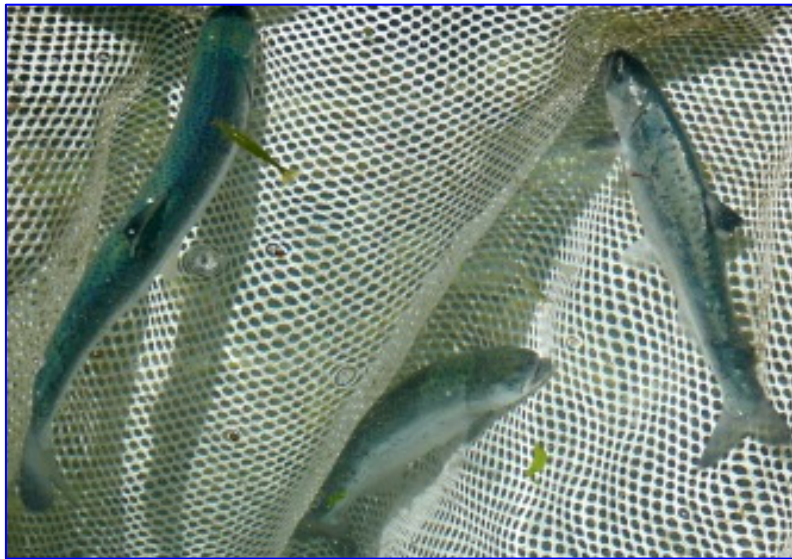


Report to the  
San Juan County Marine Resources Committee

# DEVELOPMENT AND APPLICATION OF A METHOD for the analysis of **DIETHYLHEXYL PHTHALATE** **(DEHP)** in **SAN JUAN COUNTY WATERS**



Jack Bell and Russel Barsh

Kwiàht and University of Washington Friday Harbor Laboratories

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**KWIÀHT**

Center for the Historical Ecology of the Salish Sea  
PO Box 415, Lopez, WA 98261

# **DEVELOPMENT AND APPLICATION OF A METHOD FOR THE ANALYSIS OF DIETHYLHEXYL PHTHALATE (DEHP) IN SAN JUAN COUNTY WATERS**

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## **SUMMARY**

This report provides local decision-makers with preliminary data and monitoring cost-benefit considerations relevant to San Juan County's storm water monitoring plan.

An analytical method was developed for the determination of the ubiquitous plasticizer diethylhexyl phthalate (DEHP) in San Juan County waters, using high performance liquid chromatography with ultraviolet absorbance detection (LC/UV method). In preliminary tests low parts-per-billion (ppb) levels of DEHP were found in all samples: 0.2 ppb in Friday Harbor seawater, 0.4 ppb in Lopez village bioswale water, 0.6 ppb in Lopez store parking lot runoff, and 1.6 ppb in East Sound storm sewer water. The National Toxics drinking water criterion to protect human health for DEHP is a minimum reporting level of 0.6 ppb and a maximum contaminant level of 6 ppb (EPA, 2002). The recommended maximum concentration of DEHP in water to protect aquatic life is also 0.6 ppb (Canadian Water Quality Guidelines, 1987). Local input waters tested therefore appear to be at the threshold of potential adverse environmental impacts, before dilution in marine waters.

The HPLC method described here is sensitive and relatively inexpensive, using an instrument that is easier to operate and maintain than mass spectrometry. It also appears to be more accurate than many previous attempts to determine DEHP in water that failed to take into account the very low solubility of this analyte in water, and its tendency to form micelles and adsorb to dissolved organic matter (DOM).

At this time we do not recommend routine monitoring of DEHP in the Islands' stormwater for the following reasons: (1) Current levels of this contaminant are below those of the Seattle area (King County, 2007), and (2) Standard multi-parameter EPA methods do not reflect advances in aquatic sciences nor focus on Contaminants of Emerging Concern (EPA, 2008).

The fates and effects of DEHP in our freshwater and marine habitats nonetheless are so poorly understood at present, that further study of bioaccumulation would be warranted, as well as strategic surveys of DEHP loading of the islands' most three impacted nearshore habitats—its urban waterfronts—every two to five years to detect any significant increase.

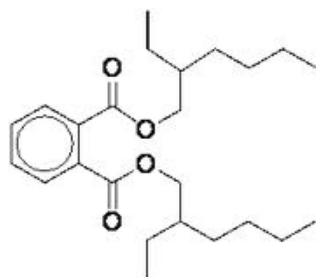
# DEVELOPMENT AND APPLICATION OF A METHOD FOR THE ANALYSIS OF DIETHYLHEXYL PHTHALATE (DEHP) IN SAN JUAN COUNTY WATERS

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## INTRODUCTION

The waters of the San Juan Islands have been assumed to be relatively pristine as compared to other Puget Sound and Canadian waters surrounding them. Recent studies suggest that island communities contribute at least some contaminants to marine waters of the archipelago, however, including pyrethroid pesticides and anionic surfactants used in homes and gardens, and polycyclic aromatic hydrocarbons from motor vehicles (Barsh et al., 2009; 2010a; 2010b).

Legacy pollutants such as lead, mercury, and the polychlorinated biphenyls (PCBs) have declined in the sediments of Puget Sound as the use of these toxics has been reduced or eliminated. In contrast, emerging pollutants such as phthalates and flame-retardants have increased (Partridge, et. al., 2009). Among numerous phthalate additives in plastics, cosmetics, some insecticides and lubricants, diethylhexyl phthalate (DEHP, Figure 1) is the dominant phthalate and the most toxic to aquatic wild life. Disruption of invertebrate and vertebrate endocrine systems is now recognized as a subtle but serious effect. Long-term exposure of several species of fish to 4-14 ppb of DEHP caused loss of young and disturbed growth regulation (Wams, 1987). DEHP was reported in the range of parts-per-trillion in open oceans 30 years ago, and tenths of a ppb in nearshore environments closer to sources such as solid waste disposal sites and sewers (Giam, et al., 1978). DEHP bioaccumulates in the food web because it is very lipophilic, however; loading of biota could be several times greater than ambient concentrations in water.



**Figure 1: Structure of DEHP, the diester of phthalic acid and 2-ethylhexanol**

Of 65 regulated chemicals DEHP had the highest percent of occurrences above the minimum reporting level (MRL) of 0.6 ppb in ground and surface waters (EPA, 1999). For this reason, among many, we chose DEHP for this initial study as a biomarker relevant to the fauna and flora of San Juan County. As a component of many plastics including PVC, DEHP can serve as an indicator of increased use of construction materials, and waste generation associated with population growth.

## EXPERIMENTAL

Methanol and dichloromethane (DCM) are Sigma-Aldrich Chromasolv grade for HPLC. Water is purified through a reverse osmosis system followed by a Millipore MilliQ system. The C18 silica powder is Sigma-Aldrich Octadecyl-functionalized silica gel, 200-400 mesh (40-75 microns), with 60-Angstrom pores, and 20-22 % carbon loading. The Honeywell B&J C18 cartridges contain 500 mg of C18 bonded-silica sealed between plastic frits in 3 mL plastic Luer-tipped syringe barrels; the void volumes are approximately 0.2 mL. The Waters HLB (Hydrophilic-Lipophilic Balance) cartridges contain 60 mg of 30 micron polymeric particles sealed with 10 micron plastic frits in 3 mL plastic Luer-tipped syringe barrels; void volumes are ~ 0.08 mL. Whatman GFC glass fiber filters (retaining  $\geq$  0.4 micron particulates) are clamped in a glass funnel assembly on top of a vacuum flask attached to a tap water aspirator. Rainin pipettors with plastic disposable tips are used for aliquotting and transfers. Unless otherwise noted, all solvents, solutions and extracts are prepared and stored in 20 mL borosilicate glass scintillation vials with polypropylene-lined white plastic screw caps. When preparing calibration standards, vials are pre-rinsed with methanol to avoid adsorption of DEHP to active glass surfaces.

Preparation of analytical standards: The di(2-ethylhexyl)phthalate (DEHP) standard is a pure, single isomer purchased from Sigma-Aldrich as a one milliliter methanol solution of 2 mg/mL DEHP in an ampoule. The solution is transferred to a 20 mL vial, and rinsed with measured volumes of methanol to provide a 1.56 mg/mL stock solution stored at  $-20\text{ }^{\circ}\text{C}$ . The DEHP stock is kept on ice while in use to minimize evaporation. Calibration standards in methanol at 10 to 1000 ppb concentrations are prepared in 20 mL vials by dilution of the DEHP stock.

HPLC instrumentation: The LC system consisted of a Waters Model 510 pump with a 100% methanol (MeOH) reservoir above the pump, a Valco 1.5 mL loop injector, a 2.1 x 220 mm cartridge column containing 5 micron C18 reverse phase silica particles (void volume ~ 0.5 mL), an ABI UV detector set at 235 nm, and an eDAQ data logger with input amplifier range of 1 V = 1 Absorbance Unit (AU).

LC operation and maintenance: The LC column backpressure is normally roughly 500 psi. When the pressure increases to 3000 psi, due to particulates from unfiltered samples, the column is replaced by a Reliasil 2 x 250 mm 5 micron ODS (C18) column with ~ 900 psi back pressure. Injections are 100 microliters ( $\mu\text{L}$ ) to increase peak height sensitivity. The injector is kept in the Inject position during the run to prevent artifacts in the next run. The large volume loop allows injection of DCM to purge the column of contaminants not eluted in a timely manner with methanol, and to reduce column backpressure. Replicate peak retention times, as a measure of pump, injector and system stability, are typically within  $\pm 0.3\%$  coefficient of variation. Plugging of columns, and/or system leaks, cause lower flow rates and longer retention times. The UV detector baseline noise should be about 0.05 millivolt (mV) peak-to-peak. The detector is manually autozeroed before each run. The following eDAQ settings prevent crashes. Recorded data: Range 1 volt, Anti-alias *On*, use

input 1, data acquisition rate 20/second. Calculated data: Digital filter, 1 Hz low pass. Peak heights are measured on the fly with the eDAQ screen cross-hair marker, or by ruler on hard copy.

Sample collection and storage: Surface seawater is collected in a one liter glass bottle with a Teflon-lined plastic screw cap, and stored at 5 °C. Samples for method development are collected off the outer edge of the Friday Harbor Laboratories outer dock. The Lopez and Orcas terrestrial water samples are collected in glass jars with plastic-lined metal lids and stored at room temperature for two days.

## **DEVELOPMENT OF THE ANALYTICAL METHOD**

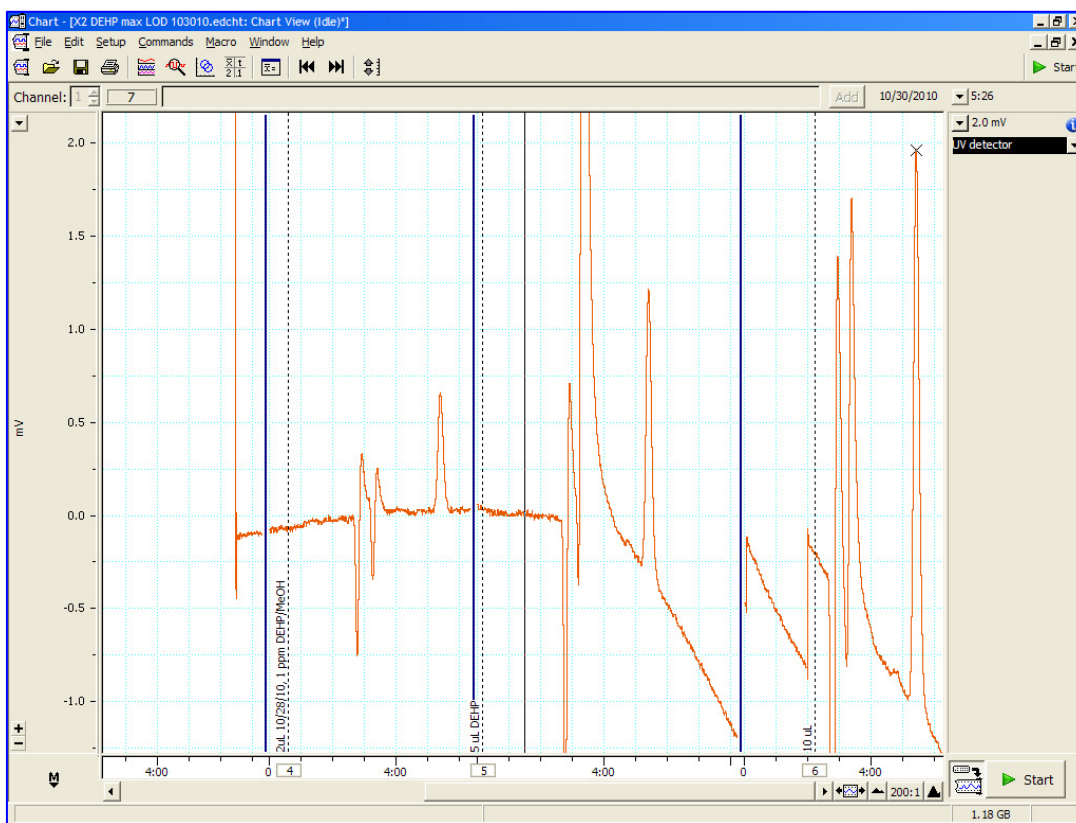
Controlling contamination: DEHP is a common additive in numerous plastics, glues, and lubricants, and is therefore a ubiquitous contaminant of the lab environment, equipment and glassware (Giam, et.al., 1975). Consequently LC is used to monitor suspected sources of contamination as measured by a peak eluting at the retention time of a DEHP standard. Low-level contamination is found in the solvents and injection syringes. Clean but suspect glassware, syringes, etc, are thus rinsed with methanol before use. Solvents, samples and standards are exposed only to polyethylene or polypropylene tubing and fittings, and glass syringes with stainless steel needles. Maintaining scrupulous attention to the solvents used and potential sources of contamination kept DEHP artifacts to a minimum.

Stability of DEHP solutions: Preliminary experiments were conducted to investigate the stability of low parts-per-billion (ppb) levels of DEHP in methanol and water. Surprisingly, given that trace acid or base in methanol should trans-esterify DEHP to its methyl ester, no loss of DEHP was detected at room temperature. However, 20 mL vials fresh from the box adsorb DEHP unless they are pre-rinsed with methanol. The very low solubility of DEHP in water, and the tendency of DEHP to bind to glass surfaces (Sullivan, etal, 1981), precludes the use of standards of greater than 1 ppb.

SPE filtration method: With a view to the potential cost savings of using readily available materials, we first developed a streamlined, solid phase extraction (SPE) procedure using bulk C18-silica powder rather than cartridges. Briefly, a stock suspension of C18-silica in methanol is prepared, and a portion aliquotted onto a glass fiber filter clamped in a glass funnel/vacuum flask apparatus. The pre-conditioned C18 particles are then transferred with the filter into a liter of unfiltered seawater (initial filtration of cloudy samples is not needed). After stirring vigorously with a magnetic stir bar to bring C18 particles in contact with any DEHP molecules, the loaded C18 particles are captured on a fresh glass fiber filter, water-rinsed to remove salts, and eluted with small portions of methanol to recover DEHP. Nominal 0.45-micron GFC glass fiber filters are the standard for separating suspended particulate matter (SPM, "seston") from dissolved organic matter (DOM), micelles, and most colloids (0.001 – 1 micron) (Hickel, 1984). GFC filter methods will be useful in the future for determining DEHP's affinity for these seawater fractions, and how this affects transport and bioavailability.

DEHP micelles do not bind to SPE phases: Both the bulk C18 procedure described above, and standard C18 and HLB cartridge procedures, produced very low recoveries of DEHP from 100 ppb-spiked seawater. We then discovered that in soil leachate water, DEHP at greater than 3 ppb is reported to form micelles, binds to DOM, and in either form passes through lipophilic SPE cartridges (De Jonge, et al, 2002). A comprehensive review of the aqueous solubilities of DEHP concludes that its solubility in seawater is 0.6 ppb, and in water is a maximum of 3 ppb (Staples, et al, 1997). By working with 1 ppb DEHP-spiked water, we improved recovery from an HLB cartridge to roughly 70 %. Conversely, 40 % methanol in the 100 ppb DEHP-spiked water provided nearly 90 % retention of DEHP on an HLB cartridge, suggesting that the DEHP micelles were broken up by methanol. This also indicates that liquid-liquid extraction should break up micelles and more likely desorb DEHP off DOM. Numerous previous studies using SPE (Mollet, 2007) may under-report DEHP contaminant levels in water.

Method protocol: A convenient scale of 100 mL water sample yielding a 1 mL SPE cartridge extract provides a 100-fold concentration factor, decreasing the limit of detection (LOD) of the LC/UV analysis to approximately 0.2 ppb in the original sample. In Figure 2 the analyte elutes at ~ 5.4 minutes, separated from the solvent and matrix contaminants eluting at 2-4 minutes. Baseline noise and drift become issues in trace pollutant analyses.



**Figure 2: LC chromatograms for determining the limit of detection of DEHP.**

By fractionating the SPE cartridge eluates of DEHP-spiked blanks and samples, we found that approximately 70 % of the DEHP elutes in the first one mL of methanol eluate. DEHP contamination varied from day to day, in a range of 0.1 to 0.2 ppb in samples. Results are therefore corrected with the ambient contamination level found when the data is collected. To confirm the identity of DEHP in extracts contaminated with matrix peaks, "peak spiking" is employed. Extracts are spiked with known amounts of DEHP to observe the appropriate additive effect on a peak with the observed retention time of DEHP. Confirmation of results by photodiode array and mass spectrometry is recommended.

Application of the method to water samples: Fresh or refrigerated Friday Harbor seawater samples were used for development of the method. They did not require filtration prior to SPE extraction, but did require additional water rinses of sample-loaded C-18 cartridges to remove salt. The cloudy terrestrial water samples required initial GFC filtration to prevent plugging of the SPE cartridges.

## RESULTS

In all calculations efforts were made to avoid false positive results. Blank peak heights are subtracted from DEHP peak heights to provide a net DEHP peak height. In calculating recoveries the higher number is used. Response factors (net peak height/ppb DEHP) vary with changes in LC column characteristics and are therefore determined for each sample tested.

Soluble DEHP found in water samples: During development of this LC/UV method, Friday Harbor marine water was tested three times and found to contain approximately 0.2 ppb of apparent DEHP, which is very close to the method's limit of detection (LOD). Another test found 3 ppb DEHP, most probably from DEHP contamination. In one test of the East Sound storm sewer water we found 1.6 ppb apparent DEHP. In two tests of the new Lopez village store parking lot runoff water, we found 0.67 ppb and 0.60 ppb apparent DEHP (average of 0.64 ppb). In one test of Lopez village bioswale water we found 0.4 ppb apparent DEHP. It is reasonable to conclude that local storm sewer inputs may be as high as 1-2 ppb

## DISCUSSION

These results reflect only the fully soluble DEHP (less than 3 ppb in fresh water and less than 1 ppb in seawater). Micellar and DOM-bound DEHP pass through SPE cartridges and will not be detected by the LC/UV method we described here. Vigorous liquid-liquid extraction with an aggressive solvent such as hexane should extract all forms of DEHP, possibly including that bound to suspended particulate matter (SPM, "seston"). However, fully soluble DEHP may account for most or all of the bioavailable DEHP in aquatic habitats.

By regulatory criteria referenced in the Summary, our results suggest that storm water runoff in San Juan County is currently at the threshold of concern for aquatic wildlife. Dilution in marine waters lowers DEHP concentrations to the levels we observed as

contamination in laboratory environments (0.1 ppb to 0.2 ppb), consistent with reported ubiquitous, worldwide contamination by DEHP.

As to the toxic effects of these DEHP levels on wildlife in the San Juan Islands, much remains to be explored (cf. Nadzialek, et. al., 2010). Due to its strongly lipophilic character, DEHP should accumulate in the tissues of individual animals and in aquatic food webs. We do not know the extent to which bioaccumulation is occurring in the islands' freshwater or nearshore habitats. A magnification effect of 5-10 would raise DEHP loads in freshwater and marine animals to the 4-14 ppb range in which estrogenic effects have been observed in fish (Wams, 1987).

Further study to explore bioaccumulation of DEHP in island biota would seem to be warranted, insofar as it would determine whether current concentrations of this analyte in water threatens local food webs. Switching from an aqueous matrix to animal tissue would require additional extraction and clean-up steps already developed and tested as part of a previous study of pesticides and PAHs in freshwater fish, insects and crustaceans (Barsh et al., 2010b). A single investigation with a sample of 100-200 animals should suffice to answer the question whether bioaccumulation is great enough to reclassify DEHP from a threshold concern in the islands, to a current threat.

In addition, it would be prudent to sample and test storm water inputs for DEHP on a periodic basis, for example every 2-5 years, to detect any significant increase in loading of this contaminant. An increase to the 4-14 ppb range would reasonably justify intervention, as well as adding this analyte to routine storm water monitoring activities in the future.

#### **ACKNOWLEDGMENTS**

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