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# Prevalence of Eight Monoester Phthalates in the Okavango Delta, Northern Botswana

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**Abstract**

The Okavango Delta, a seasonal wetland, covers 5,000-12,000 $km^2$ in northern Botswana. The Delta is rich with animal and plant life, providing fresh water to many species of birds, fish, and large mammals. Water samples were taken from the Okavango Delta using solid phase extraction techniques and analyzed by liquid chromatography tandem mass spectrometry (LC/MS/MS). Water samples were collected and analyzed for eight different phthalate monoesters. These molecules are active in the endocrine system as antiandrogens and suppress testosterone synthesis. Phthalate diesters are mass produced and used in personal care products, construction materials, and pesticides. Phthalate diesters leach out of these products, enter biological systems through inhalation, ingestion, and dermal absorption, and then are quickly metabolized to the endocrine active monoester form. Human exposure to phthalates is nearly unavoidable and ecosystems are being contaminated from encroaching anthropogenic activity. Analysis has shown 7 phthalate monoesters are present above background levels. It is important to understand the contamination that is present in the water system and whether these compounds are affecting sexual development in wildlife.

## 1. Introduction

The Okavango Delta in Northern Botswana was the 1000th UNESCO world heritage site due to its diverse and rare ecosystem. Located in the Kalahari Desert, the Delta is a landlocked wetland varying from 5,000-12,000 $km^2$ based on season and rainfall. Hundreds of species of birds, fish and large mammals depend on the water system to survive. In addition, tourism in the area, which is a large part of the local economy, depends on the wildlife.

The headwaters of the Okavango Delta, in the mountains of Angola, flow south across the Caprivi Strip of Namibia to reach the Okavango Panhandle. The Panhandle stretches roughly 60 miles from the northern border of Botswana, south, until the waters spread across the Okavango Wetlands. As Angola recovers from a long civil war, the economy is growing rapidly. The Basin of the Delta in Angola is a large area highly suitable for irrigated agriculture and hydropower[1]. This presents problems for the Delta as surface waters carry pollutants south. Foregin investment groups have made large land purchases in this area and intend to develop the area for its potentially profitable agriculture and power resources. This is important to consider as pollution in this area will bring contaminants into the Okavango Delta. Environmental policy and regulation Angola is lacking and insufficient for its size. Pollutants are being carried into the rivers of Angola and eventually collecting in the Okavango wetlands. Pollutants include pesticides, phthalates, and arsenic. Toxic metabolites of the pesticide dichlorodiphenyltrichloroethane (DDT) were found in the Delta at concentrations over 10 times higher than the maximum safe levels set by the European Community[2]. Icosane ($C_{20}H_{42}$), an indicator of oil contamination has also been detected[2]. Arsenic in the Delta occurs naturally but is being washed out of bedrock when water systems are diverted or boreholes drilled. Arsenic has been detected at levels above the World Health Organizations provisional limit of 10 µg/L [3].

Phthalates are a compound of growing concern and serve as a marker for human impact in an ecosystem. Every year several million tons of phthalates are produced worldwide. Diester phthalates are chemicals used to manage flexibility

in plastics, in the production of construction materials, cosmetics, and solvents on the industrial scale. These molecules are found in many consumer products. Phthalates are not bound covalently in the matrix of plastics and construction materials and eventually will leach out over time[4]. However, human exposure to phthalates is largely due to the use of lotions, makeup, and other personal care products. Phthalates enter the body by inhalation, ingestion, or dermal absorption[5]. Monoester phthalates, the metabolites of diesters, have been detected in most humans and indicate exposure is almost unavoidable[6]. A phthalate monoester base structure is shown in Figure 1, with various lipophilic side chains at the R position. The molecules monomethyl phthalate (mMP), monoethyl phthalate (mEP), mono isobutyl phthalate (miBP), mono butyl phthalate (mBP), mono-2-ethylhexyl phthalate (mEHP), and mono benzyl phthalate (mBzP) as well as the metabolites of mEHP, mono-2-ethyl-5-oxohexyl phthalate (mEOHP), and mono-2-ethyl-5-hydroxyhexyl phthalate (mEHHP), were quantified by multiple reaction monitoring using liquid chromatography with a triple quadrupole mass spectrometer (LC/MS/MS).

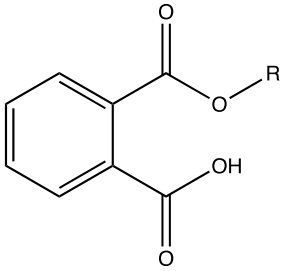


**Figure 1:** Phthalate monoester base structure

Phthalate diesters are rapidly hydrolyzed by esterases in the liver to the monoester phthalate, which are biologically active in the endocrine system. Hydroxylation and oxidation by CYP450 enzymes leads to conjugation by glucuronosyltransferase (UGT) with the cofactor uridine diphosphate glucuronic acid (UDP) to make a glucuronide conjugate. The increased hydrophilicity of the conjugate leads to faster excretion through the kidneys. The metabolic fate of the diester phthalate is shown in Figure 2.

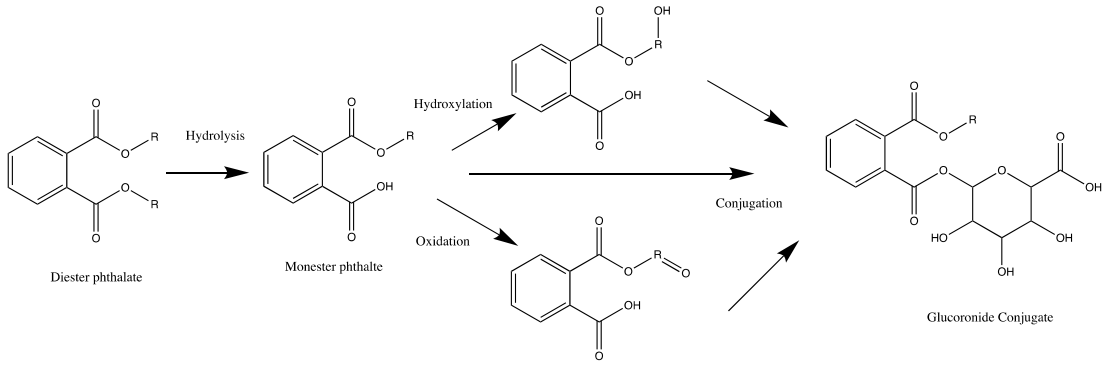


Figure 2: Partial metabolic pathway for monoester phthalates

When the more hydrophilic glucuronide conjugate is excreted via urine or feces, the glucuronide bond may be cleaved by hydrolysis or microbial actions and return to the environment as an active compound. However, this is an unlikely source as the half-life in a probiotic system like sewage is only a few days. The half-life of dibutyl phthalate in natural surface/ground waters, like those of The Delta, is 1-28 days[7]. Hydrolysis of the diester in the waters of the Okavango Delta is also possible. The mechanism is either acid catalyzed in the in the Okavango Delta proper (pH≅ 6.8), or base catalyzed in Lake Ngami (pH≅8). Hydrolysis may be slow due to lack of a strong catalyst in the Okavango Delta proper. Hydrolysis may not happen before the phthalate is broken down microbially or by UV light.

Monoester phthalates act as antagonist of the androgen signaling pathway and suppress testosterone synthesis[8]. Monobutyl phthalate (mBP), and mono 2-ethylhexyl phthalate (mEHP) disrupt the androgen-signaling pathway during fetal development in rats[9]. Monoester phthalates do not readily bond to androgen receptors (AR), instead, the presence

of monoester phthalates interferes with normal testosterone binding at AR sites. Males are most affected by this, and exposure leads to developmental defects in sex organs[7]. Estrogenic activity of monoester phthalates have been studied but activity was relatively low compared to the anti androgenic properties[10,11].

In humans, prenatal phthalate exposure has led to feminization of boys[12]. The highly sensitive nature of hormone signaling and regulation during fetal development is vulnerable to disruption by low concentrations of EAC like phthalates. This is concerning as different phthalates are active in combination, and their activity cumulative[9]. Multiple phthalates are usually found together so a suite of phthalate analytes will better represent activity in an ecosystem.

This study quantifies eight phthalate monoesters in water samples collected from the Okavango Delta. Another study has identified some diester phthalates but they have not been quantified[2]. The vast abundance of diester phthalates becomes an issue for analysis because contamination may come from many sources (equipment, deodorant, lotions, fragrances etc.). Analysis of the monoesters by solid phase extraction (SPE) is an improvement for determining concentration and activity as any contamination of the diesters during sample collection and preparation, would be filtered out during (LC/MS/MS) analysis. In the SPE method used, the diester phthalates that may come from contamination could not reach the monoester form.

## 2. Methods/Experimental

Sampling locations are shown on the map in Figure 3. Samples were taken in the panhandle from the northernmost part at the border to the southernmost part where the panhandle stretches into the alluvial fan of the Delta, this area is marked A. There are many villages and tourist destinations down the west side of the panhandle. Further south, samples from area **B** stretch from the west side of Chief's Island to the southern end of the Delta near Maun. This area is highly populated with wildlife. Samples from area **C** are were taken in the city of Maun on the Thamalakane river. This area should have the heaviest anthropogenic activity of any sample sites. Lake Ngami is a temporary lake West of Maun.

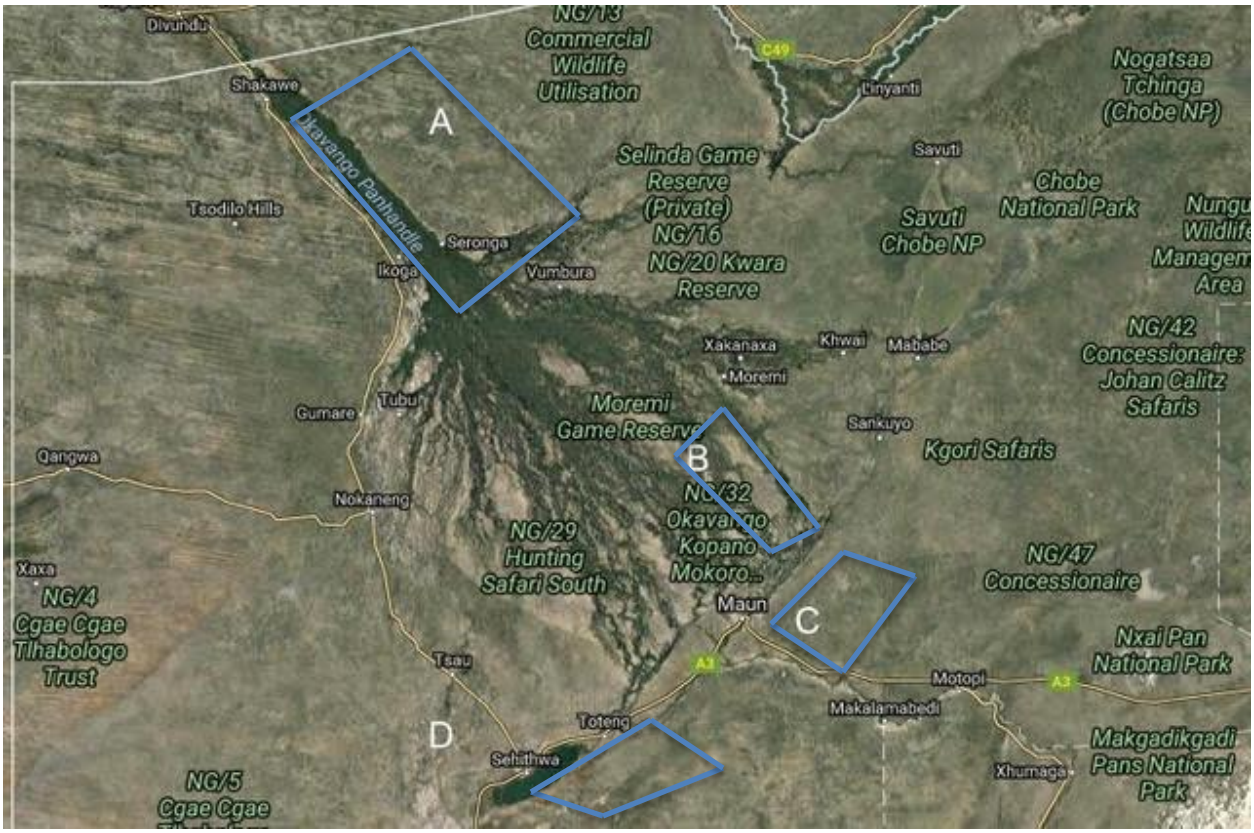


Figure 3: Map of samples sites on the Okavango Delta. Samples sites are organized into four areas listed A,B,C,D.

Table 1: Sampling locations and the corresponding sample numbers.

| Sample # | Area | Location |
|---|---|---|
| 16,17,18,19.20,21,22,23, 24, 25,26,27,28,29, 30,31,32,33 | A | Okavango Panhandle (From border of Namibia 60 miles south to alluvial fan.) |
| 2,3,4,5,6,7,8,9,10,11,12,13,14,15 | B | Chiefs Island (Okavango wetlands) |
| 1,37,38,39,40,41,42,43,44,45,46 | C | Maun (37,38,39 are in the city and others are outside the city.) |
| 34,35,36 | D | Lake Ngami |

Lake Ngami is much different than the Okavango wetlands proper. The pH here is about 9.4 during July and the water is filled with algae and dissolved solids. This is area **D** and also has a fair amount of human activity. The sample numbers are shown along with their location in **Table 1.**

## 2.1. Field Sampling Method

All solid phase extraction cartridges (SPE Oasis HLB cartridge 6cc) were treated with 5 mL of 95% ethanol and 5 mL distilled water to activate SPE cartridge. For the 46 samples, 200 mL of water was removed from the Delta and treated with 6 mL of 5% acetic acid (vinegar). The vinegar provided a way to keep the carboxylic acid protonated because a negative charge on that carboxylic acid would overwhelm the Van der Waals interactions and the monoester phthalate would not hold in the SPE packing. The 200 mL sample was then pushed through the SPE cartridge using a plastic syringe and the sample location was recorded. For the four method controls all the SPE cartridges were treated with 5 mL 95% ethanol and 5 mL distilled water. Then 6 mL 5% acetic acid was added to 200 mL samples of distilled water and pushed through the SPE cartridge with the same syringe.

### *2.1.1 spe methods*

Oasis HLB solid phase extraction (SPE) cartridges were used in field sampling and as a means to concentrate the original sample. When the sample was extracted and pushed through the SPE cartridge the monoester phthalates were suspended in the packing by Van Der Waals interactions between the lipophilic chain of the phthalate and the benzene ring in the active site of the SPE cartridge. The intermolecular interactions are shown in **Figure 4.** The cartridges were returned to the lab for extraction and analysis. Elution using acetonitrile then ethyl acetate is ideal as the weak polar properties of these molecules will replace the analytes at the active sites in the cartridge, but not lipophilic enough to elute unwanted molecules.

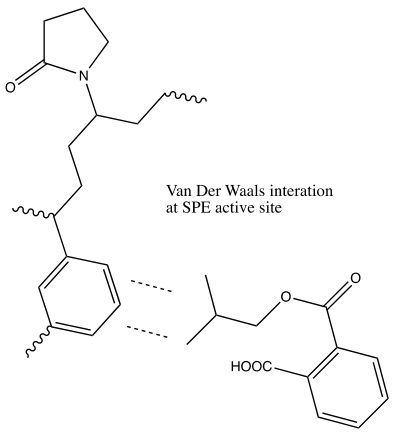


Figure 4. Anlayte binding in an Oasis HLB SPE Cartridge

## 2.2 Extraction Procedure [13]

All SPE cartridges were spiked with 50 µL of $^{13}C_4$ isotopic phthalate internal standards solution. Extraction of the analytes began by putting the SPE cartridges on a Vacuum manifold and washing them with 3 mL formic acid (0.1M) then 1 mL $H_2O$ at a rate of 1ml/min. Air was then pulled through the cartridge for 2 minutes to dry the cartridges. The analytes were then eluted with 2 mL acetonitrile, then 2 mL ethyl acetate. The samples were then dried using a Zymark TurboVap LV Evaporator until all solvents evaporated under a stream of $N_2$ (2 psi) for 2 hours. The dried samples were then resuspended in 0.2 mL of HPLC grade water, mixed well, and transferred to vials for LC/MS/MS analysis.

## 2.3 Instrumental Analysis

The sample (10µL) was injected into a Shimazdu UFLC liquid chromatograph coupled to a Shimazdu LCMS-8040 triple quadrupole mass spectrometer using multiple reaction monitoring in the negative ion mode. Retention times of the analytes are listed in **Table 2**. A developed method using high performance liquid chromatography coupled to triple quadrupole mass spectrometers using electrospray ionization (HPLC-ESI-MS/MS)[14] was used to analyze the samples. A Betasil phenyl HPLC column (3 µm, 150 x 2.1 mm from Thermo Scientific) was used to separate analytes with a pump flow of 0.2 mL/min. Mobile phases used were 0.005% formic acid in $H_2O$ and 100% acetonitrile using gradient elution.

Table 2. Retention times for analytes

| Analyte | Retention Time (min) |
|---|---|
| mMP | 4.50 |
| mEP | 5.90 |
| mEOHP | 10.50 |
| miBP | 10.70 |
| mBP | 11.00 |
| mEHHP | 11.60 |
| mBzP | 13.80 |
| mEHP | 24.23 |

## 2.4 Statistical analysis

Peak areas of analytes were compared with peak areas of isotopic internal standards (ITSD) as a ratio. Area ratio (area analyte/area ITSD) was calculated and applied to a linear regression line from calibration curves ($R^2$>0.95) to calculate concentration in the sample. The concentration factors (approx. 1000x) were calculated from field sampling records. The dilution factors were applied to get actual concentration of samples in ng/L. The limit of detection (LOD) was calculated by finding the average background concentration of each analyte in the four method blanks, adding 3σ, and setting that as the LOD. This gave a 99.9% confidence interval to be above background levels for the values listed in the results.

# 3. Results

46 water samples were taken from the northernmost point of the panhandle down to the southernmost point of the alluvial fan in Maun. Seven analytes were detected across sampling locations with the highest response in the area around Maun. Values for each sample are listed in ng/L. However, the original sample was concentrated 1000x to µg/L. The values listed are after the concentration factor of about 1000 was applied to each sample. The LOD is listed in µg/L which was the concentration of the samples injected into the LC/MS/MS. After the LOD was subtracted from the measured values, the concentration factor was applied to get the real concentration of phthalates in the Delta in ng/L, or parts per trillion (ppt).

## 3.1 mMP

Monomethyl phthalate was found in 10 of the 46 samples above the background level of 0.86 ppt with a LOD of 1.32 ppt. The values range from 1.1-36.0 ppt with an average of 6.84 ppt among detected samples. mMP was detected with the lowest concentrations compared to other analytes found. mMP is shown in Figure 5 and the mMP concentrations are listed in Table 3.

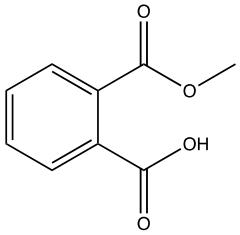


Figure 5: mMP

Table 3: Concentrations of 10 samples that show mMP. All concentrations are in ng/L. All values are corrected for proper concentration factor. The LOD has been subtracted to give real values shown.

| Location | Concentration mMP | Location | Concentration mMP |
|---|---|---|---|
| Panhandle | 2.6 | Maun | 1.3 |
| Chiefs Island | 36.0 | Maun | 3.6 |
| Chiefs Island | 1.1 | Maun | 2.3 |
| Chiefs Island | 2.11 | Lake Ngami | 10.9 |
| Maun | 1.6 | Lake Nagami | 6.9 |

## 3.2 mEP

Monoethyl phthalate was found in 9 of 46 samples above the background levels of 15.2 ppt with a LOD of 90.3 ppt. The concentrations range from 304.8-15940.8 ppt with an average value of 2713.4 ppt. High variation in the method control led to a higher LOD for this analyte. Diethyl phthalate has been used in lotions, sunscreen, bugspray and other personal care products. This may be a potential source of contamination leading to a high LOD. Despite the high LOD, 9 samples contained concentrations well above the LOD. mEP had the highest average value of any analyte among detected samples. mEP is shown in Figure 6 and the mEP concentrations are listed in Table 4.

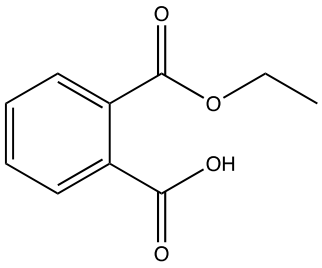


Figure 6: mEP

Table 4: Concentrations for nine values that contain mEP. All concentrations are in ng/L. *Sample 23 needs to be diluted and rerun to avoid extrapolation. All values are corrected for proper concentration factor. The LOD has been subtracted to give real values shown.

| **Location** | **Concentration mEP** | **Location** | **Concentration mEP** |
|---|---|---|---|
| Panhandle | 15940.8 | Maun | 3922.1 |
| Chiefs Island | 653.1 | Maun | 3326.4 |
| Chiefs Island | 304.8 | Lake Ngami | 664.9 |
| Maun | 1138.0 | Lake Ngami | 556.3 |
| Maun | 718.3 | | |

## 3.3 miBP

miBP was found above the background levels of 1.8 with a the LOD of 0.9 ppt in 22 of the 46 samples taken. Values range from 1.9 - 141.1 ppt with an average value of 33.6 ppt. miBP was present mostly in the southern part of the Delta, Maun, and Lake Ngami. miBP is shown in Figure 7 and the miBP concentrations are listed in Table 5.

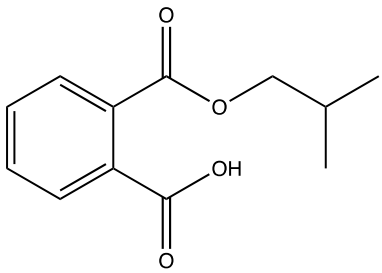


Figure 7: miBP

Table 5: Concentration of 22 detected samples for miBP. All values are in ng/L. All Values are corrected for concentration factor. The LOD has been subtracted to give real values shown. Locations are concentrated in the southern part of the Delta, Maun and Lake Ngami.

| Location | Concentration miBP | Location | Concentration miBP |
|---|---|---|---|
| Panhandle | 1.9 | Chiefs Island | 2.8 |
| Panhandle | 2.1 | Chiefs Island | 2.1 |
| Panhandle | 2.6 | Maun | 141.1 |
| Chiefs Island | 47.1 | Maun | 42.6 |
| Chiefs Island | 46.2 | Maun | 68.1 |
| Chiefs Island | 34.2 | Maun | 85.4 |
| Chiefs Island | 2.1 | Maun | 50.8 |
| Chiefs Island | 5.7 | Maun | 2.3 |
| Chiefs Island | 7.0 | Lake Ngami | 14.2 |
| Chiefs Island | 3.4 | Lake Ngami | 78.7 |
| Chiefs Island | 3.0 | Lake Ngami | 95.0 |

## 3.4 mBP

mBP was detected above the background levels of 2.5 ppt with a LOD of 21.3 ppt in 19 of the 46 samples. Values range from 3.3 – 781.3 ppt with an average among detected samples of 223.1 ppt. Most of the samples site that show mBP also show miBP, especially the sites with high concentrations. mBP is shown in Figure 8 and the mBP concentrations are listed in Table 6.

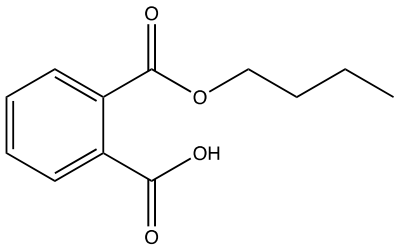


Figure 8: mBP

Table 6: Concentrations of 19 samples that detected mBP. All values are in ng/L. All values are corrected for the proper concentration factor. The LOD has been subtracted to give real values shown. Most sample sites that detect mBP also detect miBP (Maun, Lake Ngami, and the Southern area of the Delta.).

| Location | Concentration mBP | Location | Concentration mBP |
|---|---|---|---|
| Panhandle | 19.9 | Maun | 781.3 |
| Panhandle | 38.7 | Maun | 262.5 |
| Panhandle | 20.4 | Maun | 397.3 |
| Chiefs Island | 289.7 | Maun | 19.3 |
| Chiefs Island | 367.0 | Maun | 621.5 |
| Chiefs Island | 31.5 | Maun | 317.7 |
| Chiefs Island | 3.3 | Lake Ngami | 324.7 |
| Chiefs Island | 13.1 | Lake Ngami | 66.0 |
| Chiefs Island | 279.8 | Lake Ngami | 362.0 |
| Chiefs Island | 18.8 | | |

## 3.5 mBzP

mBzP was the only analyte not detected over background levels of 5.1 ppt with a LOD of 5.7 ppt. mBzP is shown in Figure 9.

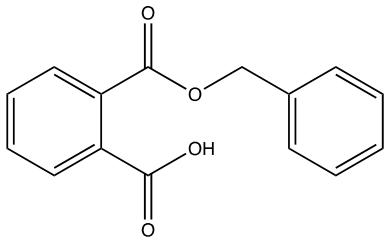


Figure 9: mBzP

## 3.6 mEHP

mEHP was detected above background levels of 39.0 ppt with the LOD of 70.5 ppt in 12 of the 46 samples. The values range from 44.0-356.2 ppt with an average value of 114.2 ppt. The LOD was very high for this analyte because of high variation in the method blanks. However, the values listed here are well above the LOD. mEHP is shown in Figure 10 and the mEHP concentrations are listed in Table 7.

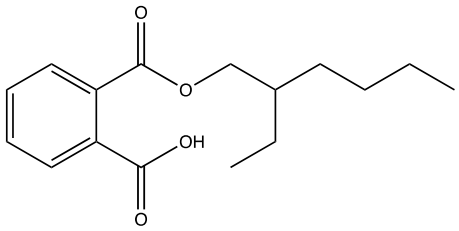


Figure 10: mEHP

Table 7: Twelve samples that contained mEHP. All values are in ng/L. All values are corrected for proper concentration factor. The LOD has been subtracted to give real values shown.

| Location | Concentration mEHP | Location | Concentration mEHP |
|---|---|---|---|
| Panhandle | 60.6 | Chiefs Island | 356.2 |
| Panhandle | 112.5 | Chiefs Island | 64.9 |
| Panhandle | 44.0 | Chiefs Island | 80.4 |
| Panhandle | 62.8 | Chiefs Island | 152.4 |
| Panhandle | 107.0 | Maun | 76.1 |
| Chiefs Island | 161.1 | Lake Ngami | 92.7 |

## 3.7 mEOHP

mEOHP is an oxidized metabolite of mEHP and represents a metabolized phthalate. 33 of the 46 samples show mEOHP present above background levels of 0.2 ppt with a LOD of 0.45 ppb. Value ranged from <1 - 29.3 ppt with an average value among detected samples as 3.3 ppt. Detected samples came from all sample locations and indicate mEOHP present throughout the Delta. mEOHP is shown in Figure 11 and the mEOHP concentrations in each sample are listed in Table 8.

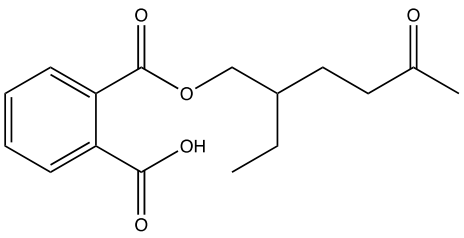


Figure 11: mEOHP

Table 8: Concentrations of 33 detected samples for mEOHP. Concentrations are in ng/L. mEOHP is present throughout the Delta. All values are corrected for the proper concentration factor. The LOD has been subtracted to give real values shown

| **Location** | **Concentration mEOHP** | **Location** | **Concentration mEOHP** |
|---|---|---|---|
| Panhandle | 3.1 | Chiefs Island | 0.4 |
| Panhandle | 1.0 | Chiefs Island | 10.6 |
| Panhandle | 0.6 | Chiefs Island | 8.8 |
| Panhandle | 4.6 | Chiefs Island | 0.7 |
| Panhandle | 0.8 | Chiefs Island | 0.5 |
| Panhandle | 3.5 | Chiefs Island | 2.0 |
| Panhandle | 1.7 | Chiefs Island | 2.1 |
| Panhandle | 3.3 | Maun | 4.0 |
| Panhandle | 1.0 | Maun | 0.3 |
| Panhandle | 29.1 | Maun | 29.3 |
| Panhandle | 1.0 | Maun | 0.7 |
| Panhandle | 1.2 | Maun | 0.2 |
| Panhandle | 1.1 | Maun | 1.6 |
| Chiefs Island | 9.3 | Maun | 1.3 |
| Chiefs Island | 1.7 | Maun | 1.1 |
| Chiefs Island | 0.8 | Lake Ngami | 0.5 |
| Chiefs Island | 4.7 | | |

## 3.8 mEHHP

mEHHP was found above background levels of 1.9 ppt with a LOD of 0.12 ppt. in 41 of the 46 samples. Concentrations ranged from 2.0-9.6 ppt with an average of 3.4 ppt among detected samples. mEHHP is another metabolite of mEHP and is found at similar concentrations, and abundance, as mEOHP. This is logical as both are equally likely routes of metabolism for monoester phthalates by oxidative enzymes. mEHHP is shown in Figure 12 and the mEHHP concentrations are listed in Table 9.

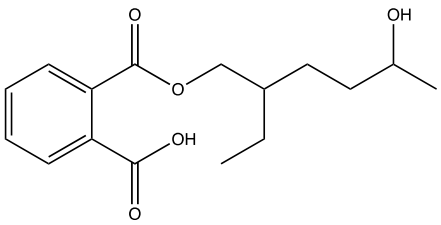


Figure 12: mEHHP

Table 9: concentrations of 41 samples that contained mEHHP. All concentrations are in ng/L. Values are corrected for proper concentration factors. mEHHP and mEOHP have similar abundance and concentrations. The LOD has been subtracted to give real values shown.

| Location | Concentration mEHHP | Location | Concentration mEHHP |
|---|---|---|---|
| Panhandle | 2.8 | Chiefs Island | 2.1 |
| Panhandle | 2.6 | Chiefs Island | 5.6 |
| Panhandle | 2.4 | Chiefs Island | 2.4 |
| Panhandle | 2.3 | Chiefs Island | 9.7 |
| Panhandle | 2.9 | Chiefs Island | 2.0 |
| Panhandle | 5.2 | Chiefs Island | 9.6 |
| Panhandle | 2.0 | Chiefs Island | 2.3 |
| Panhandle | 2.4 | Chiefs Island | 2.6 |
| Panhandle | 3.0 | Chiefs Island | 4.1 |
| Panhandle | 3.8 | Chiefs Island | 3.1 |
| Panhandle | 4.8 | Maun | 3.2 |
| Panhandle | 3.1 | Maun | 2.0 |
| Panhandle | 3.2 | Maun | 2.4 |
| Panhandle | 3.1 | Maun | 5.9 |
| Panhandle | 4.0 | Maun | 2.4 |
| Panhandle | 2.9 | Maun | 3.1 |
| Panhandle | 3.1 | Maun | 2.6 |
| Chiefs Island | 2.1 | Maun | 2.7 |
| Chiefs Island | 4.1 | Maun | 2.7 |
| Chiefs Island | 2.0 | Lake Ngami | 2.7 |
| Chiefs Island | 5.1 | | |

## 4. Locations

All sampling locations tested positive for the seven analytes listed below. Highest response was at Lake Ngami and in and around the city of Maun. It should be noted that the metabolites mEHHP and mEOHP were found across all sampling locations with the highest average response from those samples. The values listed in Table 11 are the percent of samples that had a positive response from samples taken at that location.

Table 10: Values listed below are the percent of samples taken from that location that had a positive response for the corresponding analyte.

| Analyte | Maun | Panhandle | Chiefs Island | Lake Ngami |
|---|---|---|---|---|
| mMp | 36.4% | 5.5% | 21.4% | 66.6% |
| mEP | 36.4% | 5.5% | 14.2% | 66.6% |
| miBP | 54.5% | 16.7% | 71.4% | 100% |
| mBP | 54.5% | 16.7% | 50.0% | 100% |
| mEHP | 9.1% | 27.8% | 35.7% | 33.3% |
| mEOHP | 72.7% | 72.2% | 78.6% | 33.3% |
| mEHHP | 81.8% | 94.4% | 100% | 33.3% |

## 4. Discussion

Levels of phthalate monoesters were measured in a river in Tokyo between 1999 and 2000[15]. The results from that study are shown beside the results from this study for comparative purposes. Interestingly levels of four phthalate monoesters, mEP,miBP,mBP, and mEHP, were equivalent or higher than the results from a river in Tokyo. The remoteness of the Okavango Delta as compared to Tokyo should show significantly less monoester phthalates in the water. This is not the case, and is concerning since phthalates are an indicator of the reach of human contamination. The comparative results are shown in **Table 11.**

Table 11: Comparison monoester phthalate level in the Okavango Delta to a River in Tokyo. All concentrations are listed in ηg/L.

| Phthalate | LOD | % Det. | Avg. | Max. |
|---|---|---|---|---|
| mMP | 1.3 | 22% | 7 | 40 |
| mEP | 93.0 | 20% | 2700 | 16000 |
| miBP | 0.9 | 48% | 34 | 140 |
| mBP | 21.0 | 41% | 22 | 780 |
| mEHP | 71.0 | 26% | 110 | 360 |
| mBzP | 5.7 | 0% | ND | ND |

| Phthalate | LOD | Site 1 | Site 2 | Site 3 |
|---|---|---|---|---|
| mMP | 30 | ND-70 | ND-170 | ND-190 |
| mEP | 14 | ND | ND | ND |
| miBP | 12 | ND | ND | ND |
| mBP | 10 | ND-50 | ND-150 | 12-100 |
| mEHP | 10 | ND-120 | ND-120 | 23-48 |
| mBzP | 13 | ND | ND | ND |

Seven monoester phthalates (mMP, mEP, mBP, miBP, mEOHP, mEHHP, mEHP) were identified in the Okavango Delta from low ppt to low ppb concentrations. The specific sources of this contamination is unknown, but this suggests increasing anthropogenic activity. Monoester phthalates are active in combination, so sample sites with detections of multiple analytes will have higher activity on the ecosystem[5]. The oxidized metabolites mEOHP and mEHHP have very similar concentrations and locations around the Delta, this indicates metabolism of DEHP in wildlife, hydrolysis, or bacterial activity across the Delta. mEOHP has an average among detected samples of 3.3 ng/L and mEHHP has an average of 3.4 ng/L. Both analytes have common detected sample sites and were detected across all sample areas. mBP and miBP are known to be present in pesticide formulas and the detection of these compounds suggest contamination from pesticide use. mEP was found at the highest concentrations but with the fewest number of detection due to a high LOD. Many people wash themselves in the Delta, and with such an arid climate, people may use a lot of moisturizers which would wash off into the water. Interestingly, mEP was found in populated areas and remote areas as well. Future work will include analyzing 50 new samples that were obtained by the author over the summer of 2017. The Brock research group will analyze these samples for monoester phthalates and 11 over the counter medications using a LC/MS/MS method that is in development. This will show changes in concentration over time and what we can expect for the future of the Okavango Delta.

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