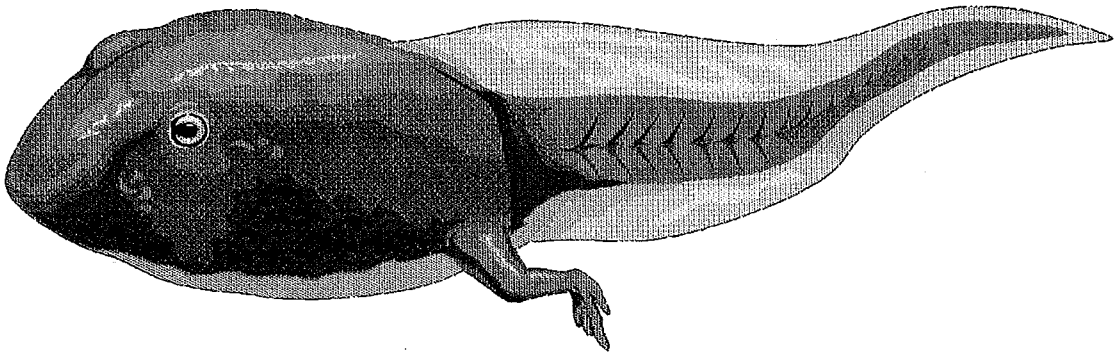
IVL-RAPPORT

The Influence of Sediment-Associated Phthalate Esters (DEHP and DIDP) on Hatching and Survival of the Moorfrog, *Rana arvalis*



Lena Wennberg, Helena Parkman, Mikael Remberger, Tomas Viktor and Camilla Williams

Stockholm, maj 1997
B 1260

IVL
INSTITUTET FÖR VATTEN- OCH LUFTVÅRDSFORSKNING

The Influence of Sediment-Associated Phthalate Esters (DEHP and DIDP) on Hatching and Survival of the Moorfrog, *Rana arvalis*

1997-01-10

B- 1260

Lena Wennberg
Helena Parkman
Mikael Remberger
Tomas Viktor
Camilla Williams

IVL

Institutet för Vatten- och Luftvårdsforskning
Swedish Environmental Research Institute

Organisation/Organization
Institutet för Vatten- och Luftvårdsforskning

Adress/Address
Box 21060
100 31 STOCKHOLM

Telefonnr/Telephone
08-729 15 00

RAPPORTSAMMANFATTNING
Report Summary

Projekttitel/Project title
The Influence of Sediment-Associated Phthalate Esters (DEHP and DIDP) on Hatching and Survival of the Moorfrog, *Rana arvalis*

Anslagsgivare för projektet/Project sponsor
Hydro Plast AB and Neste OXO AB

Rapportförfattare, author
Lena Wennberg, Helena Parkman, Mikael Remberger, Tomas Viktor and Camilla Williams

Rapportens titel och undertitel/Title and subtitle of the report
The Influence of Sediment-Associated Phthalate Esters (DEHP and DIDP) on Hatching and Survival of the Moorfrog, Rana arvalis

Sammanfattning/Summary
Larsson and Thurén (1987), studied the influence of DEHP on hatching of the moorfrog (*Rana arvalis*). Eggs from the moorfrog were exposed to sediment contaminated with DEHP in a laboratory model systems. Hatching success was decreased when the eggs were exposed to DEHP contaminated sediments. Approximately 50 % of the eggs hatched when exposed to sediments that contained 150 µg DEHP/ g fresh weight. The sediment was fortified with DEHP dissolved in ethanol. Doubts have been raised as to whether this spiking method may have caused localised high concentrations of DEHP giving the possibility of physical effects. Furthermore, the presence of ethanol, or other organic solvents, may alter the bioavailability and hence the toxicity of the compound. Concerns have also been raised regarding the statistical validity of the work.

The aims of the project were to (i) repeat the frog-study by Larsson and Thurén (1987), using a more appropriate method to fortify the sediments and possibilities to use improved statistical analysis of results and (ii) investigate if the organic matter content of the sediment alters the toxicity of DEHP. This is important for translating the results from this study to other environments (or; in a hazard assessment).

Contamination of DEHP and DIDP in sediment did not cause any observed effects on hatching and survival of frog eggs and tadpoles. Results indicated no statistically significant effects on hatching at a sediment concentration of 600 µg/g dw . Based on the results a NOEC value would be >600 µg DEHP/g dw (150 µg DEHP/g fresh weight) and >600 µg DIDP/g dw (150 µg DIDP/g fresh weight).

The phthalates did not effect growth of the tadpoles. Some differences in growth (not statistically significant) were noticed but were probably caused by spatial differences in the exposure system. The bioaccumulation of DEHP in the tadpoles was proportional to the concentrations in the sediments, up to a 'plateau'-concentration of 300 µg DEHP/g lipid contents in the frogs. Increased organic contents in the sediments decreased the bioaccumulation. The biota to sediment accumulation factor defined as the quotient of DEHP in the tadpoles / DEHP in sediment was much lower (more than tenfold) than in the study of Larsson and Thurén. DIDP was not detected in the tadpoles.

Nyckelord samt ev. anknytning till geografiskt område, näringsgren eller vattendrag/Keywords
Phthalate esters, DEHP, DIDP, sediment, toxic effects, moorfrog, Rana arvalis

Bibliografiska uppgifter/Bibliographic data
IVL Rapport B 1260

Beställningsadress för rapporten/Ordering address
IVL, Publikationsservice, Box 21060, S-100 31 Stockholm, Sweden

Table of Contents

Foreword

This study was initiated and financed by Hydro Plast AB and Neste Oxo AB. The study was performed at IVL during May and June 1996. Project leader was Lena Wennberg. Helena Parkman was responsible for all tasks about sediments and Mikael Remberger for all analyses. For the handle and care of frog eggs and tadpoles Camilla Williams and Tomas Viktor were responsible. At the hectic start of the experiment we had great help from Ann-Sofie Allard, Carina Bergqvist, Björn Palvall and Jonas Röttorp.

Sammanfattning

Larsson och Thurén (1987), studerade påverkan på kläckning av grodägg från åkergroda (*Rana arvalis*) som exponerats för DEHP (dietylhexylftalat) via sediment. Resultatet från denna studie tyder på att DEHP kan påverka kläckningen av grodägg. Av äggen kläcktes ca 50 % vid koncentrationen 150 µg DEHP/g sediment (våtvikt). I denna studie tillsattes DEHP sedimentet genom att först lösa ämnet i etanol. Det finns tveksamheter i detta förfaringssätt, eftersom närvaro av etanol, eller andra lösningsmedel, kan förändra biotillgängligheten och därmed toxiciteten hos det testade ämnet. Metoden kan också medföra att DEHP inte är jämnt fördelad i sedimentet utan att lokala höga koncentrationer kan orsaka fysikaliska effekter på äggen.

Målet med den studie som nu är gjord är att (i) upprepa Larsson och Thuréns studie med en lämpligare metod för att preparera sedimentet med ftalat, i syfte att klarlägga tveksamheterna i denna tidigare studie och (ii) undersöka om innehållet av organiskt material i sedimentet påverkar DEHPs toxicitet hos grodägg.

Under våren 1996 upprepades Larsson och Thuréns studie med följande förändringar: experimenttemperaturen valdes till 10 °C och ftalatinblandningen i sedimentet skedde enligt en metod utarbetad av Brown *et al.* (1996). Förutom DEHP testades även DIDP (diisodecylftalat). Koncentrationer mellan 0 till 600 µg ftalat/g sediment (ts) testades.

Ingen påverkan av DEHP eller DIDP på kläckning av grodägg eller överlevnad av grodyngel kunde iakttagas. Enligt detta försök bör ett NOEC värde vara > 600 µg DEHP/g sediment (torrsubstans) eller omräknat >150 µg DEHP/g sediment (våtvikt). Det samma gäller för DIDP.

Ftalaterna påverkade inte heller tillväxten hos grodynglen. De skillnader i tillväxt (ej statistiskt signifikant) som noterades vid olika ftalatkoncentrationer, orsakades troligen av temperaturskillnader i experimentlokalen.

Bioackumulationen av DEHP i grodynglen var proportionell mot halten i sedimenten, upp till ett platåvärde av ca 300 µg/g fett i grodorna. Ökad organisk halt i sedimenten innebar minskad ackumulation i grodorna. Förhållandet DEHP i groda/DEHP i sediment var betydligt lägre (mer än en tiopotens) än i Larsson och Thuréns studie. DIDP kunde ej detekteras i grodorna.

Summary

Larsson and Thurén (1987), studied the influence of DEHP on hatching of the moorfrog (*Rana arvalis*). Eggs from the moorfrog were exposed to sediment contaminated with DEHP in a laboratory model systems. Hatching success was decreased when the eggs were exposed to DEHP contaminated sediments. Approximately 50 % of the eggs hatched when exposed to sediments that contained 150 µg DEHP/ g fresh weight. The sediment was fortified with DEHP dissolved in ethanol. Doubts have been raised as to whether this spiking method may have caused localised high concentrations of DEHP giving the possibility of physical effects. Furthermore, the presence of ethanol, or other organic solvents, may alter the bioavailability and hence the toxicity of the compound. Concerns have also been raised regarding the statistical validity of the work.

The aims of the project were to (i) repeat the frog-study by Larsson and Thurén (1987), using a more appropriate method to fortify the sediments and possibilities to use improved statistical analysis of results and (ii) investigate if the organic matter content of the sediment alters the toxicity of DEHP. This is important for translating the results from this study to other environments (or; in a hazard assessment).

Contamination of DEHP and DIDP in sediment did not cause any observed effects on hatching and survival of frog eggs and tadpoles. Results indicated no statistically significant effects on hatching at a sediment concentration of 600 µg/g dw . Based on the results a NOEC value would be >600 µg DEHP/g dw (150 µg DEHP/g fresh weight) and >600 µg DIDP/g dw (150 µg DIDP/g fresh weight).

The phthalates did not effect growth of the tadpoles. Some differences in growth (not statistically significant) were noticed but were probably caused by spatial differences in the exposure system.

The bioaccumulation of DEHP in the tadpoles was proportional to the concentrations in the sediments, up to a 'plateau'-concentration of 300 µg DEHP/g lipid contents in the frogs. Increased organic contents in the sediments decreased the bioaccumulation. The biota to sediment accumulation factor defined as the quotient of DEHP in the tadpoles / DEHP in sediment was much lower (more than tenfold) than in the study of Larsson and Thurén. DIDP was not detected in the tadpoles.

Introduction

The Ecocycle Commission (Kretsloppsdelegationen) in Sweden proposed that the plasticized PVCs of today should be phased out in the near future. The plasticizers used in PVC consist predominantly of phthalates, and di-(ethylhexyl)-phthalate (DEHP) is one of the most used forms. Large amounts of these substances are used in Swedish production, 10 900 tonnes of DEHP was used in plastic production in Sweden 1992.

Phthalate esters "plasticizers" such as DEHP and DIDP (di-isodecyl-phthalate) have low water solubility and are therefore expected to predominantly be bound to organic particles. The "true" water solubility of DEHP is expected to be less than 1 µg/l whereas the water solubility of DIDP is even lower. In aquatic ecosystems, sediment serve as a sink for phthalates. In a study by Parkman and Remberger (1995), sediments were sampled from different types of aquatic environments in Sweden and analysed for seven phthalates and two mixtures of phthalates, including DEHP and DIDP.

All nine substances and mixtures were detected in Swedish sediment. The amounts of phthalates increased with increasing anthropogenic influence. DEHP-concentrations at two point sources were 33 and 47 µg/g dw respectively, and at one of the point sources the concentration of DIDP was 20 ng/g dw. DIDP was not detected in any other sediment sample. DEHP varied between 0.01 and 0.4 µg/g dw in remote lakes, while other phthalates were mostly undetectable.

The transport of DEHP in the aquatic environment is mainly dependent on the transport of particles and colloids. Short-chain phthalates with lower K_{ow} will be less adsorbed to sediment and particulate organic matter. Hydrolysis is considered to be of less importance for the fate of DEHP. Biotic degradation is the major fate process for long chained phthalates in the aquatic environment (KemI, 1995). Degradation half-lives of DEHP range from about one week to a couple of months. The degradation is slower at low temperature (<10 C) and much slower under anaerobic conditions. Branched and long chained phthalates adsorb very strongly to organic material and may become less available for biodegradation, which results in accumulation in sediments (KemI, 1995).

The phthalate esters with alkyl chain lengths of more than four carbon atoms are not regarded as acutely toxic to pelagic organisms, due to the low water solubility (Adams *et al.*, 1995). In chronic toxicity tests, on the other hand, it was found that the number of immobilised daphnia increased as water solubility decreased. (Rhodes *et al.*, 1995). This effect is probably caused by physical entrapment. The effects of phthalates to benthic organisms have only been investigated in a few studies.

At the Brixham Environmental Laboratory a study of phthalate effects on midges was performed (Brown *et al.*, 1996). Larvae of the midge *Chironomus riparius*, were

exposed for 28 days to sediment spiked with DEHP or DIDP at nominal concentrations of 100, 1000, and 10000 mg/kg dw. The number of animals developing successfully and emerging as adult insects was recorded. There was no effect at all at any of the concentrations tested for either of the test substances. Therefore, no observed effect concentration (NOEC) was 10 000 mg DEHP/kg dw and 10 000 mg DIDP/kg dw. These results confirm that chironomide larvae are not sensitive to phthalates, which was suggested by Streufert *et al.* (1980), who found that chironomide larvae could accumulate phthalates from the water (about 300 times the concentration in the water) without being affected.

Larsson and Thurén (1987), studied the influence of DEHP on hatching of the moorfrog (*Rana arvalis*). Eggs from the moorfrog were exposed to sediment contaminated with DEHP in laboratory model systems. Hatching success decreased when the eggs were exposed to DEHP contaminated sediments. Approximately 50 % of the eggs hatched when exposed to sediments that contained 150 µg DEHP/g fresh weight. The sediment was fortified with DEHP dissolved in ethanol. Doubts have been raised as to whether this spiking method may have caused localised high concentrations of the DEHP giving the possibility of physical effects. This hypothesis may possibly be supported by the fact that the only effect attributable to the DEHP was on the number of eggs hatching with no effect on the times of hatching and no recorded abnormalities of the hatched tadpoles. The study also showed an apparent plateau of effect on hatching success with increasing DEHP concentration although the DEHP body burden of the tadpoles increased with increasing exposure to DEHP. Thus, the effect seen may not be due to a direct toxic mechanism. The presence of ethanol, or other organic solvents, may also alter the bioavailability of the compound and by that, the toxicity of the compound. Some concerns have been raised regarding the statistical validity of the effects noted in the paper.

The aims of the project were to (i) repeat the frog-study by Larsson and Thurén (1987), using a more appropriate method to fortify the sediments and an improved statistical analysis of results and (ii) investigate if the organic matter content of the sediments alter the toxicity of DEHP. This is important for the translating of the results from this study to other environments (or; in a hazard assessment).

Materials and Methods

Three test approaches were conducted: 1) frog-eggs exposed to a natural sediment fortified with different concentrations of DEHP, 2) frog-eggs exposed to DEHP-contaminated sediments with different organic contents and 3) frog-eggs exposed to a natural sediment fortified with different concentrations of DIDP.

Test species

The test species, the moorfrog (*Rana arvalis*) is a common species of frog in northern Europe and most of Sweden, except at high altitudes, while it is considered as a endangered species in southern Europe. The moorfrog is a generalist and has been observed in oligotrophic as well as eutrophic environments. It lays its eggs in the beginning of April in small lakes and ponds. The eggs are laid in water, but after 2-3 days the egg clump sinks to the bottom and continue to develop there. Normally, more than 90 % of the eggs hatch (Sven-Åke Berglind, pers. comm.). After hatching the tadpoles swim and burrow at the surface of the sediment. Therefore, exposure to contaminated sediment can have a greater influence on the hatching of frog eggs and in tadpole development than does water exposure alone.

Experimental sediments

Original sediments

The sediment was collected in lake Stensjön in Hälsingland (in the middle of Sweden). Sediment from lake Stensjön, was analysed by Parkman and Remberger (1995). The contents of phthalate and other substances such as mercury are low, and the organic content is 22.5 % of dw which is similar to the sediment used by Larsson and Thurén (1987), which had an organic content between 21-33 % dw. Dry weight were determinated from the loss during 24 h at 105°C.

Four sediments (including Stensjön), with different organic contents measured as "loss of ignition" (LOI) for 2 h at 550°C (2,9 - 76,6 %) were selected. Those were Lake Grändalssjön (south of Stockholm, 42 % LOI) river Göta Älv (close to Trollhättan, 2,9 % LOI), River Svartån (close to Ormaryd, 76,6 % LOI).

Fortifying sediment prior to experiment

In the study of Larsson and Thurén (1987), the sediment was fortified with DEHP dissolved in ethanol. This method, using water miscible solvents, is commonly employed when lipophilic compounds, with low water solubility, are dissolved/emulsified in water and sediment. However, the presence of ethanol, or other organic solvents, may alter the bioavailability of the compound and by that the toxicity. The presence of an readily degradable compound, for instance ethanol, may also enhance the oxygen demand of the sediment. To avoid uncertainty and problems in using organic solvents, the following method was adopted (Brown *et al.*, 1996): (i) the phthalate ester was dissolved in acetone and carefully mixed with air-dried uncontaminated sediment (ii) the solvent was carefully evaporated under reduced pressure in an evaporator (Rotovap) (iii) this artificially contaminated sediment

("spiking sediment") was added to the fresh uncontaminated test sediment (in 4 litre glass jars) at eight concentrations; 0 (acetone blank), 15, 30, 50, 100, 150, 300 and 600 µg DEHP/g dw and DIDP respectively, and was thoroughly blended. A control without phthalates and acetone was included.

Another series of bottles were arranged in the same way, contained the three additional sediments with different organic contents, (300 µg DEHP/g dw). Thereafter, were the sediment samples agitated on a shaking board at ambient temperature for one week in order to facilitate homogeneity and equilibrium. The pore water was analysed after 3, 6 and 8 days (by centrifugation; 1000 G, 30 min) in order to establish if equilibrium was achieved. The "spiking sediments" were analysed in order to calculate the expected concentrations in the experimental sediments (*cf.* Analyses).

One batch of the sediment (2200 g fw) was fortified according to the ethanol-spiking method used by Larsson and Thurén (1987). It was not possible to completely follow the experimental protocol of Larsson and Thurén (1987), since the spiking volume of ethanol was not reported. In the present study, the sediment was spiked with 300 µg DEHP/g dw, with DEHP dissolved in ethanol (2.75 ml). The sediment was carefully blended (1 hour) but not equilibrated after the addition of the test compound.

Experimental set-up

After the equilibration period, sediment from the different concentration series were divided in five replicates (400 g) and added to beakers (3 litres) and subsequently synthetic lake water (approximately 2 litres) was added. The series spiked with DEHP dissolved in ethanol was immediately (without equilibration) divided in five replicates and transferred to beakers. The beakers were left for approximately four days, in order to let the sediment settle properly, before the eggs were added.

The water was not changed during the study. The systems were continuously and gently aerated during the experimental period. The air was cleaned by activated charcoal.

Each beaker received approximately 50 eggs, randomly chosen from egg clumps, collected in the field. In figure 1 the experimental beaker is shown. The study took place in a controlled environmental chamber at approximately 10°C, on a 12 h light: 12 h dark photoperiod. The experimental period was 29 days and started when the eggs were placed in the beaker. Oxygen and pH were measured daily in 20 different beakers. After the eggs had hatched, also the temperature was measured daily in 20 beakers. Ammonia and nitrite-nitrate were measured in beakers when the frog larva seemed "unhealthy" as

evidenced by decreased tadpole motility and traces of flocks in the water, probably caused by fungi/bacteria infection,

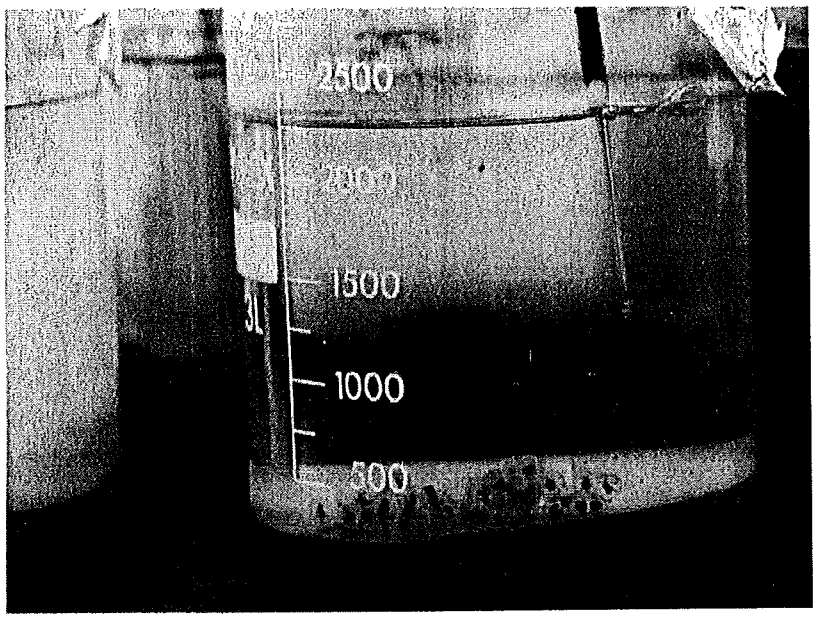


Figure 1. Experimental beaker with frog eggs.

The response variables (endpoints) were embryo hatching, and tadpole survival and growth. These variables were monitored once a week. The amount of DEHP in overlaying water, sediment and tadpoles was measured at the end of the experiment.

The toxicity for aquatic organisms of many compounds is dependent only on the cumulative body burden (Verhaar *et al.*, 1995; Veith *et al.*, 1983). The effect is controlled by the bioavailability of the compound which, in turn depends on the organism / water or sediment partitioning properties of the compound (Abernethy and MacKay, 1988). Therefore, the bioaccumulation of the phthalate esters were determinated in the tadpoles.

Analyses

General considerations

Contamination of samples from the air is a common problem for many volatile and semi volatile compounds and has been studied carefully for PCB and phthalate esters (Wallace *et al.*, 1996; Alcock *et al.*, 1994; Furtmann, 1993).

Many articles of laboratory equipment consist of plastic that may contain phthalate esters, generally DBP and DEHP, which slowly evaporates and consequently indoor air

contains elevated levels of phthalate esters compared to outdoor air (Furtmann 1993) This means that phthalate esters are frequently artefacts in chemicals, solvents and equipment. This causes contamination in sample preparations as well as artefacts in sample collection.

It has recently been shown that the most important sources for contamination of phthalate esters (DBP, DEHP) were solvents, glassware, Pasteure pipette filler and Teflon®-faced screw caps made of melamine plastics (Parkman and Remberger, 1995).

In order to minimise the likelihood for contamination of the sample, the following preparations are recommended: (i) effective cleaning procedures of the equipment, (ii) protect the cleaned equipment from recontamination from the air by means of aluminium foil and (iii) avoid, if possibly, all material made of rubber as well as plastic (iv) use high quality solvents and chemicals (v) use a minimum of equipment and a minimum of steps in sample collection and analysis. Safe material are glass, metal and Teflon®.

The phthalate ester di-(propyl)-phthalate (DPP) was purchased from Tokyo Kasei Organic Chemicals (Tokyo, Japan) while di-allyl-phthalate (DAlP) was obtained from Aldrich (Steinheim, Germany). The commercial product di-(etylhexyl)-phthalate (DEHP, 99.6%) was kindly donated by Neste Oxo (Stenungsund, Sweden). The di-isodecyl-phthalate (DIDP) was obtained from Fluka (Buchs, Switzerland). The DIDP is not a pure compound but rather a mixture of isomeric compounds.

The certified standard mixture, used to calibrate the analytical instrument, was purchased from Ultra Scientific (North Kingstown, USA).

Water was produced with a Milli-Q water cleaning equipment.

Chemicals, solvents, and equipment were selected and treated as previously described (Parkman and Remberger 1995).

All solvents used, hexane (Riedel-deHaën, Hannover, Germany), acetonitrile (J. T. Baker, Gross-Gerau, Germany) and *tert*-buthyl-methyl ether (TBME; Rathburn Chemical Ltd., Peeblesshire, Scotland), were of the highest quality available with established low content of phthalate esters. Hexane and TBME were cleaned before used, by filtration on an acidic alumina column (Merck, Darmstadt, Germany). All solvents were checked before used by means of GC.

The cleaning procedure for all equipment of glass and metal was carried out as follows: (i) detergent washed, rinsed with tap water and distilled water in a dish washing machine and (ii) all glassware were wrapped in aluminium foil, in order to prevent recontamination, and cleaned in a oven overnight at 400°C. This procedure was reliable in order to eliminate and keep the equipment free from phthalate esters.

In order to prevent contamination from the Pasteur pipette filler, a plug of glasswool was added onto the upper part off the pipette before incinerated at 400°C. Immediately before use, activated charcoal (Merck 35-50 mesh) was added onto the top of the glasswool plug.

Equipment made of Teflon® was carefully detergent cleaned, rinsed with distilled water and soaked for one hour in methanol or 2-propanol (J. T. Baker). Finally, after drying, the equipment were rinsed with hexane and wrapped in clean (incinerated) aluminium foil.

The problem with screw caps, containing phthalate esters, was solved by covering the Teflon-lining and screw-thread with clean aluminium foil (Parkman and Remberger, 1995).

Alumina (neutral) and sodiumsulfate were extracted repeatedly with TBME and finally muffled at 400°C overnight.

Extraction

The following compartments of the test system were analysed: (i) sediments (ii) water and (iii) tadpoles.

Method blanks were included at every analyse occasion, to monitor contamination during the extraction and clean-up procedure. These blanks followed the same working-up protocol as the samples.

Water

Water samples were analysed as follows: the samples were fortified with surrogate standard (di-allyl-phthalate ester) and extracted twice with hexane. The combined extract were, without further clean-up, concentrated by means of a gentle stream of nitrogen. Finally a solution of pentachlorobensene was added as internal standard before injected on the gaschromatograph.

Sediment

Sediment samples were analysed, with minor modifications, according to Parkman and Remberger (1995, 1996).

Briefly, the sediment sample (*c.* 1-1.5 g fw) was weighed into test tubes and centrifuged (1000 g, 30 min). The water phase was discarded. Surrogate standard (di-allyl-phthalate) and acetonitrile was added and carefully mixed with the sample. The extraction was first performed in an ultrasonic cleaning bath for ten minutes. The sample was then allowed to stand in refrigerator overnight.

Next day, the acetonitrile phase was withdrawn after centrifugation and the extraction was repeated with hexane / acetonitrile. The combined extract was washed two times with water in order to remove the acetonitrile. The hexane phase was saved and chromatographed on a alumina column (see below).

Tadpoles

The tadpoles (*c.* 4-11 g, fw) were thawed, mixed with acetonitrile / hexane, spiked with surrogate standard (di-allyl-phthalate and di-propyle-phthalate) and thoroughly homogenised with a Polytron. The samples were extracted over night and were then centrifuged (1000g, 10 min) and the organic phase sacrificed. The extraction was repeated with acetonitrile / hexane but the extraction time was 60 min. The combined extracts were diluted with Milli-Q water and the acetonitrile was removed by extraction. The extracts were subjected to clean-up on a alumina column.

Clean up on alumina column

The raw extract of sediment, and tadpoles may contain many interfering compounds. Therefore the phthalates were separated from these with the aid of a open-column of alumina.

The extract (in hexane) was transferred onto the top of the column. The column was drained to the head of the gel and then eluted with hexane (discarded). The phthalates were eluted with hexane/TBME.

Finally a solution of pentachlorobensene was added as internal standard before the GC-analyses.

Instrument

The analyses were carried out with a HP 5890A Model gas chromatograph equipped with a electron capture detector (ECD) and a model HP 7673 auto sampler. A fused silica capillary column DB-5 (30 m), with an ID of 0.25 mm and a film thickness of 0.25 μm was used.

The detector signal from the gas chromatograph was acquired and integrated with a personal computer with the chromatography data program Turbochrom^{TM}.

Quantification

Certified standard mixture of phthalate esters was used to calibrate the GC-instrument.

The phthalates were quantified by comparison of their retention time and peak areas to authentic reference compounds. The isomeric mixture of compounds in the DIDP could not be resolved on the analytical column used but was detected in the diagram as a

"hump" of unresolved peaks. Therefore, it was not possible to evaluate the concentration by normal quantification procedure as when the analyte is eluated as single peak. The technique to semi-quantify DIDP implies comparison of the total integration of the hump from the sample to the commercial product. The major problem is the difficulty to establish a correct baseline and exclude interfering peaks in the rather broad retention window for the analytes.

Method detection limit (MDL) was defined as the minimum concentration of the analyte that can be measured with 99% confidence to be greater than zero and corresponds to 3σ above the blank. The determination of MDL was based on the result from the method blanks.

Results

During the experimental period the temperature in the air was 10.2 °C ± 0.34. The pH-value varied between 4.7 and 7.7 (see appendix I). pH was measured every day in 20 different beakers, if the pH-value decreased below 5.5, NaOH was added to increase the pH to 5.5. A pH-value below 5.5 was observed at 79 times (total number of observations 464) (see appendix I).

Oxygen saturation and temperature were measured daily in 20 different beakers (see appendix I). The oxygen saturation varied between 45 % and 108 %, with a mean value of 86 % and the water temperature between 7.1 and 11.8 °C. During the run of the experiment, it was found that there were weak temperature gradients in the room resulting in different mean temperatures in the beakers for different treatments. The range was typically 9.8 - 11.2 °C. The average temperatures, for each treatment in the DEHP-concentration series is shown in figure 2 (Note! The temperature was not measured equally many times in every beaker, in some beakers the temperature was only measured once). Ammonium and nitrite were measured if the tadpoles looked unhealthy. Nitrite was never detected. Ammonium was detected, but did never reach concentrations that is considered toxic at the actual pH.

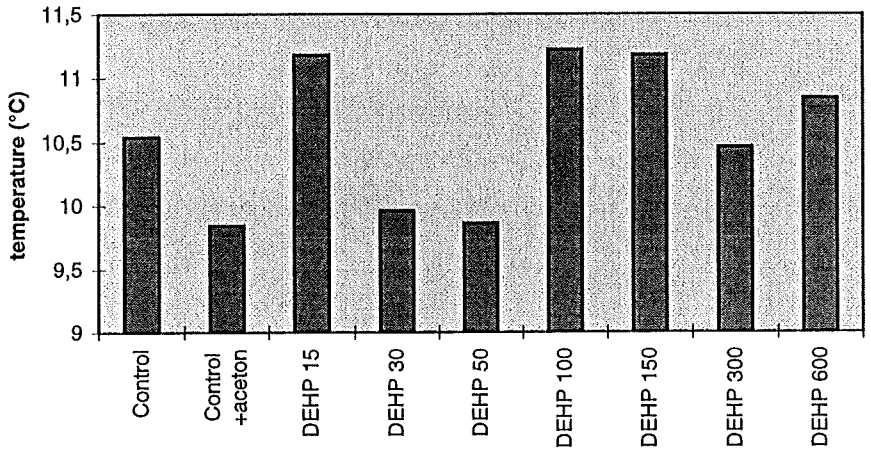


Figure 2. Average temperature measured for each treatment in the DEHP-concentration series. (Note! The averages are based on unequally many measurements.)

Hatching success

Hatching success was monitored carefully one and two weeks after the eggs were placed in the beaker, and at the end of the test (after 28-29 days). (After three weeks it was not possible to count the hatched tadpoles exactly, in several beakers, without taking them out. Since such handling could severely disturb the animals, we excluded that count). No hatching of tadpoles had occurred after one week of exposure. The result after two weeks of exposure are illustrated in figure 3 and 4.

At the end of the experimental period all tadpoles and the remaining of eggs and embryos were counted. Unfortunately most of the remaining eggs and embryos had started to decay and could not be recovered.

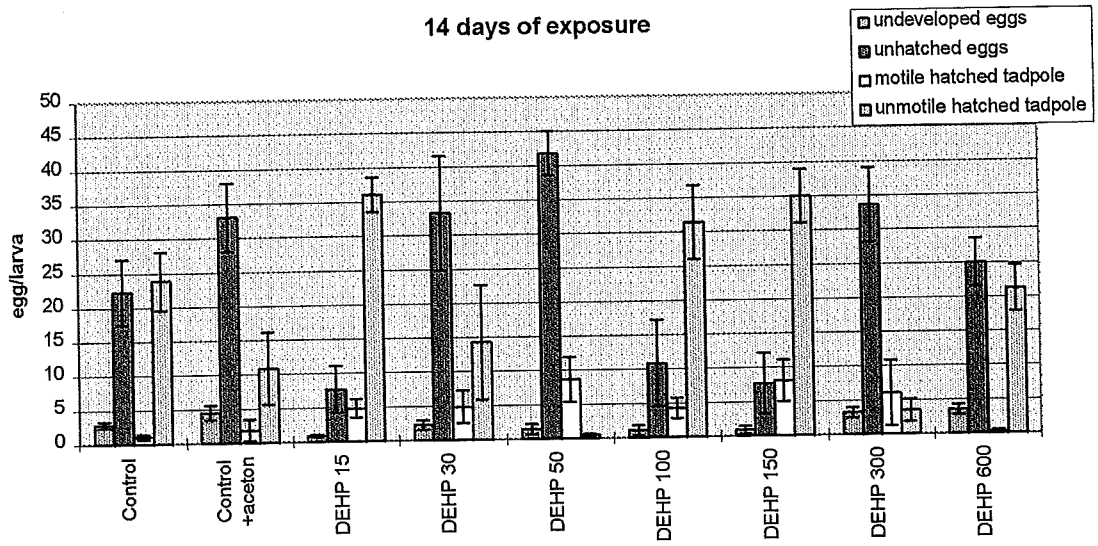


Figure 3. Hatching success in beakers with DEHP spiked sediment. The bars represent mean values of five replicates. The nominal DEHP-concentrations were 15, 30, 50, 100, 150, 300 and 600 µg DEHP/g dw (Standard Error= high-low lines).

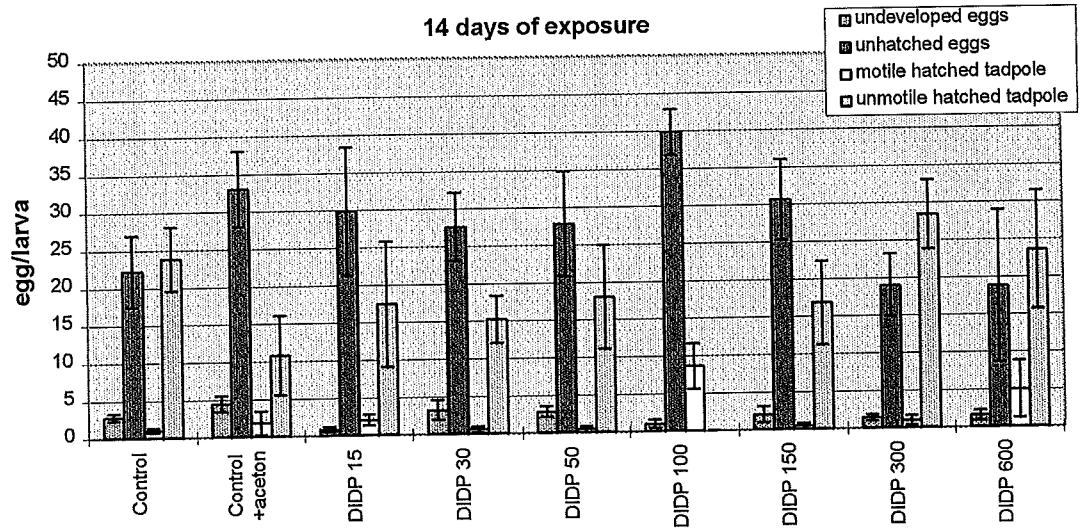


Figure 4. Hatching success in beakers with DIDP spiked sediment. The bars represent mean values of five replicates. The nominal DIDP-concentrations were 15, 30, 50, 100, 150, 300 and 600 µg DIDP/g dw (Standard Error= high-low lines).

Survival of tadpoles

Larva mortality in the experiment were low, except in a few beakers with a fungi or bacterial infection. Fungi or bacterial infection are common problems when using natural sediments in test system. In figure 5, 6 and 7 results of survival are expressed as living frog larva at the end of the experiment (we could not count the dead, because

decomposed very fast). In some beakers with DEHP-spiked sediment (DEHP 30C and 30D and DEHP 50A and 50C) we found fungi, which may cause some of the frog larva death. If the fungi/bacteria caused death in a beaker during the experiment, we excluded the beaker from the results, but if we found fungi/bacteria in a beaker when the experiment was terminated, it was included in the result. Therefore, beakers DEHP 30C and 30D as well as DEHP 50 were included while DEHP 50C was excluded in the results. The fungi/bacteria effect could explain the large spread in mean values for concentration DEHP 30 and DEHP 50.

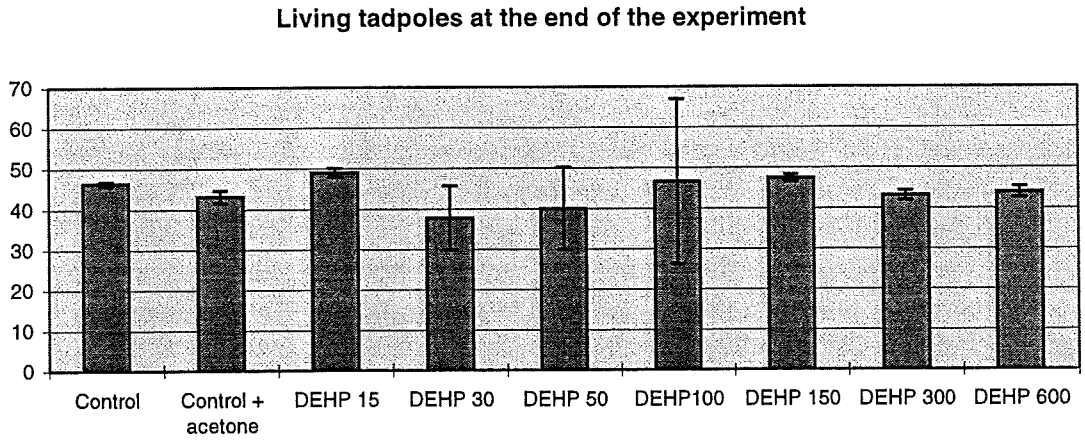


Figure 5. Living tadpoles at the end of the experiment. Each bar represent the mean value of five beakers (Standard Error= high-low lines).

In some of the beakers with sediment spiked with DIDP we observed mortality probably caused by fungi or bacteria. The same procedure with including and excluding beakers were made as with the DEHP-series. In beaker DIDP 15C (excluded), DIDP 30B and 30C (both included); DIDP 100A (included) and 100E (excluded), DIDP 150A (excluded) and DIDP 300 C(excluded) we found fungi. The fungi/bacteria affect could explain the large spread in mean values in the DIDP-series.

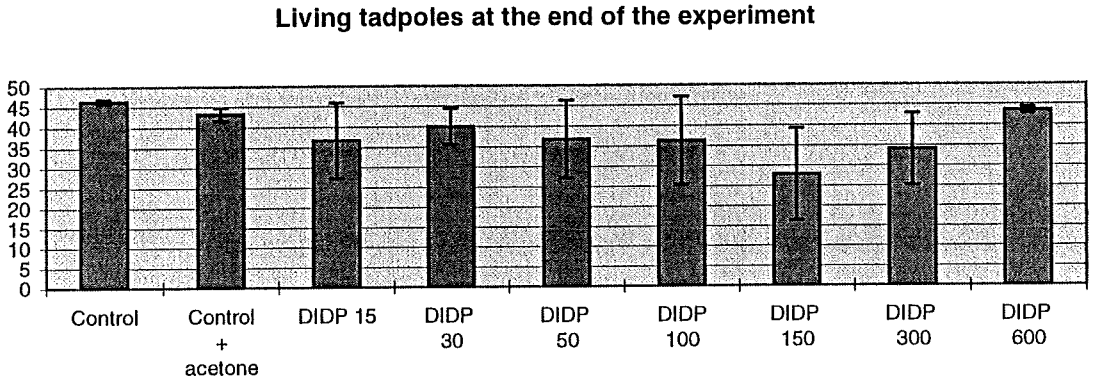


Figure 6. Living tadpoles at the end of the experiment. Each bar represents the mean value of five beakers (Standard Error = high-low lines).

Figure 7 illustrates the result from DEHP-spiked sediment with different organic carbon. Sediment from Trollhättan has the lowest content of organic carbon (approximately 3 %) and Ormaryd has the highest content (aprox. 77 %). The figure also include results from sediment with DEHP spiked with Ethanol (according to Thuren and Larsson, 1987).

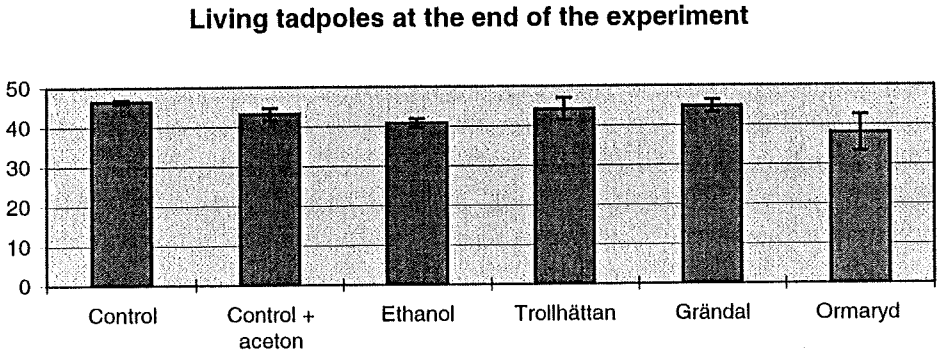


Figure 7. Living tadpoles at the end of the experiment. Each bar represent the mean value of five beakers (Standard error = high-low lines). The DEHP-content in the last four treatments were 300 µg/g dw. The bar named "Ethanol" represent the spiking-method used by Thurén and Larsson, 1987. Grändalsjön, Ormaryd and Trollhättan represent sediment with different content of organic carbon.

Analyses

Recovery

The recovery data reported below concern absolute recovery (%) for the whole analytical protocol. The analytical results reported in the experiments were adjusted according to the surrogate standard (DAlP). The recovery rate for DEHP in water samples were 82 %, in sediments 85 % and tadpoles 78 %. Recovery data of DIDP could only be evaluated for sediment samples and was 78 %.

Method detection limit

Due to the restricted amount of water samples and tadpoles available for analysis, the MDL for DEHP was higher in this study than reported previously (7 ng/l; Parkman and Remberger 1996). In this study the MDL for DEHP in the water samples were 0.1 µg/l and tadpoles 1 mg/kg fat assuming 15 mg fat/sample or equivalent with *c.* 7 g fw tissue.

It was not possible to detect and quantify DIDP in the water samples and tadpoles. There are three reasons for this. First, the sample amounts available were restricted, second, the concentrations of DIDP in tadpoles and water samples were low (*cf.* DEHP).The third reason is that the DIDP-product is, as mentioned above, an isomeric mixture with branched C10 alkyl chains all with more or less different retention times on the GC-column. Consequently, the mixture was eluting from the analytical column in a time interval as a "hump" of unresolved peaks and not as a distinct single peak as e.g. DEHP. This also means that the amount of DIDP injected into the GC-instrument is divided or "diluted" into a great number of peaks, which obviously cause a corresponding high MLD.

Thus, the MDL fore DIDP in the water samples (50 ml) was calculated to be about 10 µg/l which is far above the most likely solubility value for DIDP (1 µg/l, Staples *et al.*, 1997). DIDP could indeed be detected in the tadpoles but only from the sediments fortified with the highest concentrations (300 and 600 µg/kg dw) but serious interferes, probably from natural biogenic compounds, made it impossible to reliably quantify DIDP. The MDL fore DIDP in tadpoles was calculated to be 100 mg/kg fat or 0.2 mg/kg fw (6.5 g fw, 0.2% fat, table 7).

Water samples were taken at the end of the experiment. No DIDP could be detected in any of the water samples, while DEHP was found even in the beakers with DIDP-contaminated sediments (table 1).

The low concentrations of DEHP in the water phase is consistent with its high affinity to the sediment (Williams *et al.*, 1995) and the expected true aqueous solubility of the

compound (3 µg/l, Staples *et al.*, 1997). But in this study, the concentrations in the water did not correlate well to the sediment concentrations. According to Williams *et al.*, (1995) the expected concentrations in our experiments should be 0.2-8 µg/l. Furthermore, the difference in the concentrations of DEHP in the water phase from the DEHP and DIDP experiments was not statistically significant at the 0.05 level. Therefore, it was concluded that the concentration of DEHP found in the water phase was probably a result of contamination from the laboratory environment.

Table 1. DEHP concentrations in water samples at the end of the experiment.

Sample Name	DEHP (µg/l)	Sample Name	DEHP (µg/l)
Control	0.80		
Controll Aceton	1.5		
DEHP 15	0.14	DIDP 15	1.2
DEHP 30	0.26	DIDP 30	1.2
DEHP 50	0.30	DIDP 50	1.7
DEHP 100	0.25	DIDP 100	1.2
DEHP 150	0.47	DIDP 150	0.7
DEHP 300	1.00	DIDP 300	1.7
DEHP 600	1.3	DIDP 600	1.6

In table 2 the DEHP concentrations in water are presented when having different content of organic carbon in the sediment, or another solvent (ethanol instead of acetone) when spiking the sediment with phthalates.

Table 2. DEHP concentrations in water samples at the end of the experiment.

Sample Name	DEHP µg/l
Trollhättan	1.5
Grändalsjön	2.0
Ormaryd	2.3
DEHP Ethanol	1.3

Sediment samples

The analyses of the artificially contaminated sediments, "spiking sediments", showed that the concentrations agreed with the intended concentrations. The average concentration were 91% of the expected (calculated) concentrations.

The sediment and water partition of the added phthalate esters (DEHP) did not reach an apparent equilibrium during the week of agitation *e.g.* the concentration in the pore

water, in the Ormaryd sediment, increased between day 3 and 8 from 28 µg/l to 65 µg/l This observation could be an effect of slow leaching of dissolved organic carbon from the sediment (Williams *et al.*, 1995), increasing DEHP's apparent water solubility. However, the amount of DEHP in the pore water phase was insignificant and correspond to less then 0.02 % of DEHP in the sediment.

The calculated partition coefficient, $\log K_{oc}$ (oc=organic carbon), fore the sediment/water system was *c.* 5.4, based on the analysed concentration in pore water (52 µg/l) and sediment (14600 mg/kg oc.) after 3 days of agitation of the Trollhättan sediment (2.9 organic materials or 1.7% oc.; *cf.* Experimental sediment). This calculation was only made for the Trollhättan sediment since only this sediment fits OECD sediment/soil recommended for adsorption/desorption experiments. OECD is recommending a organic carbon content in the sediment of 0.5-1.5% (dw). The other sediments used in this study contained between 22.5-76.6% organic materials.

Table 3. DEHP content in sediment at the end of the experiment

Sample name	DEHP (µg/g dw)	% of spike	DEHP (µg/g LOI)
Control	2.5		11.1
Control + acetone	2.4		10.7
DEHP 15	13	97.8	57.8
DEHP 30	28	93.5	124
DEHP 50	41	90.2	182
DEHP 100	77	85.4	342
DEHP 150	137	91.3	609
DEHP 300	223	82.4	991
DEHP 600	433	80.0	1924

Table 4. DIDP content in sediment at the end of the experiment

Sample name	DIDP µg/g dw	% of spike	DIDP (µg/g LOI)
DIDP 15	20	133	88.9
DIDP 30	29	99	129
DIDP 50	46	93	204
DIDP 100	91	91	404
DIDP 150	123	81	547
DIDP 300	339	113	1057
DIDP 600	657	110	2920

Samples from the sediment were taken at the end of he experimental period. In table 3, 4 and 5 the results from the sampling are listed. The concentrations measured for DEHP agree very well with the concentrations we aimed at reaching (78-102 %) except in one case, the sediment from Grändalssjön (244 %). (This is a very unlikely value and could be due to contamination or that the sediment was accidentally spiked twice). The concentrations of DIDP agree with the concentrations we aimed at (81-133 %) with the spiking.

Table 5. DEHP content, at the end of the experiment, in the sediments with different content of organic carbon .

Sample name	DEHP µg/g dw	% of spiked	DEHP (µg/g LOI)
Trollhättan	205	78	7069
Grändalsjön	699	244	1664
Ormaryd	255	97	333
DEHP Ethanol	305	102	1356

Tadpoles

Tadpoles were sampled at the end of the experimental period and analysed for DEHP or DIDP. The results are shown in tables 6-8. The relation between DEHP in tadpole and DEHP in sediment is shown in figure 8 and the relation between tadpole concentrations and organic content in the sediments is shown in figure 9 and 10. We were not able to quantify any detected DIDP in the tadpoles (*cf.* Analyses).

Table 6. DEHP content in tadpoles at the end of the experiment.

Sample Name	DEHP (µg/g lipid content)	Lipids (% of fresh w.)	Fresh weight (g)
DEHP 0	0	0.233	7.283
DEHP 15	18	0.22	10.18
DEHP 30	29	0.244	6.213
DEHP 50	51	0.35	3.915
DEHP 100	112	0.249	8.31
DEHP 150	152	0.173	10.09
DEHP 300	329	0.191	7.098
DEHP 600	291	0.162	9.032

Table 7. DIDP content in tadpoles at the end of the experiment.

Sample Name	DIDP (µg/g lipid content).	Lipids (% of fresh w.)	Fresh weight (g)
DIDP 15	<100	0.274	4.632
DIDP 30	<100	0.135	5.714
DIDP 50	<100	0.151	5.298
DIDP 100	<100	0.235	3.822
DIDP 150	<100	0.142	8.564
DIDP 300	nd. *	0.217	5.355
DIDP 600	nd. *	0.201	9.095

(*) DIDP was detected but serious interference, probably from natural biogenic compounds, made it impossible to reliably quantify DIDP.

Table 8. DEHP content in tadpoles at the end of the experiment.

Sample Name	DEHP (µg/g lipid content)	Lipids (% of fresh w)	Fresh weight (g)
Trollhättan	460	0.193	9.322
Grändalsjön	232	0.083	11.14
Ormaryd	298	0.521	4.614
DEHP Ethanol	280	0.298	5.231

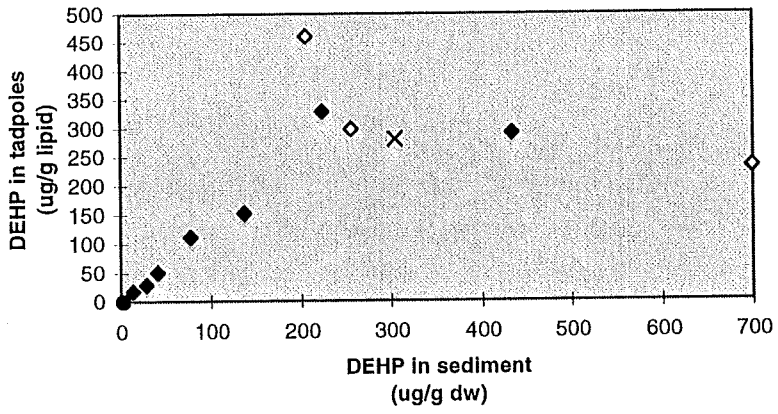


Figure 8: DEHP concentrations in tadpoles (µg/g lipid contents) in relation to sediment concentrations (µg/g dw) at the termination of the experiment. (♦ = the DEHP-concentrations series, • =control, X= ethanol as solvent, ◈= sediments with different organic contents.

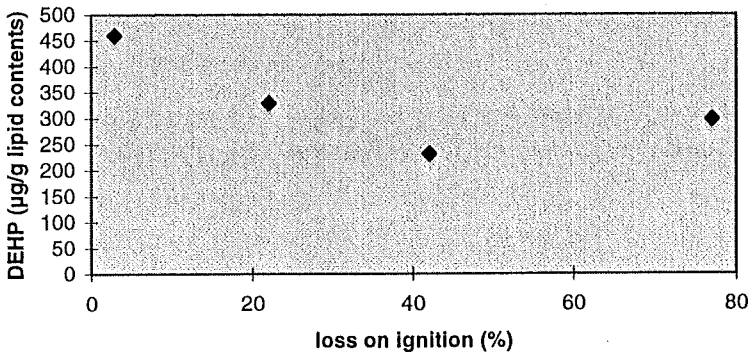


Figure 9: The relation between DEHP in tadpoles and organic contents (loss on ignition) in the sediments. All sediments were spiked with DEHP to 300 µg/g dw.

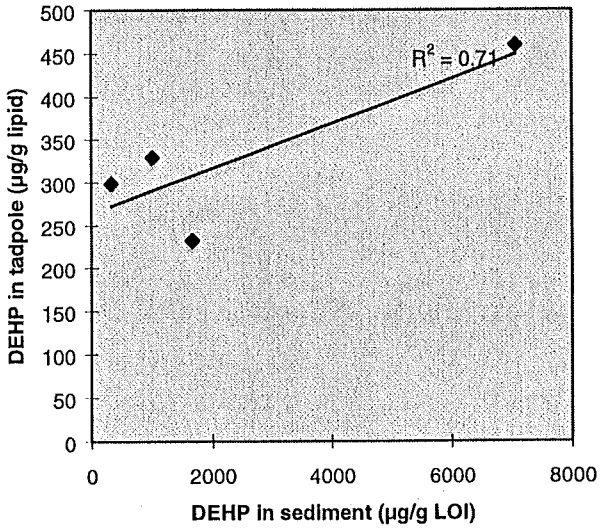


Figure 10. DEHP in tadpoles, per lipid contents, in relation to DEHP in sediment, per organic contents (LOI).

Discussion

The addition of DEHP and DIDP to sediment did not cause any proved effects on hatching and survival of frog eggs and tadpoles. In the study by Larsson and Thurén (1987) DEHP contaminated sediment caused decreased hatching success of frog eggs. Successful hatching appeared to decrease with increasing levels of DEHP up to an apparent plateau at a DEHP concentration of 200 µg/g f.w. At this concentration approximately 30 % of the eggs hatched. Above this concentration level no further decrease in hatching occurred. Approximately 50 % of the eggs hatched when exposed to sediments that contained 150 µg DEHP/g fresh weight. This could be compared to our study with no statistically proved effects on hatching at a comparable concentration (600 µg/g dw).

There are two important differences between the earlier and the present study. The first difference is the spiking method. Larsson and Thurén used a method where DEHP was dissolved in ethanol and then mixtured with fresh sediment. In present study we spiked the sediment according to Brown *et al.*, (1996). The presence of ethanol, or other organic solvents, may alter the bioavailability of the compound and by that, the toxicity of the compound. The spiking method used by Larsson and Thurén may have caused localised high concentrations of the DEHP giving the possibility of physical effects.

The second difference between the two studies is the experimental temperature. In the Larsson and Thurén study the temperature was 5 °C. This temperature is not relevant for hatching of frog eggs. In the natural environment of the moor frog, egg laying, development and hatching takes place when the temperature is approximately 10-15 °C, and in the egg clump the temperature could even be higher. However, in order to resemble ecological conditions we chose 10 °C as experimental temperature.

Another hypothesis to explain the differences between hatching success in the two studies could therefore be the difference in temperature. If the temperature is "normal" during the experimental period hatching is not affected by a stress caused by phthalates (as in our study), but when the temperature is lower than "normal", this extra stress from contaminants (phthalates, DEHP) could cause negative effects on hatching success, as in the Larsson and Thurén study. The fact that the controls (with and without ethanol) in the Larsson and Thurén study showed no notable mortality among the tadpoles, support the theory that low temperature alone is not a stress factor that could explain mortality.

The phthalates did not affect growth of the tadpoles. Some differences in growth (not statistically proved) could be noticed and was probably caused by differences in temperature in different parts of the experimental location (see appendix 1). These temperature variations is probably also the reason for differences that can be noticed for development stage for the tadpoles after two weeks (compare figures 2 and 3).

In some of the test beakers we noticed bacterial and/or fungi infestation which caused mortality among the tadpoles. Infestation of bacteria or fungi is a common problem in tests where natural sediments are used. We have removed those beakers where all tadpoles were dead (DEHP 50C and DIDP 15C, 150A, 300C) from the reported result. The beakers with only a few dead tadpoles and/or visible amount of bacteria/fungi were not excluded from the result.) because of the theory that some organic substances may cause effects on the immuno system. However, there was no clear concentration-response between infestation of bacteria/fungi and test material concentration.

The DEHP concentrations in sediment and water measured in the present study are very high compared to concentrations measured in the Swedish environment. In the studies of Parkman and Remberger (1995, 1996) DEHP concentrations between 0.008 and 40 µg/g dw were measured in surface sediments, and the highest values were recorded close to point sources. In recipients in connection with major cities in Sweden the concentrations were 0.3 - 2.5 µg/g dw. Also concentrations measured in Rhine River (1.8 - 18.3 µg/g dw), Germany (Furtmann, 1993), are comparatively low. DEHP concentrations in water between 0.007 and 3.1 µg/L have been measured en Swedish environments (Larsson and Thurén 1986; Parkman and Remberger 1996), while concentrations between 0.08 and 10.3 where measured in Rhine River (Furtmann, 1993).

The DEHP accumulation in the tadpoles correlated with DEHP in the sediments (figure 8), up to 300 µg/g lipid (at a sediment concentration of 300 µg/g dw) where the accumulation seem to level off. This levelling off might be explained by a threshold value where the excretion of DEHP from the animals increases. In Larsson and Thuréns (1986) study the accumulation in the tadpoles seemed to correlate both with concentrations in the water and in the sediments, and no levelling off was found (although much higher concentrations were used). However, the correlation between DEHP in tadpoles and water might be a false relationship since the concentrations in sediments and water also covaried. The water concentrations reported in the present study did not correlate at all with sediment concentrations and were probably highly dependent on the occurrence of particles in the water. Neither in a field study of phthalates in Swedish aquatic environments was any correlation found between water and sediment concentrations (Parkman and Remberger, 1996). The low concentrations of phthalate esters in the water phase are consistent with the results of Williams *et al.*, (1995). According to their theoretical calculations the expected concentration of DEHP in water above a sediment containing 300 µg DEHP/g dw, should be about 4 µg/l.

If the water solubility data (Staples *et al.*, 1997) and the high sediment-water partition coefficient (Williams *et al.*, 1995) are recognised, the reported concentrations by Larsson and Thurén (1987), of DEHP in the water phase seems to be extremely high. It is not possible to evaluate the reason for this unexpected result, due to the incomplete

description of experimental set-up. Larsson and Thurén (1987), further stated that there was a liner relationship between the concentration of DEHP in the sediment and the water phase. From these data a organic carbon normalised partition coefficient (K_{oc}) may be calculated since the dry weight and organic content were reported. The outcome of this calculation was a K_{oc} of about 97 000 L/kg. This is a low value according to Williams *et al.* (1995) who reported a partition coefficient of 482 000 L/kg. In the present investigation a partition coefficient of 284 000 L/kg was determinated (logKoc 5.4). Thus, according to Williams *et al.* (1995),the calculated concentration in the water phase in the Larsson and Thurén study, with a sediment concentration of 600 µg DEHP/g dw, should be $\leq$ 20 µg DEHP/l.

The conclusion of this might be that the sorption capacity of the sediments in Larsson and Thurén study was altered plausibly as an effect of the added ethanol.

The bioaccumulation of DEHP seemed to correlate inversely with the organic contents in the sediments (figure 9), as was presupposed by Larsson and Thurén (1986). A higher organic contents probably reduce the uptake of DEHP in the tadpoles. The DEHP is probably bound to the organic material in the sediments, why a higher contents of organic material results in a higher degree of dilution of the compound. This can also be expressed as: the bioaccumulation in the tadpoles is determined by DEHP per organic contents in the sediments (illustrated in figure 10).

The concentrations in tadpoles measured in this study are reported per lipid contents. This is common for organic compounds, since they accumulate mainly in the fat tissue of organisms. If the tadpole concentrations from our study are recalculated per fresh weight, the values from the concentration series range between 0 and 0.63 µg per gram fresh weight and the highest concentration is found in the tadpoles that have been exposed to Ormaryd sediment (1.55 µg/g fw). These concentrations in the tadpoles are much lower than the concentrations measured by Larsson and Thurén (1986), (up to 250 µg/g fw), also when we spiked the sediment with their method at corresponding DEHP concentration. This might indicate that DEHP was biotransformed in the tadpolest o a higher degree in our study (due to the higher temperature?) or that the bioavailability of DEHP was lower in the present study, compared to Larsson and Thurén (1987), due to different spiking methodologies

Anyway, it was not possible, with the present experimental set-up, to evaluate if the apparent low accumulation of the phthalate esters in the tadpoles were caused by an effective biotransformation or a low bioavailability of the DEHP. This can only be reliably evaluated by means of chemical analysis of both the mother compound and the expected transformation products or by using labelled test compounds (Albro and Lavenhar 1989; Barron *et al.*, 1995).

References

Abernethy, G., S., and Mackay, D., 1988. "Volume fraction" correlation for narcosis in aquatic organisms: The key role of partitioning. Environmental Toxicology and Chemistry, 7, 469-481.

Adams, J., W., Biddinger, R., G., Robillard and Gorsuch, W., J., 1995. A summary of the toxicity of 14 phthalate esters to representative aquatic organisms. Environmental Toxicology and Chemistry, 9, 1569-1574.

Albro, W. P. and Lavenhar, R. S., 1989, Metabolism of di(ethylhexyl)phthalate, Drug metabolism rewiewes. 21(1), 13-34.

Barron, G. M., Albro, W. P. and Hayton, L. W., 1995. Niotransformation of di(ethylhexyl)phthalate by rainbow trout. Environmental Toxicology and Chemistry, 14, 873-876.

Brown, D., Thompson, R. S., Stewart, K. M., Croudace, C. P., and Gillings, E., 1996. The effect of phthalate ester plasticisers on the emergence of the midge, *Chironomus riparius*, from treated sediments. Chemosphere, 32, 2177.

Furtmann, K., 1993: Phthalate in der aquatichen Umwelt. Landesamt für wasser und Abfall Nordrhein - westfalen, Auf dem Draap 25, 40221, Dusseldorf 1 Report nr 6/93.

Howars, H., P., Banerjee, S. and Robillard, H., K., 1985. Measurement of water solubilities, octanol/water partition coefficients and vapour pressures of commercial phthalate esters. Environmental Toxicology and Chemistry, 4, 653-661.

KemI, 1995. Hazard Assessments - Chemical Substances Selected in the Swedish Sunset Project. Supplement to KemI report 13/94. The Swedish National Chemicals Inspectorate, Report no 12/95

Larsson, P. and Thurén, A., 1987. Di-2-ethylhexylphthalate inhibits the hatching of frog eggs and is bioaccumulation by tadpoles. Environmental Toxicology and Chemistry, vol.6, pp. 417-422.

Neilson, A.H., Allard, A.-S., Fischer, S., Malmberg, M. and Viktor., T., 1990. Incorporation of a subacute test with zebra fish into a hierarchical system for evaluating the effect of toxicants in the aquatic environment. Ecotoxicol. Environ. Saf. 20: 82-97.

Neilson, A.H., Allard, A.-S., Reiland, S., Remberger, M., Tärnholm, A., Viktor, T. and Landner, L., 1984. Tri- and tetra-chloroveratrole, metabolites produced by bacterial O-methylation of tri- and tetra-chloroguaiacol : an assessment of their bioconcentration potential and their effects on fish reproduction. Can. J. Fish. Aquatic Sci. 41 : 1502-1512.

Parkman, H. and Remberger, M., 1995. Phthalates in Swedish sediments. IVL-Report B 1167.

Parkman, H. and Remberger, M., 1996. Phthalate esters in water and sediments in major cities and remote lakes in Sweden. For: Hydro Plast AB, Stenungsund, Sweden.

Rohdes, E., J., Adams, J., W., Biddinger, R., G., Robillard, A., K. and Gorsuch, W., J., 1995. Chronic toxicity of 14 phthalate esters to *Daphnia magna* and Rainbow trout (*Oncorhynchus mykiss*). Environmental Toxicology and Chemistry, 14, 1967-1976.

Staples, A. C., Peterson, R. D, Parkerton, F. T. And Adams, J. W., 1997. A litterature review: The environmental fate of phthalate esters. To appear in Chemosphere.

Streufert, J., M., Jones, J., R and Sanders, H. O., 1980. Toxicity and biological effects of phthalate esters on midge (*Chironomus plumosus*). Transactions, Missouri Academy of Science 14: 33-40.

Veith, D., G., Call, J., D. and Brooke, T. L., 1983. Structure-Toxicity relationship for the Fathead Minnow, Pimephales Promelas: Narcotic industrial chemicals. Can. J. fish. Aquat. Sci., 40, 743-748.

Verhaar, M., J., H., Busser, M., J., F. and Hermens, M., L., J., 1995. Surrogate parameter for the baseline toxicity content of contaminated water: Simulating the bioconcentration of mixtures of pollutants and counting molecules. Environmental and Technology, 29, 726-734.

Williams; D. M.; Adams, W. J., Parkerton, T. F., Biddinger, G. R. and Robillard, K. A, 1995: Sediment sorption coefficient measurements for four phthalate esters: experimental results and model theory. Environmental Toxicology and Chemistry, Vol 14, 1477-1486.

Date	Beaker	pH	O2	Water temperature degree Celcius	Notice	NaOH number of droplets	Air temperature degree Celcius	NH4-N, mg/l	NO2-N
1996-05-08	Control+acetone C	7,11	98						
1996-05-08	DEHP50D	6,79	94						
1996-05-08	DIDP15E	6,49	93						
1996-05-08	DIDP15D	6,49	91						
1996-05-08	DIDP100A	6,46	90						
1996-05-08	DEHP30E	6,44	90						
1996-05-08	DEHP30D	6,39	90						
1996-05-08	DEHP30C	6,58	86						
1996-05-08	DIDP100E	6,54	80						
1996-05-08	DIDP100D	6,57	84						
1996-05-09	Control+acetone C	6,27	103				6,2		
1996-05-09	DEHP50D	6,29	105						
1996-05-09	DIDP15E	6,33	107						
1996-05-09	DIDP15D	6,3	105						
1996-05-09	DEHP30C	6,36	103						
1996-05-09	DEHP30D	6,29	103						
1996-05-09	DIDP100A	6,44	101						
1996-05-09	DIDP100E	6,53	100						
1996-05-09	DIDP100D	6,41	101						
1996-05-10	DIDP100C	5,93	104				9,2		
1996-05-10	DIDP100B,	5,9	104						
1996-05-10	DEHP300B	6,06	105						
1996-05-10	DEHP50C	5,8	103						
1996-05-10	DEHP50B	5,85	103						
1996-05-10	DEHP50A	5,72	104						
1996-05-10	DEHP300E	6,04	104						
1996-05-10	DEHP600B	6,11	103						
1996-05-10	Control+acetone A	6,19	103						
1996-05-10	DEHP50E	5,96	103						
1996-05-10	DIDP50E	5,83							
1996-05-10	DEHP300A	5,89							
1996-05-10	Control+acetone E	6							
1996-05-10	Control+acetone D	5,83							
1996-05-10	DIDP30B	5,61							
1996-05-10	Control+acetone B	5,92							
1996-05-10	KontrollC	6,16							
1996-05-10	DEHP300C	5,85							
1996-05-10	DEHP30B	5,88							
1996-05-10	DIDP50D	5,8							
1996-05-11	DIDP30B	5,71					9,2		
1996-05-11	DIDP50C	5,8	106						
1996-05-11	DIDP50B	5,72	108						
1996-05-11	DIDP30D	5,8	105						
1996-05-11	DIDP30C	5,63	105		Dropped sampling device in beaker				
1996-05-11	DIDP150D	6,02	105						
1996-05-11	DIDP30A	5,73	105						
1996-05-11	DIDP50A	5,83	105						
1996-05-11	DIDP150B	6,02	104						
1996-05-11	DIDP150A	6,02	105						
1996-05-11	DIDP30E	5,37	106		12 droppiets 0,1 M NaOH to pH 5,49	12			
1996-05-12	DIDP30E	5,55	98				9,5		
1996-05-12	DEHP300D	6,17	99						
1996-05-12	DEHP600A	6,06	99						
1996-05-12	DIDP150E	6,04	99						
1996-05-12	DEHP30A	6,12	99						
1996-05-12	Gran A	6,83	99						
1996-05-12	DEHP600E	6,14	101						
1996-05-12	DEHP600D	6,11	101						
1996-05-12	DEHP600C	6,1	101						
1996-05-12	DIDP150C	6,03	100						
1996-05-12	Controll B	6,41	100						
1996-05-13	Grån C	6,49	88		Change of oxygenequipment		10,2		
1996-05-13	Grån B	6,55	89						
1996-05-13	Troll D	7,18	92						
1996-05-13	Orm B	7,11	94						
1996-05-13	DIDP15A	5,88	96						
1996-05-13	DEHP150E	6,14	95						
1996-05-13	Troll E	7,15	98						
1996-05-13	Orm D	7,11	96						
1996-05-13	DEHP150B	5,83	97						
1996-05-13	DEHP150A	5,97	97						
1996-05-13	DIDP30E	5,52	98		remeasured				

Date	Beaker	pH	O2	Water temperature degree Celcius	Notice	NaOH number of droplets	Air temperature degree Celcius	NH4-N, mg/l	NO2-N
1996-05-14	DIDP30E	5,38			12 droplets Na OH pH 5,62	12	9,7		
1996-05-14	DEHP100A	5,72	101						
1996-05-14	DEHP100D	5,68	101						
1996-05-14	DEHP100C	5,67	101						
1996-05-14	DEHP100B	5,6	93						
1996-05-14	Troll A	7,18	95						
1996-05-14	EtOH A	5,08	99			24			
1996-05-14	DEHP15D	5,64	100						
1996-05-14	DEHP15C	5,7	100						
1996-05-14	DEHP15B	5,67	100						
1996-05-14	DEHP15A	5,92	100						
1996-05-14	EtOH B	5,02				50			
1996-05-14	EtOH C	4,92				50			
1996-05-14	EtOH D	5,13				25			
1996-05-14	EtOH E	4,93				55			
1996-05-15	DIDP600D	6,67	95				9,4		
1996-05-15	DIDP600E	6,3	85						
1996-05-15	Gran D	6,53	90						
1996-05-15	DEHP150C	5,97	91						
1996-05-15	DIDP15C	5,75	91						
1996-05-15	DIDP300E	5,57	82						
1996-05-15	Grån E	6,39	68						
1996-05-15	DIDP300C	6,01	80						
1996-05-15	DIDP300B	5,81	71						
1996-05-15	DIDP300A	5,56	91						
1996-05-16	DIDP15B	5,86	91				9,8		
1996-05-16	EtOH C	5,45	90		5 drp NaOH	5			
1996-05-16	EtOH B	5,5	91						
1996-05-16	EtOH D	5,5	89						
1996-05-16	DIDP600A	6,1	93						
1996-05-16	EtOH E	5,53	90						
1996-05-16	Troll B	7,43	92						
1996-05-16	Troll C	7,45	95						
1996-05-16	DIDP300D	6,12	94						
1996-05-16	DIDP600B	6,16	95						
1996-05-17	Orm E	7,52	97		Just over the eggs				
1996-05-17	Orm C	7,49	98		Just over the eggs				
1996-05-17	DEHP150D	6,12	78		Just over the eggs				
1996-05-17	DIDP600C	6,3	87		Just over the eggs				
1996-05-17	Orm A	7,53	78		Just over the eggs				
1996-05-17	Controll E	5,97	71		Just over the eggs				
1996-05-17	DEHP15E	6,11	61						
1996-05-17	DEHP100E	6,33	61						
1996-05-17	Control B	5,81	67						
1996-05-17	Control A	5,86	73						
1996-05-17	DIDP50A	5,3	97		Orange sediment surface. Ariation stopped. + NaOH	20			
1996-05-17	DIDP50B	4,7			Strongly orange coloured water. Fe + NaOH	30			
1996-05-17	DIDP50C	5,2			+ NaOH	20			
1996-05-17	DIDP50D	4,92			+ NaOH	30			
1996-05-17	DIDP50E	4,8			+ NaOH	30			
1996-05-17	DIDP30D	5,2			+ NaOH	20			
1996-05-17	DIDP15C	5,43			+ NaOH	2			
1996-05-17	DIDP150B	5,28			+ NaOH	20			
1996-05-17	DIDP150A	5,18			+ NaOH	20			
1996-05-17	DIDP30E	5,12			Added 150 ml water	20			
1996-05-17	DIDP30D	6,2							
1996-05-17	DIDP30C	5,12			. + NaOH	30			
1996-05-17	DIDP150D	5,01			+ NaOH	30			
1996-05-17	DIDP30A	5,25			+ NaOH	20			
1996-05-17	DIDP30B	5,34			+ NaOH	10			
1996-05-17	DIDP50D	5,56							
1996-05-17	DIDP50C	5,35			+ NaOH	10			
1996-05-17	DIDP50B	5,35			+ NaOH	10			
1996-05-17	DIDP30B	5,21			+ NaOH	20			
1996-05-17	DEHP0AB	5,4			+ NaOH	10			
1996-05-17	Control C	5,44			+ NaOH	5			
1996-05-17	DEHP300C	5,26			+ NaOH	20			
1996-05-17	DIDP50B	5,44			+ NaOH	10			
1996-05-17	DIDP150C	5	53		+ NaOH	40			
1996-05-17	Control D	5,39	67		+ NaOH	25			

Date	Beaker	pH	O2	Water temperature degree Celcius	Notice	NaOH number of droplets	Air temperature degree Celcius	NH4-N, mg/l	NO2-N
1996-05-17	Grän C	6,04	63						
1996-05-17	Grän B	6,09	70						
1996-05-17	Grän A	6,13	62						
1996-05-17	DEHP600E	5,35	67		+ NaOH	15			
1996-05-17	DEHP600D	5,44	63		+ NaOH	30			
1996-05-17	DEHP600C	5,35	67		Orange sedimentsurface + NaOH	25			
1996-05-17	DEHP300D	5,32	71		+ NaOH	30			
1996-05-17	DEHP600A	5,28	68		+ NaOH	30			
1996-05-17	DIDP150E	5,22	58		+ NaOH	40			
1996-05-17	DEHP30A	5,38	58		+ NaOH	15			
1996-05-17	DIDP50A	5,4	53		+ NaOH	20			
1996-05-17	DIDP150B	5,46	55		Orange sediment + NaOH	5			
1996-05-17	DIDP150A	5,3			+ NaOH	15			
1996-05-17	DIDP30E	5,6	75		Decreased ariation. Orange sediment.+ NaOH				
1996-05-17	DIDP30D	5,34	66		Orange sediment+NaOH	15			
1996-05-17	DIDP30C	5,56	79		Orange sediment. Decreased ariation. +NaOH				
1996-05-17	DIDP150D	5,48	61		Slightly orange sediment. +NaOH				
1996-05-17	DIDP30A	5,36	71		Sligthly orange sediment surface+ NaOH	15			
1996-05-17	DEHP30B	5,45	57		Sligthly orange sediment +NaOH	10			
1996-05-17	DIDP50D	5,36	70		Sligthly orange sediment surface+ NaOH	20			
1996-05-17	DIDP50C	5,34	62		Orange sediment +NaOH	17			
1996-05-17	DIDP50B	5,44	60		Deep orangecoloured sediment+water+ NaOH	10			
1996-05-17	DIDP30B	5,4	56			13			
1996-05-17	Control+acetone B	5,43	63		Sligthly orange sediment	10			
1996-05-17	Control C	5,39	76			15			
1996-05-17	DEHP300C	5,43	60		Orangecoloured sediment. Slightly orange water	10			
1996-05-18	DIDP50E	5,8							
1996-05-18	DEHP300A	5,62							
1996-05-18	Control+acetone E	5,76							
1996-05-18	Control+acetone D	5,63							
1996-05-18	Control+acetone C	5,98	68		10,8 C in water close to the door, 11,8 C in water in the back of chamber, 10,0 C front wall		12,8		
1996-05-18	DEHP50D	5,75	70						
1996-05-18	DIDP15E	5,77	65						
1996-05-18	DIDP15D	5,84	65						
1996-05-18	DIDP100A	5,64	64						
1996-05-18	DEHP30E	5,86	70						
1996-05-18	DEHP30D	5,86	66						
1996-05-18	DEHP30C	5,82	64						
1996-05-18	DIDP100E	5,85	62						
1996-05-18	DIDP100D	5,92	66						
1996-05-18	DIDP100C	5,93	69						
1996-05-18	DIDP100B	5,85	80						
1996-05-18	DEHP300B	6	66						
1996-05-18	DEHP50C	5,72	67						
1996-05-18	DEHP50B	5,71	67						
1996-05-18	DEHP50A	5,68	68						
1996-05-18	DEHP300E	5,69	66						
1996-05-18	DEHP600E	5,79	67		orange water				
1996-05-18	Control+acetone A	5,83	68						
1996-05-18	DEHP50E	5,38	63		orange sediment surface	15			
1996-05-19	TROLLD	7,03	62	11,8			11,4		
1996-05-19	ORMB	7,41	68	11,8	ariation stopped				
1996-05-19	DIDP15A	5,65	64	11,7					
1996-05-19	DEHP150E	6,32	62	11,8					
1996-05-19	TROLLE	7,07	59	11,5					
1996-05-19	ORMD	7,43	69	11,7					
1996-05-19	DEHP150B	5,78	58	11,7	slightly orange cotoured sediment surface				

Date	Beaker	pH	O2	Water temperature degree Celcius	Notice	NaOH number of droplets	Air temperature degree Celcius	NH4-N, mg/l	NO2-N
1996-05-19	DEHP150A	5,96	56	11,7	slightly orange coloured sediment surface				
1996-05-19	DEHP100A	5,75	53	11,5	ariation stopped				
1996-05-19	DEHP100D	5,75	60	11,5	slightly orange coloured sediment surface				
1996-05-19	DEHP100C	5,71	59	11,6	slightly orange coloured sediment surface				
1996-05-19	DEHP100B	5,66	60	11,8					
1996-05-19	TROLLA	7,02	56	11,5	ariation stopped				
1996-05-19	ETOHA	5,71	61	11,4	ariation stopped				
1996-05-19	DEHP15D	5,51	59	11,6	ariation stopped	13			
1996-05-19	DEHP15C	5,48	54	11,6	ariation stopped	3			
1996-05-19	DEHP15B	5,71	55	11,1					
1996-05-19	DEHP15A	5,99	55	11,4					
1996-05-19	DIDP600D	6,01	57	11,4					
1996-05-19	DIDP600E	6,13	56	11,2					
1996-05-19	DIDP30B	5,66							
1996-05-19	DIDP50C	5,67							
1996-05-20	GRÅND	6,45	52	11,4			11,8		
1996-05-20	DEHP150C	5,87	54	11,4					
1996-05-20	DIDP15C	5,64	54	11,4					
1996-05-20	DIDP300E	5,61	52	11,5					
1996-05-20	GRÅNE	6,45	50	11,1					
1996-05-20	DIDP300C	5,94	50	11,2					
1996-05-20	DIDP300B	5,77	49	11,1					
1996-05-20	DIDP300A	5,56	50	11,2					
1996-05-20	DIDP15B	5,73	49	11,1					
1996-05-20	ETOHC	5,59	49	10,9					
1996-05-20	ETOHB	5,71	50	11					
1996-05-20	ETOHD	6	49	11,3					
1996-05-20	DIDP600A	5,94	50	11					
1996-05-20	ETOHE	5,86	49	10,9					
1996-05-20	TROLLB	7,23	50	11					
1996-05-20	TROLLC	7,24	50	11,1					
1996-05-20	DIDP300D	5,91	54	10,9					
1996-05-20	DIDP600B	6,14	54	10,6					
1996-05-20	ORME	7,55	53	10,8					
1996-05-20	ORMC	7,54	53	11			9,1		
1996-05-22	DEHP150D	5,7	89	11,1	New battery in oxygen apparatus		8,1-13,2		
1996-05-22	DIDP600C	6,8	91	11					
1996-05-22	ORMA	7,26	91	11,1					
1996-05-22	Control E	5,67	94	11,3	decreased araiation				
1996-05-22	DEHP15E	5,65	92	10,7					
1996-05-22	DEHP100E	6,07	94	11					
1996-05-22	Control B	5,38	90	10,9		15			
1996-05-22	Control A	5,41	91	11		12			
1996-05-22	Control+acetone C	5,51	92	10,1					
1996-05-22	DEHP50D	5,46	93	10		5			
1996-05-22	DIDP15E	5,35	95	10,3	decreased ariation	15			
1996-05-22	DIDP15D	5,3	92	10,5	decreased ariation	15			
1996-05-22	DIDP100A	5,41	92	10,6		10			
1996-05-22	DEHP30E	5,29	87	10,3	increased ariation	15			
1996-05-22	DEHP30D	5,49	93	10,4					
1996-05-22	DEHP30C	5,59	94	10,6					
1996-05-22	DIDP100E	5,15	68	10,8	Ariation stopped	30			
1996-05-22	DIDP100D	5,24	74	10,5	Ariation stopped	20			
1996-05-22	DIDP100C	5,23	67	10,4	Ariation stopped	20			
1996-05-22	DIDP100B	5,16	74	10,5	Ariation stopped	30			
1996-05-23	DEHP30B	5,87	98	10,5			9,2		
1996-05-23	DEHP50C	5,5	99	10,4		5			
1996-05-23	DEHP50B	5,66	99	10,3					
1996-05-23	DEHP50A	5,39	99	10,1		15			
1996-05-23	DEHP300E	5,43	99	10,7		10			
1996-05-23	DEHP600B	5,48	99	10,6		5			
1996-05-23	Control+acetone A	5,67	98	10,4					
1996-05-23	DEHP50E	5,52	98	10,6		5			
1996-05-23	DIDP50E	5,26	98	10,9		30			
1996-05-23	DEHP300A	5,26	97	10,7		30			
1996-05-23	Control+acetone E	5,48	92	9,9		5			
1996-05-23	Control+acetone D	5,4	90	9,8		15			
1996-05-23	DIDP30B	5,54	95	10,7					
1996-05-23	Control+acetone B	5,53	98	10,6					

Date	Beaker	pH	O2	Water temperature degree Celcius	Notice	NaOH number of droplets	Air temperature degree Celcius	NH4-N, mg/l	NO2-N
1996-05-23	Control C	5,69	99	10,5					
1996-05-23	DEHP300C	5,7	102	10,7					
1996-05-23	DEHP30B	5,54	102	11,1					
1996-05-23	DIDP50D	5,52	102	10,9					
1996-05-23	DIDP50C	5,43	102	10,6		10			
1996-05-23	DIDP50B	5,61	102	10,6					
1996-05-23	Control B	5,66					11,7		
1996-05-23	Control A	5,68							
1996-05-23	DEHP50D	5,5				5			
1996-05-23	DIDP15E	5,57							
1996-05-23	DIDP15D	5,54							
1996-05-23	DIDP100A	5,66							
1996-05-23	DEHP30E	5,61							
1996-05-23	DIDP100E	5,7							
1996-05-23	DIDP100D	5,61			Orange coloured water, decreased ariation				
1996-05-23	DIDP100C	5,75							
1996-05-23	DIDP100B	5,8							
1996-05-24	DIDP30D	5,73	97	11,3			12,9	0	0
1996-05-24	DIDP30C	5,69	97	11				0	0
1996-05-24	DIDP150D	5,59	99	10,8				0	0
1996-05-24	DIDP30A	5,72	102	10,5				0	0
1996-05-24	DIDP50A	5,46	101	11,4				0	0
1996-05-24	DIDP150B	5,61	101	11,1				0	0
1996-05-24	DIDP150A	5,75	102	11				0	0
1996-05-24	DIDP30E	5,68	101	10,8				0	0
1996-05-24	DEHP300D	5,74	101	11,4				0	0
1996-05-24	DEHP600A	5,65	100	11,2				0	0
1996-05-24	DIDP150E	5,54	101	11				0	0
1996-05-24	DEHP30A	5,9	104	10,9				0	0
1996-05-24	Grån A	6,44	104	11,4				0	0
1996-05-24	DEHP600E	5,54	104	11,2				0	0
1996-05-24	DEHP600D	5,6	104	11				0	0
1996-05-24	DEHP600C	5,59	105	11				0	0
1996-05-24	DIDP150C	5,56	105	11,4				0	0
1996-05-24	Control D	5,62	103	11,3				0	0
1996-05-24	Grån C	6,14	104	11,1				<0,5	0
1996-05-24	Grån B	6,24	102	11				<0,5	0
1996-05-24	ORME				5dH				
1996-05-24	TROLLA				3dH				
1996-05-25	TROLLD	7,27	85	11,1			12,7	<0,5	
1996-05-25	ORMB	7,65	87	11,1				1	0
1996-05-25	DIDP15A	5,94	87	11,2				0	
1996-05-25	DEHP150E	6,7	88	11,6				1	
1996-05-25	TROLLE	7,31	94	11,2				1	0
1996-05-25	ORMD	7,71	96	11,3	brownish water			1	
1996-05-25	DEHP150B	6,14	96	11,5				0	
1996-05-25	DEHP150A	6,21	97	11,5				0	
1996-05-25	DEHP100A	5,79	93	11,4				0	
1996-05-25	DEHP100D	5,79	92	11,4				0	
1996-05-25	DEHP100C	5,73	95	11,7				0	
1996-05-25	DEHP100B	5,69	97	11,8				0	
1996-05-25	TROLLA	7,24	98	11,4				1	
1996-05-25	ETOHA	6,34	63	11,4	ariation stopped			1	
1996-05-25	DEHP15D	5,82	89	11,6				<0,5	
1996-05-25	DEHP15C	5,75	93	11,8				0	
1996-05-25	DEHP15B	5,65	101	11,4				0	
1996-05-25	DEHP15A	5,84	97	11,4				0	
1996-05-25	DIDP600D	6,34	98	11,4				0	
1996-05-25	DIDP600E	6,47	97	11,7				0	
1996-05-26	GRÅND	6,4	105	9,9			7,8	1	0
1996-05-26	DEHP150C	6,55	104	9,8				0	
1996-05-26	DIDP15C	5,88	103	9,8				0	
1996-05-26	DIDP300E	5,91	104	9,5				0	
1996-05-26	GRÅNE	6,35	78	9,8				1	0
1996-05-26	DIDP300C	6,09	78	9,5				0	
1996-05-26	DIDP300B	5,96	78	9,7				0	
1996-05-26	DIDP300A	5,77	45	10	increased ariation			0	
1996-05-26	DIDP15B	5,23	51	9,8	increased ariation	30		0	
1996-05-26	ETOHC	6,53	56	9,6	ariation stopped			1	0
1996-05-26	ETOHB	6,84	77	9,4				1	0
1996-05-26	ETOHD	6,93	79	9,8				1	0
1996-05-26	DIDP600A	6,26	55	9,7				<0,5	

Date	Beaker	pH	O2	Water temperature degree Celcius	Notice	NaOH number of droplets	Air temperature degree Celcius	NH4-N, mg/l	NO2-N
1996-05-26	ETOHE	6,84	74	9,5				1	0
1996-05-26	TROLLB	7,32	75	9,6				0	
1996-05-26	TROLLC	7,27	74	9,8				0,5	
1996-05-26	DIDP300D	5,95	98	9,2				0	
1996-05-26	DIDP600B	6,25	97	9,3				0	
1996-05-26	ORME	7,06	65	9,4	ariation stopped				
1996-05-26	ORMC	7,57	97	9,3					
1996-05-27	DEHP150D	6	92	9,4			9,7	<0,5	
1996-05-27	DIDP600C	6,51	98	9,3				0,5	
1996-05-27	Orm A	7,48	97	9,2				0	
1996-05-27	Control E	5,58	94	9,6				0	
1996-05-27	DEHP15E	5,56	92	9,1				0	
1996-05-27	DEHP100E	6,36	95	9				0	
1996-05-27	Control B	5,49	94	9,2		5			
1996-05-27	Control A	5,56	95	9,2					
1996-05-27	Control+acetone C	5,63	97	8,1					
1996-05-27	DEHP50D	5,57	93	7,8					
1996-05-27	DIDP15E	5,76	80	7,6					
1996-05-27	DIDP15D	5,65	89	7,1					
1996-05-27	DIDP100A	5,75	82	8,3					
1996-05-27	DEHP30E	5,7	81	8,2					
1996-05-27	DEHP30D	5,64	84	8,2					
1996-05-27	DEHP30C	5,7	85	8,2					
1996-05-27	DIDP100E	5,85	80	9,2					
1996-05-27	DIDP100D	5,83	78	8,7					
1996-05-27	DIDP100C	5,78	80	8,5					
1996-05-27	DIDP100B,	5,84	79	8,8					
1996-05-29	DEHP300B	6,03	94	9					
1996-05-29	DEHP50C	5,66	94	9,1					
1996-05-29	DEHP50B	5,57	95	9,1					
1996-05-29	DEHP50A	5,65	97	9					
1996-05-29	DEHP600B	5,68	100	9,4					
1996-05-29	Control+acetone A	5,73	100	9,4					
1996-05-29	DEEHP300A	5,82	101	9,5					
1996-05-29	Control+acetone D	5,62	101	9,4					
1996-05-29	DIDP30B	5,86	100	9,8					
1996-05-29	Control+acetone B	5,79	101	9,5					
1996-05-29	Control C	5,65	101	9,5					
1996-05-29	DEHP300C	5,87	101	10,4					
1996-05-29	DEHP30B	5,66	101	9,9					
1996-05-29	DIDP50D	5,92	103	9,9					
1996-05-29	DIDP50C	5,98	102	9,8					
1996-05-29	DIDP50B	5,86	82	9,5	ariation stopped				
1996-05-29	DIDP30D	5,79	101	10,2					
1996-05-29	DIDP30C	5,77	95	10,1					
1996-05-29	DIDP150D	6,14	94	9,9					
1996-05-29	DIDP30A	5,77	100	9,5					
1996-05-30	DIDP50A	6,23	74	11,1			11,4		
1996-05-30	DIDP150B	6,4	98	11					
1996-05-30	DIDP150A	6,53	97	10,9					
1996-05-30	DIDP30E	5,96	97	10,8					
1996-05-30	DEHP300D	6,01	95	11,1					
1996-05-30	DEHP600A	5,5	46	10,8	ariation stopped	5			
1996-05-30	DIDP150E	6,31	95	10,9					
1996-05-30	DEHP30A	6	90	10,8					
1996-05-30	Grän A	6,55	97	11					
1996-05-30	DEHP600E	6,04	98	10,9					
1996-05-30	DEHP600D	5,95	98	10,9					
1996-05-30	DEHP600C	5,88	100	11					
1996-05-30	DIDP150C	5,97	71	11					
1996-05-30	Control D	5,84	81	11,1					
1996-05-30	Grän C	6,31	79	11					
1996-05-30	Grän B	6,49	85	11					
1996-05-30	TROLLD	7,41	86	11,1					
1996-05-30	ORMB	7,64	77	10,9					
1996-05-30	DIDP15A	6,42	80	11,1					
1996-05-30	DIDP100E	6,03	60	11,5	ariation stopped				
1996-05-31	TROLLE	7,35	94	11,1			10		
1996-05-31	ORMD	7,7	95	11,3					
1996-05-31	DEHP150B	6,66	94	11,3					
1996-05-31	DEHP150A	6,29	87	11,7	ariation stopped				
1996-05-31	DEHP100A	6,01	93	11,3					
1996-05-31	DEHP100D	6,12	92	11,4					

Date	Beaker	pH	O2	Water temperature degree Celcius	Notice	NaOH number of droplets	Air temperature degree Celcius	NH4-N, mg/l	NO2-N
1996-05-31	DEHP100C	6,01	94	11,5					
1996-05-31	DEHP100B	6,01	91	11,6					
1996-05-31	TROLLA	7,21	94	11,4					
1996-05-31	ETOHA	7,16	94	11,4					
1996-05-31	DEHP15D	6,15	91	11,5					
1996-05-31	DEHP15C	6,08	92	11,7					
1996-05-31	DEHP15B	6,05	96	11,4					
1996-05-31	DEHP15A	6,32	95	11,4					
1996-05-31	DIDP600D	6,54	96	11,4					
1996-05-31	DIDP600E	6,51	97	11,5					
1996-05-31	GRÅND	6,2	83	11,7	ariation stopped				
1996-05-31	DEHP150C	6,38	96	11,2					
1996-05-31	DIDP15C	6,17	95	11,2					
1996-05-31	DIDP300E	5,89	98	10,9					
1996-06-01	GRÅNE	6,27	97	11,1			9,7		
1996-06-01	DIDP300C	6,48	95	11,1					
1996-06-01	DIDP300B	6,4	95	10,9					
1996-06-01	DIDP300A	6,4	96	11,1					
1996-06-01	DIDP15B	6,08	94	11,2					
1996-06-01	ETOHC	7,13	95	11,1					
1996-06-01	ETOHB	7,09	94	11,1					
1996-06-01	ETOHD	7,2	95	11					
1996-06-01	DIDP600A	6,64	92	10,6					
1996-06-01	ETOHE	7,13	76	10,9					
1996-06-02	TROLLB	7,53	95	11,1			10,1		
1996-06-02	TROLLC	7,17	92	11,1					
1996-06-02	DIDP300D	6,71	96	10,8					
1996-06-02	DIDP600B	6,73	93	10,8					
1996-06-02	ORME	7,62	96	10,8					
1996-06-02	ORMC	7,54	98	10,8					
1996-06-02	DEHP150D	6,55	85	10,5					
1996-06-02	DIDP600C	6,86	97	10,6					
1996-06-02	Orm A	7,57	97	10,6					
1996-06-02	DIDP50E	6,38	71	10,7	All tadpoles dead, slightly ariated				0
1996-06-02	Control B	5,96	78	10,6					
1996-06-02	Control A	5,66	83	10,6					
1996-06-03	Control+acetone C	6,17	94	10,2			11,4		
1996-06-03	DEHP50D	6,18	96	10					
1996-06-03	DIDP15D	5,97	100	10,1					
1996-06-03	DIDP100A	5,92	88	10,3					
1996-06-03	DEHP30D	5,62	58	10,2					
1996-06-03	DEHP30C	6,14	100	10,3					
1996-06-03	DIDP100D	6,11	68	10,3					
1996-06-03	DIDP100C	6,34	102	9,8					
1996-06-03	DIDP100B,	6,23	80	10					
1996-06-03	DEHP300B	6,33	102	10,2					
1996-06-03	DEHP50C	6,2	96	10,1					
1996-06-03	DEHP50B	6	99	10,1					
1996-06-03	DEHP50A	6,02	100	10					
1996-06-03	DEHP600B	6,02	93	10,5					
1996-06-03	Control+acetone A	6,12	101	10,2					
1996-06-03	DEHP300A	6,11	100	10,3					
1996-06-03	Control+acetone D	5,98	102	10,3					
	mean	5,99	85,86	10,60		8	10,21		
	min	4,7	45	7,1			6,2		
	max	7,71	108	11,8			12,9		
	number	464	408	259		79	23	58	