rhodes *et al.*, 1995). A search on critical micellar concentrations of phthalates yielded no literature information on this subject. By several authors the adverse effects that are observed, are attributed to physical effects instead of internal toxicity of DEHP (e.g. Adams *et al.*, 1985; Scholz, 1995ab; Rhodes *et al.*, 1995; Brown & Thompson, 1982; Brown, 1998). For example, daphnia's are found entrapped at the water surface. Brown *et al.* (1998) showed that no toxicity was observed when DEHP was tested for acute and chronic toxicity to *Daphnia magna* at concentrations well above its water solubility (1 mg/L), mixed with a dispersant at a ratio of DEHP:dispersant equals 1:10. The dispersant concentration is according to the authors well below the critical micellar concentration. The authors conclude that the toxic effects that are observed in the literature for the higher molecular weight phthalates are due to surface entrapment and not to inherent toxicity. In the present report we did not make the distinction between physical and toxicological effects. It is concluded that DEHP as a single compound does not lead to chronic toxicity at concentrations below the water solubility, for the organisms and exposure times tested.
For *oligochaeta* and microbial processes in soil no toxicity of DEHP is found at the highest tested concentration. One study shows an influence on the respiration inhibition at a concentration of 49000 mg/kg d.w., which is considered unlikely high (a minimal concentration in the pore water, using the highest K_{oc} of $51 \cdot 10^4$ and a sediment of 10% o.c. on a dry weight base, can be calculated of 1 mg/L). A study in sediment on hatching success from the moorfrog *Rana arvalis* reports a NOECs of 10 mg/kg fresh weight (Larson & Thurén, 1987). It has to be noted that there exists a study which has not been published in the open literature (Wennberg et al., 1997), in which the results obtained by Larson and Thurén could not be reproduced. However, there were some differences between both studies which might explain the differences. The exposure duration in the Wennberg study was 29 d, in the Larson study 60 d. At the end of the Wennberg test, there were problems with counting remaining eggs and embryos and thus with determining the endpoint of hatching succes. In addition there were problems with fungi or bacterial infection in the

Wennberg study. For the reasons sketched above, we did not include the study by Wennberg et al. (1997) in the MPC-derivation.

3.2. Derivation of MPCs

3.2.1. Dibutylphthalate

For acute and chronic toxicity, the sensitivity of saltwater species does not fall outside the range of the sensitivity of freshwater species (see figure 3.1.), therefore no separate MPC is derived for both ecosystems.
For three taxonomic groups (algae, crustaceans and fish) NOECs are available, therefore the refined assessment method (2.4.2.) cannot be applied. The lowest NOEC is 0.1 mg/l for Oncorhynchus mykiss (Table 3.1.). Applying an assessment factor of 10 (see Table 2.1.) yields an MPC for the aquatic environment of 0.01 mg/l.
For sediment and soil no toxicity data are available. Therefore, the MPC for sediment and soil is derived by multiplication with the K_{oc} (see Table 1.2.). Variation of the Koc is higher for suspended solids ($1.2 \cdot 10^3$-$16 \cdot 10^4$ l/kg) than for soil/sediment ($1.4 \cdot 10^3$-$15 \cdot 10^3$ l/kg) (Staples 1997a). Part of the variation is explained by difficulties of measuring the truly dissolved fraction of the compounds in the water (Staples *et al.*, 1997a; Schrap *et al.*, 1995). To prevent underestimation of the risk, the low value of the range of K_{oc}'s reported is chosen ($1.2 \cdot 10^3$ l/kg). Thus, an MPC for sediment and soil is obtained of 12 mg/kg organic carbon. For a standard soil/sediment (10 % organic matter, ≈5.8% organic carbon) this yield a MPC of 0.7 mg/kg standard soil or standard sediment.

3.2.2. Di-n-ethylhexylphthalate

As discussed in 3.1.2., chronic and acute aquatic toxicity data are not useful for deriving MPCs, as effects are not observed at concentrations near aqueous solubility. Starting point is therefore the NOEC for *Rana arvalis* of 10 mg/kg fresh weight (Larsson & Thurén, 1987). The organic carbon content of the sediment is 5.3-13% of the wet weight, the wet weight/dry weight ratio of the sediment is unknown. It is assumed that the composition of the test sediment equals standard sediment. As for the other organisms and microbial processes NOECs were much higher (see appendices), a factor of 10 was applied yielding an MPC for sediment and soil of 1.0 mg/kg fresh weight.
This MPC is used to derive an MPC for the aqueous compartment. Normally in the project 'Setting Integrated Environmental Quality Standards', MPCs for sediments are derived from aqueous MPCs, now we use the same procedure the other way around. An sediment/soil MPC of 1.0 mg/kg fresh weight (Table 3.2.) equals 17.5 mg/kg o.c. (10% organic matter ~ 5.7% organic carbon). In the current report the K_{oc} as reviewed by Staples *et al.*, 1987a, ranges between $87 \cdot 10^3$-$51 \cdot 10^4$ l/kg for soil/sediment, and between $22 \cdot 10^3$-$1 \cdot 10^6$ l/kg for suspended solids. The low value of the K_{oc} reported for sediments and soils, i.e. $87 \cdot 10^3$ l/kg o.c., was chosen to calculate the aqueous MPC. Using this K_{oc}, an MPC in organic carbon of 17.5 mg/kg o.c. is in equilibrium with 0.20 µg/l.

Table 3.2. Overview of the maximum permissible concentrations and negligible concentrations that are derived for water and standard soil/sediment (10% organic matter).

Compound	MPC_{water} (µg/l)		NC_{water} (µg/l)	$MPC_{soil/sediment}$ (mg/kg fresh weight)		$NC_{soil/sediment}$ (mg/kg fresh weight)
Dibutylphthalate	10	*EPA/10*	0.1	0.7	*EP*	0.007
Di-n-ethylhexyl-phthalate	0.19	*EP*	0.002	1.0	*EPA/10*	0.01

EPA/10: Preliminary effects assessment is used with an assessment factor of 10. EP: this value is the result of harmonization via the equilibrium partitioning method

4. COMPARISON WITH MONITORING DATA

It should be noted that due to their ubiquitous presence, artificial contamination with phthalates during experimentation might well interfere with analyses (Hendriks *et al.*, 1998). This hinders the interpretation of measured concentrations. When evaluating monitoring data it should be kept in mind that high control values are often obtained in the analysis of phthalates, despite precautionary measures as rinsing of glassware and distillation of solvents. For DBP and DEHP contamination problems during the chemical analysis are higher than for other phthalate compounds (pers. comm. E. van der Velde, RIVM).
Recently the first results have become available from an extensive and ongoing project in which concentrations of suspected endocrine disrupters are monitored in surface waters, influents and effluents from sewage treatment plants, and effluents from some industrial plants (A. Belfroid, IVM, in preparation, and E. van der Velde, RIVM, personal communication). Besides phthalates, many other compounds were measured such as natural estrogens (17α-estradiol, 17β-estradiol, estrone), the synthetic oestrogen used as contraceptive (17α-ethinyloestradiol), bisphenol A, alkylphenolethoxylates and alkylphenols. For an overview on the results on concentrations of the phthalates in surface waters, see Table 4.1.

Table 4.1. Concentrations of phthalates in Dutch surface waters, december 1997

Compound	Lobith (µg/l)	Eijsden (µg/l)	Maassluis (µg/l)	Haringvlietsluizen (µg/l)
DMP	0.1	0.2	0.3	0.5
DEP	DL	1.4	1.7	2.6
DBP	0.4	0.5	0.5	0.4
BBP	DL	DL	DL	DL
DEHP	0.6	0.9	1.6	1.9
DOP	DL	DL	DL	DL
DPP	DL	DL	DL	DL
DMPP	0.1	0.2	0.2	0.2
DCHP	DL	DL	DL	DL

DL: Concentration under the detection limit. Surface water was filtered over 1.2 µm.
DMP: Dimethyl phthalate; DEP: Diethyl phthalate; DBP: Dibutyl phthalate; BBP: Butylbenzyl phthalate; DEHP: Di(2-ethylhexyl)phthalate; DOP: Dioctyl phthalate; DPP: Dipropyl phthalate; DMPP: Di(2-methylpropyl) phthalate; DCHP: Dicyclohexyl phthalate

In a literature study (Belfroid, 1998), concentrations in surface water appear to be in the µg/l range. Concentrations of DBP and DEHP often are much (up to 100 times) higher than for the other phthalate compounds. A part of these measured compounds may be bound, and therefore not bioavailable. Concentrations in particulate matter or sediment are in the mg/kg range. In Dutch surface waters, from 1982 until 1991, concentrations between <0.1 to 4.0 µg/l with a peak of 50 µg/l are reported for DEHP, and for DBP concentrations of 0.1-1.3 µg/l are reported (Ritsema, 1987; Ritsema *et al.*, 1989; references mentioned in Belfroid, 1998). In suspended particulate matter from IJsselmeer, Rhine, Meuse and Westerschelde in 1986, concentrations of 10-100 mg/kg are reported for DEHP and concentrations of 0.2-0.9 mg/kg for DBP.
Hendriks *et al.* (1998) report on phthalate levels in mussel and eel from the Rhine and Meuse. They found total phthalate levels in eel of around 0.01 µmol/kg lipid and in mussel of approximately 1 µmol/kg lipid.

It is concluded that measured concentrations of DBP are seldom above the MPCs for water and sediment as derived in chapter 3. However, reported concentrations in water for DEHP are 3 to 20 times higher than the MPC, and for sediments reported concentrations are also higher that the MPC for sediment which is derived in chapter 3.

5. ENDPOINTS RELATED TO ENDOCRINE DISRUPTION; A NEED FOR INCORPORATION OF ADDITIONAL INFORMATION IN THE DERIVATION OF MPCs?

Endocrine disruptive chemicals have effects similar to those produced by endogenous hormones. As endocrine disrupters have a broad structural diversity, it is suggested that effects are exerted by a number of possible pathways, such as binding to or activation of receptors; receptor mediated interaction with responsive elements on the DNA; or interaction with hormone metabolism or production (Gillesby & Zacharewski, 1998).
As phthalates are commonly mentioned as chemicals with possible endocrine disruptive effects (e.g. Colborn *et al.*, 1993; Gillesby & Zacharewski, 1998), tests in which phthalates are used as test compounds and which have endpoints related to endocrine disruption are reviewed and discussed in this chapter. Furthermore, the significance of these tests for the derivation of MPCs is discussed both for phthalates specifically and in general.

5.1. Data obtained from *in vitro* experiments

In vitro assays to screen for (anti-)estrogenic/androgenic potency can roughly be classified in one of the following groups (it is referred to this numbering in Table 5.1.):

1. Receptor binding affinity tests, in which the binding affinity to a receptor is measured with competitive ligand binding techniques. This assay type does not discern agonists from antagonists.
2. Cellular proliferation assays, which measure proliferation in cell lines that are dependent on hormones.
3. Gene expression tests, which measure the gene expression after exposure to contaminants by determining amounts of mRNA, or gene products (e.g. an enzyme). Sometimes, the cell lines have been constructed with help of recombinant techniques (e.g. Balaguer *et al.*, 1996).

In test types 2 and 3 receptor binding of the ligand as well as further events such as interaction with the responsive element on the DNA, DNA transcription and the resulting production of proteins are measured, while in test type 1 only ligand binding is measured. A more in-depth overview on this subject can be found in Ankley *et al.* (1998). They concluded that *in vitro* test-systems cannot replace *in vivo* test as the sole base for screening chemicals for potential (anti-) estrogenic or androgenic action. The major objections to using solely *in vitro* systems are the differences in metabolism, bioavailability and toxicokinetics between *in vitro* and *in vivo* test systems. In addition, intercellular interaction and mechanisms related to endocrine homeostasis are absent in *in vitro* systems. However, *in vitro* test systems can give a prediction about the possibility that compounds are able to exert endocrine disruptive effects. I.e., if they are not positive in any of the *in vitro* tests, it is not likely that the tested substances will be endocrine disrupters *in vivo*. Therefore, as a result of new legislation that passed the U.S. Congress (the Food Quality Protection Act and the Safe Drinking Water Act) these kind of *in vitro* tests are used in so-called 'high-throughput screening' of a high number of chemicals.

Those results that have been published on the endocrine disruptive potency of phthalates in *in vitro* assays are listed in Table 5.1.
For several of the phthalate compounds (DIDP, DMP, DOP, DIHP, DINP, DIDP, see note to Table 5.1. for the abbreviations used) no effects were found at the highest tested concentration in any of the tests. For other compounds (DBP, DEP, DHP, BBP, DEHP, DIBP and MEHP) endocrine disruptive effects are reported. The relative potency related to the potency of the natural estrogen, 17ß-estradiol, is always low (10^{-4}-10^{-8}). This relative potency appears the highest for BBP (10^{-4}-10^{-6}). For DBP the reported relative potencies are 10^{-5}-10^{-7}, for DEHP the reported relative potencies are 10^{-5}. Some authors report on differences in potency between the phthalates tested (e.g. Jobling *et al.*, 1995), others find no clear potency differences (Knudsen & Potinger, 1999).
The three major groups in which the *in vitro* tests are classified, do not always show the same picture. For DBP endocrine disruptive effects are obtained in all types of *in vitro* systems, while for DEHP effects are observed in receptor binding assays but not in the proliferation or gene expression assays. Apparently, DEHP is able to bind to the estradiol receptor, but further events such as binding of the ligand-receptor complex to the DNA and resulting transcription and protein production do not take place. For DBP these further events do appear to take place.
Different authors do not always report similar results in spite of the use of a comparable test system (see for example the receptor binding assays for DEHP). It should be noted that the actual concentrations added to these kind of *in vitro* systems can drop relatively fast for these hydrophobic compounds due to evaporation, sorption to the walls of the test system and sorption to the biological material (cells, membranes) used (unpublished results, G. de Maagd). Most authors only report nominal concentrations. These nominal concentrations can differ substantial from the actual concentrations in the test system; the magnitude of this difference will depend on the test system used. This may at least partly explain the sometimes contradictory results when studies are compared.
It was shown by Knudsen & Pottinger (1999) that phthalates do not bind to the testosterone receptor and the cortisol receptor in rainbow trout.
Despite some inconsistencies in the data, the results do give rise the idea that some phthalate compounds (the white part of Table 5.1.) are able to act as xeno-estrogens, and thereby as endocrine disrupters.

Table 5.1. Endocrine disruptive effects of phthalates in *in vitro* test systems

com-pound	test type[a]	cell type	effect (mM)	remark	Reference
DBP	1	trout hepatocyte	EC80=0.001 EC50=1	REP: $6.7 \cdot 10^{-6}$	Jobling *et al.*, 1995
	1	rat uterine	0.047: IC50	REP: $2.7 \cdot 10^{-5}$	Zacharewski *et al.*, 1998
	1	trout hepatic cytosol	EC 10-25 at 0.17	REP: $2 \cdot 10^{-5}$	Knudsen & Pottinger, 1999
	2	MCF7/ZR-75	0.01	± 50% increase in cell number	Harris *et al.*, 1997
	2	yeast	0.01	weak support of growth	Zacharewski *et al.*, 1998
	3	MCF7	0.01-0.1	28% of the maximum response by E2	Jobling *et al.*, 1995
	3	yeast	no effect at 10		Coldham *et al.*, 1997
	3	yeast	10	REP: $1 \cdot 10^{-7}$	Harris *et al.*, 1997
	3	MCF7/HeLa	0.010	37% effect	Zacharewski *et al.*, 1998
DEP	1	trout hepatic cytosol	EC 10-25 at 0.17	REP: $2 \cdot 10^{-5}$	Knudsen & Pottinger, 1999
	2	MCF7/ZR-75	no effect at 0.010		Harris *et al.*, 1997
	3	yeast	10	REP: $2 \cdot 10^{-8}$	Harris *et al.*, 1997
DHP	1	rat uterine	0.001-10	less than 50% effect	Zacharewski *et al.*, 1998
	2	yeast	no effect at 0.010		Zacharewski *et al.*, 1998
	3	yeast	no effect at 10		Harris *et al.*, 1997
	3	MCF7/HeLa	0.010	16±5 % effect	Zacharewski *et al.*, 1998
BBP	1	rat uterine	0.036: IC50	REP: $4 \cdot 10^{-5}$	Zacharewski *et al.*, 1998
	1	trout hepatocyte	EC40=0.05 EC60=0.0001	REP: $1.4 \cdot 10^{-4}$	Jobling *et al.*, 1995
	1	trout hepatic cytosol	EC 10-25 at 0.17	REP: $2 \cdot 10^{-5}$	Knudsen & Pottinger, 1999
	2	MCF7/ZR-75	0.010	110 % increase in cell number	Harris *et al.*, 1997
	2	yeast	0.010	support of growth	Zacharewski *et al.*, 1998
	2	MCF-7	0.010	Relative proliferative effect of 90%	Soto *et al.*, 1995
	3	MCF7	0.001-0.1	45% of the maximum response of E2	Jobling *et al.*, 1995
	3	yeast	10	REP: $4 \cdot 10^{-4}$	Coldham *et al.*, 1997
	3	yeast	10	REP: $1 \cdot 10^{-6}$	Harris *et al.*, 1997
	3	MCF7/HeLa	0.010	46±14 % effect	Zacharewski *et al.*, 1998
DEHP	1	trout hepatocyte	EC75=1	REP: $1 \cdot 10^{-5}$	Jobling *et al.*, 1995
	1	rat uterine	no effect at 1		Zacharewski *et al.*, 1998
	1	trout hepatic cytosol	EC 10-25 at 0.17	REP: $2 \cdot 10^{-5}$	Knudsen & Pottinger, 1999
	2	MCF7	> 0.1	4% of the maximum response of E2	Jobling *et al.*, 1995
	2	yeast	no effect at 10		Coldham *et al.*, 1997
	2	yeast	no effect at 0.010		Zacharewski *et al.*, 1998
	2	yeast	no effect at 10		Harris *et al.*, 1997
	2	MCF 7	≥ 0.010		Blom *et al.*, 1998
	3	MCF7/HeLa	no effect at 0.010		Zacharewski *et al.*, 1998
	3	MCF7/ZR-75	no effect at 0.010		Harris *et al.*, 1997
DIBP	2	yeast	10	REP: $1 \cdot 10^{-7}$	Harris *et al.*, 1997
	3	MCF7/ZR-75	0.010	± 37 % increase in cell number	Harris *et al.*, 1997
MEHP	3	rat Sertoli	0.050		Thysen *et al.*, 1990
	3	rat Sertoli	0.1	> 50% reduction in FSH stimulated cAMP production	Llyod & Foster, 1988
	3	rat granulosa	0.1	40% reduction in FSH stimulated cAMP production	Treinen *et al.*, 1990
DIDP	2	yeast	no effect at 10		Harris *et al.*, 1997
	3	MCF7/ZR-75	no effect at 0.010		Harris *et al.*, 1997
DMP	3	yeast	no effect at 10		Harris *et al.*, 1997
DOP	1	rat uterine	no effect at 1		Zacharewski *et al.*, 1998
	2	yeast	no effect at 0.010		Zacharewski *et al.*, 1998
	3	yeast	no effect at 10		Harris *et al.*, 1997
	3	MCF7/HeLa	no effect at 0.010		Zacharewski *et al.*, 1998
DIHP	1	rat uterine	no effect at 1		Zacharewski *et al.*, 1998

com-pound	test type[a]	cell type	effect (mM)	remark	Reference
	2	yeast	no effect at 0.010		Zacharewski *et al.*, 1998
	3	MCF7/HeLa	no effect at 0.010		Zacharewski *et al.*, 1998
DINP	1	rat uterine	no effect at 1		Zacharewski *et al.*, 1998
	1	trout hepatic cytosol	no effcet at 0.17		Knudsen & Pottinger, 1999
	2	yeast	no effect at 0.010		Zacharewski *et al.*, 1998
	3	MCF7/HeLa	no effect at 0.010		Zacharewski *et al.*, 1998
DIDP	1	rat uterine	no effect at 1		Zacharewski *et al.*, 1998
	2	yeast	no effect at 0.010		Zacharewski *et al.*, 1998
	3	MCF7/HeLa	no effect at 0.010		Zacharewski *et al.*, 1998

DBP: Dibutyl phthalate; DEP: Diethyl phthalate; DHP:Dihexyl phthalate; DMP: Dimethyl phthalate; DOP:Dioctyl phthalate; BBP:Butylbenzyl phthalate; DEHP: Di(2-ethylhexyl)phthalate; DIBP: Diisobutyl phthalate; DIDP:Diisodectyl phthalate; DINP: Diisononyl phthalate; DIDP: Diisodocyl phthalate; MEHP: Mono(2-ethylhexyl)phthalate; DIHP: Diisoheptyl phthalate REP: relative potency compared to 17ß-estradiol

[a]: Numbering of tests refers to 4.1.

5.2. Data based upon *in vivo* experiments

The suspicion of potency for endocrine disruption must be confirmed in *in vivo* data. Reasons therefore are differences in metabolism, bioavailability and toxicokinetics between *in vitro* and *in vivo* test systems, and the absence of intercellular interaction and mechanisms related to endocrine homeostasis in *in vitro* systems (cf. Ankley *et al.*, 1998).

It was concluded by Ankley *et al.* (1998) that existing mammalian test methods (in general rat and mice) are in general useful for a screen of potential (anti-) estrogenic or androgenic action. However it is not yet clear if they are predictive for other classes of vertebrate wildlife. The most commonly used *in vivo* assays in mammals for estrogenic effects are the uterothrophic assay in which the uterine weight is determined, and the vaginal cornification assay (Gillesby & Zacharewski, 1998). For fish and birds, full and partial life-cycle tests seem suitable for this purpose (Gimeno, 1997; Ankley *et al.*, 1998). A number of short-term tests exist, that could be used as screening tools for (anti-)estrogens/androgens. Ankley *et al.* (1998) recommended that for invertebrates specific assays should be developed.

For an overview of endocrine disruptive effects of phthalates that are found in *in vivo* tests, see Table 4.2. Despite one test with *Daphnia magna*, only mouse and rat are used as test organisms. Endocrine disruptive effects are found in *in vivo* tests for DBP, DEHP, BBP, DEP and DHP, but not for the other phthalates tested (DOP, DIHP, DINP, DIDP). Especially the two-generation reproduction studies appear sensitive in detecting endocrine disruptive effects. None of the tests with uterine weight (often in combination with vaginal cornification) showed positive results. The distinction between phthalates which can or cannot act as endocrine disrupters appears to be the same from *in vitro* and *in vivo* tests (compare Tables 4.1. and 4.2.).

Table 5.2.: Endocrine disruptive effects of phthalates in *in vivo* tests

Com-pound	Organism	Way of exposure	Type of endpoint		Reference
DBP	mouse	sc injection, 0.1 mmol	uterine vascular permeability	no effect at 28 mg	Milligan *et al.*, 1998
	mouse	3 daily sc injections	uterine weight	no effect at 5 mg	Coldham *et al.*, 1997
	mouse	0.17, 0.39, and 1.4 g/kg bw/day	2-generation reproduction study	NOAEL 0.39 g/kg bw	Lamb *et al.*, 1997a
	rat	65, 310, and 650 mg/kg bw/day	2-generation reproduction study	NOAEL ≤ 65 mg/kg/bw	Chapin *et al.*, 1997
	rat	oral gavage for 4 d, 20, 200 or 2000 mg/kg	uterine wet weight and vaginal cornification	no effect	Zacharewski *et al.*, 1998
DEHP	rat	10 days oral gevage, 1500 mg/kg b.w. day	altered steroid production im cultured ovary cells	effect at 1500 mg/kg b.w. day	Laskey & Bermann, 1993
	rat	oral gavage for 4 d, 20, 200 or 2000 mg/kg	uterine wet weight and vaginal cornification	no effect	Zacharewski *et al.*, 1998
BBP	mouse	sc injection, 0.1 mmol	uterine vascular permeability	no effect at 0.1 mmol	Milligan *et al.*, 1998
	rat	1000µg/l water, 14d before mating to 22 d after giving birth	reduced testis weight and sperm production	EC10 1000 µg/l	Sharpe *et al.*, 1995
	mouse	3 daily sc injections	uterine weight	no effect at 5 mg	Coldham *et al.*, 1997
	rat	oral gavage for 4 d, 20, 200 or 2000 mg/kg	uterine wet weight and vaginal cornification	no effect	Zacharewski *et al.*, 1998
DEP	Daphnia magna	5.6, 11.2 and 22.40 mg /l	molting time	LOEC 22.4 mg/l	Zou & Fingerman, 1997
	mouse	0.34, 1.77, and 3.64 g/kg bw/day	2-generation reproduction study	LOAEL 3.64 g/kg bw/day	Lamb *et al.*, 1997c
DHP	mouse	0.38, 0.80, and 1.67 g/kg bw/day	2-generation reproduction study	NOAEL ≤ 0.38 mg/kg bw/day	Lamb *et al.*, 1997d
	rat	oral gavage for 4 d, 20, 200 or 2000 mg/kg	uterine wet weight and vaginal cornification	no effect	Zacharewski *et al.*, 1998
DOP	mouse	sc injection, 0.1 mmol	uterine vascular permeability	no effect at 0.1 mmol	Milligan *et al.*, 1998
	mouse	1.8, 3.6, and 7.5 g/kg bw/day	2-generation reproduction study	no effect	Lamb *et al.*, 1997b
	rat	oral gavage for 4 d, 20, 200 or 2000 mg/kg	uterine wet weight and vaginal cornification	no effect	Zacharewski *et al.*, 1998
DIHP	rat	oral gavage for 4 d, 20, 200 or 2000 mg/kg	uterine wet weight and vaginal cornification	no effect	Zacharewski *et al.*, 1998
DINP	rat	oral gavage for 4 d, 20, 200 or 2000 mg/kg	uterine wet weight and vaginal cornification	no effect	Zacharewski *et al.*, 1998
DIDP	rat	oral gavage for 4 d, 20, 200 or 2000 mg/kg	uterine wet weight and vaginal cornification	no effect	Zacharewski *et al.*, 1998
17β-es-tradiol	mouse	sc injection	uterine vascular permeability	EC50: 10^{-9}-10^{-10} mol	Milligan *et al.*, 1998

For the abbreviations used see Table 5.1.

5.3. Significance for environmental risk limits

The *in vivo* tests with DBP and DEHP listed in Table 4.2., are used to calculate corresponding concentrations in the sediment exerting adverse effects. These calculations are done as descibed in the below.
It is assumed that the sensitivity of the mammals that are tested in the *in vivo* tests (Table 4.2.) does not differ from mammalian wildlife. Analogous concentrations in the sediment can be derived, if the height of the biomagnification factor (BMF) and the biota-to-sediment accumulation factor (BSAF) is known (Starodubb *et al.*, 1996). However, information on these factors specifically for phthalates is hardly available. A worst case approach is to set both factors on 10 (g lipid/g lipid or g lipid/g o.c. respectively) (Hendriks *et al.*, 1998). In view of the fact that phthalates are easily biotransformed, this will be a gross overestimation (Staples *et al.*, 1997a).
Assuming a lipid content of the test organisms of 5%, the lowest reported NOAEL for DBP (65 mg/kg b.w.) results in a concentration in the organic carbon of 13 mg/kg o.c.. This almost equals the derived MPC (12 mg/kg o.c.).
For DEHP, the LOAEC of 1500 mg/kg b.w. results in a corresponding concentration of 300 mg/kg o.c., which is much higher than the derived MPC of 36 mg/kg o.c.. Therefore, if the concentration of DEHP in the environment is on an MPC-level, there will also be a protection against endocrine disruptive effects.
As in reality the BMF and BSAF will very likely be lower than 10 (although more research is needed on these parameters), it might be concluded that the MPCs that are derived in chapter 3 will not lead to adverse endocrine related effects.
It should be noted that there is a possibility that other organism groups than the [a]aquatic organisms which are used to underpin the MPC (chapter 3) and [b]the mammals in which specific studies are done on endocrine disruptive effects (chapter 4), will be more sensitive on the endocrine disruptive properties of phthalates for example due to the absence of metabolic pathways. However, at the moment there are no data that confirm this.

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Appendix 1. Toxicity data for freshwater organisms

1.1. Legend

Organism	species used in the test, if available followed by age, size, weight or life stage
A	Y = test substance analyzed in test solution N = test substance not analyzed in test solution or no data
Test type	S = static, R = static with renewal, F = flow through
Test water	a.m. = artificial medium, a.s.w. = artificial seawater, n.f.s = natural filtered seawater, r.t.w. = reconsituted tap water (+additional salts)
Test substance purity	percentage active ingredient
Exposure time	h = hours, d = days, w = weeks, m = months, min. = minutes
Results	> and ≥ values = highest concentration used in the test

1.2. Chronic toxicity of DBP to freshwater organisms: NOEC values

Organism	A	Test-type	Test-sub. purity	Test-water	pH	Hardness mg $CaCO_3$/l	Exp. time	Parameter[a]	Result mg/l	References
Algae										
Chlorella emersoni	N						7 d	g	2.8	Melin and Egneus, 1983
Pseudokirchneriella subspicata	Y	S					10 d	g	0.21	Adams *et al.*, 1995
Pseudokirchneriella subspicata	N						7 d	g	2.8	Melin and Egneus, 1983
Scenedesmus subspicatus	Y						7 d	g	6.1	Hüls AG, 1991
Crustacea										
Daphnia magna	Y				7.9-8.3	150-180	21 d	s	0.96	Rhodes *et al.*, 1995
Daphnia magna	Y				8.0-8.4	162	21 d	s,r	1.1	DeFoe *et al.*, 1990
Daphnia magna	N						21 d	s,r	1.0	Kühn *et al.*, 1989
Daphnia magna	Y	R	99.5%	a.m.	7.9	85	16 d	s,r	0.56	McCarthy and Whitmore, 1985
Pisces										
Oncorhynchus mykiss	Y	P			7.0-8.6	158-198	60 d	g	0.1	Rhodes *et al.*, 1995
Pimephales promelas	Y	F	99.5%	a.m.	8.2	85	20 d	g	0.56	McCarthy and Whitmore, 1985

a = s:survival; g:growth; r:reproduction

1.3. Chronic toxicity of DEHP to freshwater organisms: NOEC values

Organism	A	Test-type	Test-sub. purity	Test-water	pH	Hardness mg $CaCO_3$/l	Exp. time	Cosolvent	Crite-rion	Parameter[a]	Result mg/l	References
Algae												
Pseudokirchneriella subspicata	Y	S					6 d		NOEC	g	≥0.10	Adams *et al.*, 1995
Crustacea												
Daphnia magna	Y	S					21 d	dimethylformamide	NOEC	s,r	0.64	Adams. W.J. and Heidolph, 1985
Daphnia magna	Y	S					7 d		NOEC	g	≥1.30	Adams. W.J. and Heidolph, 1985, cited in EU draft DEHP
Daphnia magna	Y		>97%		8.0	300	21 d	acetone	NOEC	s,r	0.16	Knowles *et al.*, 1987
Daphnia magna	Y				7.9-8.3	150-180	21 d	no	NOEC	s	0.077	Rhodes *et al.*, 1995
Daphnia magna	Y				7.9-8.3	150-180	21 d	acetone	NOEC	r	≥0.29	Rhodes *et al.*, 1995
Daphnia magna	Y		98%		8.3	180	21 d	solubizer	NOEC	s,r	≥0.10	Brown and Thompson, 1982a
Daphnia magna	Y	R					21 d	solubizer	NOEC	r	≥14	Scholz, 1995c
Daphnia magna	Y						21 d		NOEC	s,g,r	1.0	Brown and Williams, 1994
Daphnia magna	N	R					21 d	solubizer	NOEC	s,g,r	>1.0	Brown et al., 1997, cited in EU draft DEHP
Pisces												
Brachydanio rerio	N	R					35 d	DMSO	NOEC	s,g	≥0.32	Canton *et al.*, 1984
Gasterosteus aculeatus	N						28 d	DMSO	NOEC	s,g	≥0.32	Van den Dikkenberg *et al.*, 1989
Jordanella floridae	N	S					28 d	DMSO	NOEC	s,g	≥0.32	Adema *et al.*, 1981
Oncorhynchus mykiss	Y	F					102 d	acetone	NOEC	s,r	0.005	Mehrle and Mayer, 1976, cited in EU draft DEHP
Oncorhynchus mykiss	Y	F			7.0-8.2	44.0-46.4	90 d		NOEC	s,g,r	>0.50	DeFoe *et al.*, 1990
Oncorhynchus mykiss	Y	F					70 d		NOEC	s,g,r	>0.007 3	Cohle and Stratton, 1992, cited in EU draft DEHP
Oryzias latipes	Y	F			7.0-8.2	44.0-46.4	168 d		LOEC	g	0.55	DeFoe *et al.*, 1990
Oryzias latipes	N	R					28 d	DMSO	NOEC	s,g	≥0.32	Adema *et al.*, 1981
Pimephales promelas	Y	F					56 d	acetone	NOEC	s,g	0.062	Mehrle and Mayer, 1976, cited in EU draft DEHP
Poecilia reticulata	N						28 d	DMSO	NOEC	s,g	≥0.32	Adema *et al.*, 1981
Amphibia												
Bufo fowleri	N	R					7-8 d	acetone	NOEC	s	0.06	Birge et al., 1978, cited in EU draft DEHP
Rana pipiens	N	R					7-8 d	acetone	NOEC	s	0.18	Birge et al., 1978, cited in EU draft DEHP

a = s:survival; g:growth; r:reproduction

1.4. Acute toxicity of DBP to freshwater organisms: L(E)C50 values

Organism	A	Test-type	Test-sub. purity	Test-water	pH	Hardness mg $CaCO_3$/l	Exp. time	Crite-rion	Parameter[a]	Result mg/l	Reference
Protozoa											
Tetrahymena pyriformis	N						48 h	EC50	g	7.0[a]	Jaworska *et al.*, 1995
Algae											
Pseudokirchnerliella subspicata	Y				7.6-7.9	25-50	96 h	EC50	g	0.40[b]	Adams *et al.*, 1995
Scenedesmus subspicatus	N						48 h	EC50	g	9.0[d]	Kühn and Pattard, 1990
Scenedesmus subspicatus	Y						72 h	EC50	g	2.0[d]	Scholz, 1995e
Crustacea											
Daphnia magna	Y						48 h	LC50	s	3.7	Call *et al.*, 1983
Daphnia magna	Y	S			7.6-7.9	25-50	48 h	LC50	s	3.0	Adams *et al.*, 1995
Daphnia magna	N	S, R	99.5%			85	48 h	LC50	s	5.2	McCarthy and Whitmore, 1985
Gammarus pseudolimnaeus, mature	N	S	100%	-	7.4	272	96 h	LC50	s	2.1	Mayer and Ellersieck, 1986
Insecta											
Chironomus plumosus, 3rd instar	N	S	100%	-	7.4	272	48 h	EC50		5.4	Mayer and Ellersieck, 1986
Chironomus plumosus, 3rd instar	N	S	100%	-	7.4	44	48 h	EC50		4.0	Mayer and Ellersieck, 1986
Chironomus plumosus, 3rd-4th instar	N						48 h	LC50	s	0.76	Suggatt and Foote, 1981
Paratanytarsus parthenogenica	Y	S			7.6-7.9	25-50	48 h	LC50	s	6.3	Adams *et al.*, 1995
Pisces											
Brachydanio rerio	Y	S, R					96 h	LC50	s	2.2	{Scholz 1994a #1320}
Lepomis macrochirus, 1.40 g	N	S	100%	-	7.1	44	96 h	LC50	s	0.73	Mayer and Ellersieck, 1986
Lepomis macrochirus, 1.30 g	N	S	100%	-	6.5	44	96 h	LC50	s	2.1	Mayer and Ellersieck, 1986
Lepomis macrochirus, 1.40 g	N	S	100%	-	7.5	44	96 h	LC50	s	1.6	Mayer and Ellersieck, 1986
Lepomis macrochirus, 1.40 g	N	S	100%	-	9.0	44	96 h	LC50	s	2.1	Mayer and Ellersieck, 1986
Lepomis macrochirus, 1.70 g	N	F	100%	-	7.4	272	96 h	LC50	s	1.6	Mayer and Ellersieck, 1986
Oncorhynchus mykiss, 1.10 g	N	S	100%	-	7.1	44	96 h	LC50	s	6.5	Mayer and Ellersieck, 1986
Oncorhynchus mykiss, 1.50 g	N	S	100%	-	7.4	44	96 h	LC50	s	2.6	Mayer and Ellersieck, 1986
Oncorhynchus mykiss, 2.20 g	N	F	100%	-	7.4	272	96 h	LC50	s	1.5	Mayer and Ellersieck, 1986
Oncorhynchus mykiss	Y						96 h	LC50	s	1.2-1.8	Hrudey *et al.*, 1976
Oncorhynchus mykiss	Y	F			7.6-7.9	25-50	96 h	LC50	s	1.6	Adams *et al.*, 1995
Perca flavescens, 0.80 g	N	F	100%	-	7.6	314	96 h	LC50	s	0.35	Mayer and Ellersieck, 1986
Pimephales promelas, 0.80 g	N	S	100%	-	7.1	44	96 h	LC50	s	1.30	Mayer and Ellersieck, 1986
Pimephales promelas, 1.10 g	N	F	100%	-	7.4	272	96 h	LC50	s	4.0	Mayer and Ellersieck, 1986
Pimephales promelas	N						96 h	LC50	s	2.0	McCarthy and Whitmore, 1985
Pimephales promelas	Y	S			7.6-7.9	25-50	96 h	LC50	s	1.5	Adams *et al.*, 1995

Organism	A	Test-type	Test-sub. purity	Test-water	pH	Hardness mg $CaCO_3$/l	Exp. time	Crite-rion	Parameter[a]	Result mg/l	Reference
Pimephales promelas	Y	F	99%		7.0-8.2	44-46.4	96 h	LC50	s	0.85	DeFoe *et al.*, 1990
Pimephales promelas	Y	F			7.0-8.2	44-46.4	96 h	LC50	s	1.1	DeFoe *et al.*, 1990
Pimephales promelas	Y	F			7.6-7.9	25-50	96 h	LC50	s	0.92	Adams *et al.*, 1995

a = s:survival; g:growth; r:reproduction

1.5. Acute toxicity of DEHP to freshwater organisms: L(E)C50 values

Organism	A	Test-type	Test-sub. purity	Test-water	pH	Hardness mg $CaCO_3$/l	Exp. time	Crite-rion	Parameter[a]	Result mg/l	Reference
Algae											
Pseudokirchneriella subspicata	Y	S					96 h	EC50	g	>0.1	Adams *et al.*, 1995
Scenedesmus subspicatus	Y	S					72 h	EC50	g	>130	Scholz, 1995b
Crustacea											
Daphnia magna	Y	R					48 h	LC50	s	2.0	Adams. W.J. and Heidolph, 1985
Daphnia magna	Y	S					48 h	LC50	s	>0.16	Adams *et al.*, 1995
Daphnia magna	Y	S					48 h	LC50	s	>0.32	Adams *et al.*, 1995
Daphnia magna	Y						48 h	LC50	s	>1.0	Brown and Williams, 1994
Daphnia magna	N	S					48 h	LC50	s	>1.0	Brown et al., 1997 cited in EU draft DEHP
Daphnia magna	Y						48 h	LC50	s	>0.11	Buchen and Vogel, 1995, cited in EU draft DEHP
Daphnia magna	Y						48 h	LC50	s	>0.10	Buchen and Vogel, 1995, cited in EU draft DEHP
Daphnia magna	Y						48 h	LC50	s	>0.11	Buchen and Vogel, 1995, cited in EU draft DEHP
Daphnia magna	Y						48 h	LC50	s	>0.17	Buchen and Vogel, 1995, cited in EU draft DEHP
Daphnia magna	Y						48 h	LC50	s	>0.30	Brown and Thompson, 1982a
Daphnia magna	N	S					48 h	LC50	s	11	LeBlanc, 1980, cited in EU draft DEHP
Daphnia pulex	N	S					48 h	EC50		0.13	Passino and Smith, 1987
Gammarus pseudolimnaeus, immature	N	S	100%	-	7.4	272	24 h	LC50	s	>32	Mayer and Ellersieck, 1986
Gammarus pseudolimnaeus, immature	N	S	100%	-	7.4	272	96 h	LC50	s	>32	Mayer and Ellersieck, 1986
Gammarus pulex	N	R			8.3	249	96 h	LC50	s	>0.40	Stephenson, 1983
Gammarus pulex	N	R			8.3	104	96 h	LC50	s	>0.40	Stephenson, 1983
Nitocra spinipes	N	S					96 h	LC50	s	>300	Lindén *et al.*, 1979
Insecta											
Chironomus plumosus, 3rd instar	N	S	100%	-	7.4	44	48 h	EC50		>18	Mayer and Ellersieck, 1986
Chironomus plumosus	N						96 h	LC50	s	>18	Suggatt and Foote, 1981
Chironomus tentans	N	S					48 h	LC50	s	>10	EPA, Monsanto, 1983, cited in EU draft DEHP
Pisces											
Brachydanio rerio	N						96 h	LC50	s	>0.32	Van den Dikkenberg *et al.*, 1989
Brachydanio rerio	Y	R					96 h	LC50	s	>100	Scholz, 1995a
Gasterosteus aculeatus	N		97%		6.5-8.4	170-238	96 h	LC50	s	>0.32	Van den Dikkenberg *et al.*, 1989
Ictalurus punctatus		S					96 h	LC50	s	>10	Mayer and Sanders, 1973
Ictalurus punctatus	Y	F					96 h	LC50	s	>100	Johnson and Finley, 1980, cited in EU draft DEHP
Ictalurus punctatus, 1.50 g	N	S	100%	-	7.4	44	24 h	LC50	s	>100	Mayer and Ellersieck, 1986
Ictalurus punctatus, 1.50 g	N	S	100%	-	7.4	44	96 h	LC50	s	>100	Mayer and Ellersieck, 1986

Organism	A	Test-type	Test-sub. purity	Test-water	pH	Hardness mg $CaCO_3$/l	Exp. time	Crite-rion	Parameter[a]	Result mg/l	Reference
Ictalurus punctatus, 3.10 g	N	F	100%	-	7.4	272	96 h	LC50	s	>0.2	Mayer and Ellersieck, 1986
Jordanella floridae	N						96 h	LC50	s	>0.32	Van den Dikkenberg *et al.*, 1989
Lepomis macrochirus		S					96 h	LC50	s	>10	Mayer and Sanders, 1973
Lepomis macrochirus	N	S					96 h	LC50	s	>250	Bionomics Inc., 1972, cited in EU draft DEHP
Lepomis macrochirus	Y	F					96 h	LC50	s	>100	Johnson and Finley, 1980, cited in EU draft DEHP
Lepomis macrochirus	Y	S					96 h	LC50	s	>0.20	Adams *et al.*, 1995
Lepomis macrochirus, 0.60 g	N	S	100%	-	7.4	44	24 h	LC50	s	>100	Mayer and Ellersieck, 1986
Lepomis macrochirus, 0.60 g	N	S	100%	-	7.4	44	96 h	LC50	s	>100	Mayer and Ellersieck, 1986
Lepomis macrochirus, 7.20 g	N	F	100%	-	7.4	272	96 h	LC50	s	>0.2	Mayer and Ellersieck, 1986
Oncorhynchus mykiss		S					96 h	LC50	s	>10	Mayer and Sanders, 1973
Oncorhynchus mykiss		S					96 h	LC50	s	>1000	Silvo, O.E.J., 1974, cited in EU draft DEHP
Oncorhynchus mykiss	N	S					96 h	LC50	s	540	Hrudey et al., 1976, cited in EU draft DEHP
Oncorhynchus mykiss	Y	F					96 h	LC50	s	>0.32	Adams *et al.*, 1995
Oncorhynchus kisutch, 1.50 g	N	S	100%	-	7.4	44	24 h	LC50	s	>100	Mayer and Ellersieck, 1986
Oncorhynchus kisutch, 1.50 g	N	S	100%	-	7.4	44	96 h	LC50	s	>100	Mayer and Ellersieck, 1986
Oncorhynchus mykiss, 1.50 g	N	S	100%	-	7.4	44	24 h	LC50	s	>100	Mayer and Ellersieck, 1986
Oncorhynchus mykiss, 1.50 g	N	S	100%	-	7.4	44	96 h	LC50	s	>100	Mayer and Ellersieck, 1986
Oncorhynchus mykiss	Y	F					96 h	LC50	s	>20	DeFoe *et al.*, 1990
Oryzias latipes	N						96 h	LC50	s	>0.32	Van den Dikkenberg *et al.*, 1989
Oryzias latipes	Y	F					96 h	LC50	s	>0.67	DeFoe *et al.*, 1990
Pimephales promelas		S					96 h	LC50	s	>10	Mayer and Sanders, 1973
Pimephales promelas	Y	F					96 h	LC50	s	>0.67	DeFoe *et al.*, 1990
Pimephales promelas, fingerling	N	F	100%	-	7.4	272	96 h	LC50	s	>1	Mayer and Ellersieck, 1986
Pimephales promelas	Y	F					96 h	LC50	s	>0.33	DeFoe *et al.*, 1990
Pimephales promelas	Y	S					96 h	LC50	s	>0.16	Adams *et al.*, 1995
Pimephales promelas	Y	F					96 h	LC50	s	>5	Union Carbide, 1974, cited in EU draft DEHP
Poecilia reticulata	N						96 h	LC50	s	>0.32	Van den Dikkenberg *et al.*, 1989
Salmo salar							96 h	LC50	s	>10	Zitko 1972, cited in EU draft DEHP

a: g:growth, s:survival

Appendix 2. Toxicity data for saltwater organisms

2.1. Legend

Organism	species used in the test, if available followed by age, size, weight or life stage
A	Y = test substance analyzed in test solution N = test substance not analyzed in test solution or no data
Test type	S = static, R = static with renewal, F = flow through
Test water	a.m. = artificial medium, a.s.w. = artificial seawater, n.f.s = natural filtered seawater, r.t.w. = reconsituted tap water (+additional salts)
Test substance purity	percentage active ingredient
Exposure time	h = hours, d = days, w = weeks, m = months, min. = minutes
Results	> and ≥ values = highest concentration used in the test

2.2. Chronic toxicity of DBP to saltwater organisms: NOEC values

Organism	A	Test-type	Test-sub. purity	Test-water	pH	Hardness mg $CaCO_3$/l	Exp. time	Crite-rion	Result mg/l	Reference
Algae										
Dunaliella parva	N			a.m.			7 d	NOEC	0.28[a]	Acey *et al.*, 1987

a = growth inhibition

2.3. Chronic toxicity of DEHP to saltwater organisms: NOEC values

Organism	A	Test-type	Test-sub. purity	Test-water	pH	Hardness mg $CaCO_3$/l	Exp. time	Parameter	Crite-rion	Result mg/l	Reference
Crustacea											
Palaemonetes pugio	Y	R		a.m.			28 d	m	NOEC	>1.0[a, b]	Laughlin *et al.*, 1978
Mollusca											
Mytilus edulis	Y	F	>97.5%	n.f.s			28d	m	NOEC	≥0.042[b, c]	Brown and Thompson, 1982b

m: mortality

2.4. Acute toxicity of DBP to saltwater organisms: L(E)C50 values

Organism	A	Test-type	Test-sub. purity	Test-water	pH	Hardness mg $CaCO_3$/l	Exp. time	Crite-rion	Result mg/l	Reference
Bacteria										
Vibrio fisheri	N						0.5 h	EC50	11-23	Kaiser and Palabrica, 1991
Algae										
Gymnodinium breve	N	S		a.m.			96 h	EC50	0.0034[a, c]	Wilson *et al.*, 1978
Gymnodinium breve	N	S		a.m.			96 h	LC50	0.02[a, d]	Wilson *et al.*, 1978
Gymnodinium breve	N	S		a.m.			96 h	EC50	0.2[b, c]	Wilson *et al.*, 1978
Gymnodinium breve	N	S		a.m.			96 h	LC50	0.6[b, d]	Wilson *et al.*, 1978
Crustacea										
Artemia salina	N			a.s.w.			24 h	LC50	8.0	Hudson *et al.*, 1981
Mysidopsis bahia	Y	S			7.6-7.9	25-50	96 h	LC50	0.50	Adams *et al.*, 1995
Nitocra spinipes	N						96 h	LC50	1.7	Lindén *et al.*, 1979
Pisces										
Ictalurus punctatus, 1.40 g	N	S	100%		7.1	44	96 h	LC50	2.91	Mayer and Ellersieck, 1986
Ictalurus punctatus, yolk-sac fry	N	F	100%		7.4	272	96 h	LC50	0.46	Mayer and Ellersieck, 1986

a:both results obtained from one test
b: both results obtained from one test
c: growth rate
d: cell number

2.5. Acute toxicity of DEHP to saltwater organisms: L(E)C50 values

Organism	A	Test-type	Test-sub. purity	Test-water	pH	Hardness mg $CaCO_3$/l	Exp. time	Crite-rion	Result mg/l	Reference
Pisces										
Cyprinodon variegatus	Y	F					96 h	LC50	>0.17	Adams *et al.*, 1995
Cyprinodon variegatus	N	S					96 h	LC50	>550	Heitmuller et al., 1981, cited in EU draft DEHP

Appendix 3. Toxicity data from soil and sediment tests

3.1. Legend

Organism	species used in the test, if available followed by age, size, weight or life stage
A	Y = test substance analyzed in test solution N = test substance not analyzed in test solution or no data
Test type	S = static, R = static with renewal, F = flow through
Test water	a.m. = artificial medium, a.s.w. = artificial seawater, n.f.s = natural filtered seawater, r.t.w. = reconsituted tap water (+additional salts)
Test substance purity	percentage active ingredient
Exposure time	h = hours, d = days, w = weeks, m = months, min. = minutes
Results	> and ≥ values = highest concentration used in the test

3.2. Toxicity of DBP to soil organisms: deviating tests

Organism	Soil-type	pH	% O.m.	% Clay	Temperature °C	Exposure time	Crite-rion	Result test soil mg/kg d.w.	Result stand. soil mg/kg d.w.	Reference
Oligochaeta										
Eisenia fetida	wet paper test				20	48 h	LC50	1.4 mg/cm^{2a}		Neuhauser *et al.*, 1985
Eisenia fetida	contact test					48 h	LC50	0.074 mg/kg^{b}		Callahan *et al.*, 1994

a = 'short term contact test' on wetted paper, chloroform or acetone used as solvent, but evaporated before start of the test, at least five nominal concentrations b= no information on tests, relative standard error of LC50 is 0.06 mg/kg

3.3. Toxicity of DEHP to soil organims: deviating tests

Organism	Soil type	pH	% O.M.	% Clay	Temperature °C	Exposure time	Parameter	Criterion	Result mg.kg f.w.	Reference
Rana arvalis	sediment					29 d	reproduction	NOEC	> 150 mg/kg^{a}	Wennberg et al., 1997

a: problems with infection by fungi and bacteria; problems with measurement of endpoint as during counting hatched animals, the animals are disturbed; grey literature

3.4. Chronic toxicity of DEHP to sediment organisms

Organism	Soil type	pH	% O.M.	% Clay	Temperature °C	Exposure time	Parameter	Criterion	Result mg.kg f.w.	Reference
Rana arvalis	sediment					60 d	reproduction	NOEC	10 mg/kg	Larson and Thurén, 1987

3.5. Acute toxicity of DEHP to soil organisms

Organism	Soil-type	pH	% O.m.	% Clay	Temperature °C	Exposure time	Crite-rion	Result test soil mg/kg d.w.	Result stand. soil mg/kg d.w.	Reference
Oligochaeta										
Eisenia fetida	wet paper test				20	48 h	NOEC	>25[a]		Neuhauser *et al.*, 1985

a = 'short term contact test' on wetted paper, survival, chloroform or acetone used as solvent but evaporated before start of the test, at least five nominal concentrations

3.6. Toxicity of DEHP to soil microbial processes

Process	Soil-type	pH	% O.m.	% Clay	Temperature °C	Exposure time	Crite-rion	Result test soil mg/kg d.w.	Result stand. soil mg/kg d.w.	Reference
Microbial processes										
respiration inhibition	loam				26	8 h	LOEC	49000[a]		Mathur, 1974, cited in EU draft DEHP
respiration	silt loam				25	12.9 w	NOEC	>250[b]		Kirchmann et al., 1991, cited in EU draft DEHP
nitrogen mineralization	silt loam				25	12.9 w	NOEC	>250[b]		Kirchmann et al., 1991, cited in EU draft DEHP
nitrification	silt loam				25	12.9 w	NOEC	>250[b]		Kirchmann et al., 1991, cited in EU draft DEHP

a = inhibition of respiration observed, one (nominal) concentration tested: 0.2 ml DEHP per 4 g of soil, soil not pre-exposed to phthalate esters, moisture content 66% of field capacity; b = natural soil flora, result in wet weight or dry weight is not reported, soil wetted to 'optimal' moisture content, two test concentrations 5 and 250 mg/kg, no effects observed on all three endpoints, soil had been stored for two years

Chronic toxicity of DEHP to freshwater organisms: NOEC values, sediment (mg/kg fresh weight, endpoint is hatching success)

Appendix 4. Toxicity data from deviating tests

4.1. Legend

Organism	species used in the test, if available followed by age, size, weight or life stage
A	Y = test substance analyzed in test solution
	N = test substance not analyzed in test solution or no data
Test type	S = static, R = static with renewal, F = flow through
Test water	a.m. = artificial medium, a.s.w. = artificial seawater, n.f.s = natural filtered seawater, r.t.w. = reconsituted tap water (+additional salts)
Test substance purity	percentage active ingredient
Exposure time	h = hours, d = days, w = weeks, m = months, min. = minutes
Results	> and ≥ values = highest concentration used in the test

4.2. Toxicity data on DBP from deviating tests

Organism	A	Test-type	Test-sub. purity	Test-water	pH	Hardness mg $CaCO_3$/l	Exp. time	Crite-rion	Result mg/l	Reference
Protozoa										
Tetrahymena pyriformis	N						24 h	EC50	2.2[a]	Yoshioka *et al.*, 1985, Yoshioka *et al.*, 1986
Algae										
Synechococcus lividus	N			a.m.			14 d	LOEC	0.0028[b]	Acey *et al.*, 1987
Crustacea										
Daphnia magna	Y						24 h	EC50	4.1[c]	Scholz, 1994b
Daphnia magna	N						24 h	LC50	17	Kühn *et al.*, 1989
Gammarus pulex	N	F					25d	NOEC	0.1	Thurén and Woin, 1991
Daphnia magna	Y						48 h	EC50	3.4	Scholz, 1994b
Palaemonetes pugio	Y	R		a.m.			30 d	NOEC	≥1[d]	Laughlin *et al.*, 1978
Pisces										
Ictalurus punctatus, 1.40 g	N	S	100%	-	7.1	44	24 h	LC50	3.7	Mayer and Ellersieck, 1986
Lepomis macrochirus, 1.40 g	N	S	100%	-	7.1	44	24 h	LC50	1.2	Mayer and Ellersieck, 1986
Lepomis macrochirus, 1.40 g	N	S	100%	-	7.5	44	24 h	LC50	>3.0	Mayer and Ellersieck, 1986
Lepomis macrochirus, 1.40 g	N	S	100%	-	9.0	44	24 h	LC50	>3.0	Mayer and Ellersieck, 1986
Leuciscus idus, 3.4 g	N	F	>99%	r.t.w.	~8.0	238	96 h	LC0	≥ 4.6	BASF AG, 1989
Leuciscus idus, 3.4 g	N	F	>99%	r.t.w.	~8.0	238	96 h	LC100	≤ 10	BASF AG, 1989
Oncorhynchus mykiss, 1.50 g	N	S	100%	-	7.4	44	24 h	LC50	2.8	Mayer and Ellersieck, 1986
Oncorhynchus mykiss, 2.20 g	N	F	100%	-	7.4	272	24 h	LC50	4.2	Mayer and Ellersieck, 1986
Oncorhynchus mykiss, yolk-sac fry	N	F	100%	-	7.6	314	24 h	LC50	>1.2	Mayer and Ellersieck, 1986
Oryzias latipes	N						48 h	LC50	4.3	Yoshioka *et al.*, 1986
Perca flavescens, 0.80 g	N	F	100%	-	7.6	314	24 h	LC50	>1.2	Mayer and Ellersieck, 1986
Pimephales promelas, 0.80 g	N	S	100%	-	7.1	44	24 h	LC50	3.3	Mayer and Ellersieck, 1986
Pimephales promelas, 1.10 g	N	F	100%	-	7.4	272	24 h	LC50	4.8	Mayer and Ellersieck, 1986
Pimephales promelas	N						48 h	LC50	1.5	Mayer and Sanders, 1973
Pimephales promelas	Y	F					120 h	LC50	0.92	Springborn Bionomics, 1983
Pimephales promelas	Y	F					144 h	LC50	0.92	Springborn Bionomics, 1983

a = reproductive inhibition; b = the growth rate of monodispersed cells was greatly reduced at the lowest concentration compared to the control, however growth of aggregated cells was stimulated at all DBP concentrations compared to the control; c = immobilisation; d =survival of early instar larvae (zoeae), acetone was used as a carrier at 1 ml/l, physical effects occured at higher concentrations

4.3. Toxicity data on DEHP from deviating tests

Organism	A	Test-type	Test-sub. purity	Test-water	pH	Hardness mg $CaCO_3$/l	Exp. time	Crite-rion	Result mg/l	Reference
Natural pelagic community	Y	R					30 d	NOEC	≥0.059[a]	Perez et al., 1977, cited in EU draft DEHP
Natural pelagic community	Y	R					30 d	NOEC	≥0.016[b]	Perez et al., 1977, cited in EU draft DEHP
Natural sediment flora	N	S					20 h	LOEC	25 mg/kg_{fw}[c]	Larson et al., 1986, cited in EU draft DEHP
Macrophyta										
Lemna gibba	N	R					7 d	EC50	2100[d]	Davis, 1981, cited in EU draft DEHP
Bacteria										
Vibrio fisherie	N	S	N				5-30m	EC50	800	Kaiser and Devillers. J., 1994
Algae										
Gymnodium breve	N						96 h	EC50	31000	Wilson *et al.*, 1978
Crustacea										
Daphnia magna	Y		>97%		8.0	300	21 d	NOEC	0.072[e]	Knowles *et al.*, 1987
Gammarus pulex	N	F					25 d	NOEC	0.10[b, k, l]	Thuren & Woin, 1991
Daphnia magna	Y	S					48 h	EC50	>100[e, f]	Scholz, 1995d
Daphnia magna	Y						48 h	LC50	<1.0[g, h]	Buchen and Vogel, 1995, cited in EU draft DEHP
Daphnia magna	Y						48 h	LC50	<1.0[g, h]	Buchen and Vogel, 1995, cited in EU draft DEHP
Insecta										
Chironomus riparius, 1st instar	Y	S					28 d	NOEC	>4700 mg/kg_{fw}[f]	Thompson et al., 1995, cited in EU draft DEHP
Chironomus riparius, 1st instar	Y	S	≥98%	sediment	7.8-8.3	81	28 d	NOEC	≥10000 mg/kg_{dw}[g]	Brown *et al.*, 1996
Paratanytarsus parthenogenica 2nd or 3d instar	Y	S					48 h	EC50	>0.18[e]	Adams *et al.*, 1995
Aeshna sp.	Y	S					60 d	NOEC	300 mg/kg_{fw}[h]	Woin and Larson, 1987, cited in EU draft DEHP
Pisces										
Oncorhynchus mykiss	N	F					90 d	NOEC	0.054[i]	Mayer *et al.*, 1977
Pimephales promelas	N	F					127 d	NOEC	0.10[i]	Mayer *et al.*, 1977
Salvelinus fontinalis	N	F					150 d	NOEC	0.052[j]	Mayer *et al.*, 1977

a = marine microcosm, NOEC of reduction in NH_3 in winter; b = marine microcosm, NOEC of reduction in NH_3 in summer; c = inhibition of O_2 consumption in overlaying water, ethanol used as a cosolvent; d = growth inhibition, EC50 value is the average of 7 values, ranging from 397-7582 mg/l, solvent nor concentrations were measured d = growth inhibition, EC50 value is the average of 7 values, ranging from 397-7582 mg/l, solvent nor concentrations were measured e = DNA-content, RNA/DNA ratio at day 7, acetone used as a coslvent f = delayed emergence, number of emerged adults, aceton used as a cosolventg = survival, development, emergence, aceton used as a cosolvent, DEHP for 96 - 99%

recovered from sediment after extraction with little to no loss of activity over 28 d period, nominal = actual concentration h = predation efficiency, ethanol used as a cosolvent i = growth j = growth inhibitionsection

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