

Use of the Marine Prophage Induction Assay (MPIA) to Detect Environmental Mutagens

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ABSTRACT: The prophage induction assay provides a biologically based carcinogen-screening tool for environmental samples grounded in the parallel mechanisms of carcinogenesis and prophage induction. We developed an assay using a previously characterized marine bacterial *Pseudomonas aeruginosa* isolate designated as P94-4S3 for the detection of potentially genotoxic contamination in marine and estuarine environments. To perform the assay, the lysogenic isolate was exposed to either a known genotoxic compound or an environmental sample of interest. The response was considered positive when a statistically significant amount of prophage induction occurred in comparison to negative controls. Initial development of the assay for environmental samples included testing under a range of salinities and optimizing the method for the processing of water column and sediment samples. The assay has been field-tested over 2 yr in the Rookery Bay National Estuarine Research Reserve, Florida. The Marine Prophage Induction Assay (MPIA) was performed concurrently with laboratory toxicological analysis. There was good correspondence between positive MPIA results and detection of potentially toxic compounds by laboratory analysis. Five positive laboratory detections of known toxic compounds in natural samples occurred in conjunction with positive MPIA results. Two laboratory detections of compounds that are not genotoxic were accompanied by a negative MPIA response. Eight of the sediment samples contained detectable levels of arsenic. Four of these samples demonstrated a positive MPIA response, which may be due to the oxidation state of the arsenic within the sediment. One detection of a known toxic compound by the analytical laboratory was not accompanied by a positive induction response. Nine positive induction responses occurred without concurrent laboratory detection. This was possibly due to the limited range of compounds included in the laboratory testing performed, although false positive assay results cannot be ruled out.

Introduction

Many toxic compounds are released into the environment as a result of human activity. Estuarine environments are particularly susceptible to inputs of anthropogenic compounds from multiple non-point sources including roads, river and canal drainages, agriculture, and land development. Mutagenic compounds are of the highest concern because of their potential to harm both humans and ecosystems in general. It has been observed that known mutagenic compounds and mixtures are found in both soil and aquatic environments in concentrations high enough to be a health hazard (Cerna et al. 1996). There is an ever increasing need for mutagenesis assays to screen samples containing such compounds for potential carcinogenicity (DeMarini and Brooks 1992).

Standard laboratory toxicological testing can be used to detect specific hazardous substances in environmental samples including mutagens, but there are limitations with such an approach.

Toxicological testing detects only the specific agents assayed, requiring a prior knowledge of what compounds should be monitored. Environmental pollutants tend to be complex mixtures with synergistic biological activities that are not easily predicted by chemical profiles (DeMarini et al. 1989). Biological testing enables detection of the combined effects of compounds, unknown substances, degradation products, and metabolites and their synergistic or antagonistic effects, which are difficult to predict by toxicological testing (Helma et al. 1996). Such an approach can serve as a prescreening or a routine method for environmental monitoring. When a positive response occurs, more intensive toxicological analysis can serve to identify the causative agent and hopefully the source of the contamination.

Many of the commonly used biological tests were developed based on the strong demonstrated relationship between mutagenicity in bacteria and carcinogenicity of a compound (Ames et al. 1973). The Ames test (Ames et al. 1973) and the Rec-assay using *Bacillus subtilis* (Kada et al. 1972) were developed as bacterial-based reverse mutagenicity assays, which provided alternatives to carcinogeni-

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city testing with animals. These tests were an improvement over in vivo testing but still require lengthy incubations and complicated procedures. A lack of correlation was also demonstrated between carcinogenicity and a positive Ames test in a screening performed with 133 known carcinogenic compounds (Rossman et al. 1991).

The prophage induction assay is another alternative for biological-based carcinogen screening due to the close parallel that has been demonstrated between the mechanisms of carcinogenesis and prophage induction (Ho and Ho 1979). Prophage induction was initially identified as a superior biological basis for testing because it responds to a wide variety of antimetabolites, not just direct DNA damage (Elespuru and White 1983). One of the earliest attempts to use prophage induction as an indicator for the detection of carcinogens was performed with the *Escherichia coli*/ λ system (Thompson and Woods 1975).

The Microscreen Assay, originally termed the Inductest, also uses this principle (Moreau et al. 1976). The Microscreen Assay has proven to be a more sensitive detection method for carcinogens than the Ames test (Rossman et al. 1991). This assay uses the *E. coli*/ λ phage system and has been used to investigate the mutagenic potential of therapeutic agents (Cabrera 2000; Akerele and Obaseiki-Ebor 2002). The assay has also been adapted for use in examination of river ecosystems (Vargas et al. 1995), but it may not be directly compatible with marine samples (water and sediments), due to the optimal salinity range for the growth of *E. coli*. The Marine Prophage Induction Assay (MPIA), a similar concept to the Microscreen Assay, was developed specifically for detection of mutagens in the marine environment using a marine temperate phage-host system (McDaniel et al. 2001). During initial testing the MPIA demonstrated significant positive prophage induction response to several environmentally relevant pollutants including pesticides, polychlorinated biphenyls (PCBs) and polyaromatic hydrocarbons (PAHs).

Although the MPIA was developed similarly to the Microscreen Assay, there are some procedural differences. The Microscreen Assay uses the agar overlay technique to enumerate the number of plaque forming units as a means to estimate the level of prophage induction (Adams 1959). This technique is accurate, but it is both time consuming and requires an additional uninfected host. Another disadvantage of this technique is the lack of reproducibility during use in Microscreen Assays (DeMarini and Brooks 1992). This was thought to be due to the direct toxic effect of the putative carcinogen on the viral particles, rendering some of the liberated viral particles as noninfective.

Another standard technique for viral enumeration is counting by transmission electron microscopy (TEM). This method has several drawbacks including expensive equipment, intensive time-consuming sample preparation, as well as the inability to use this technique for work in the field (Noble and Fuhrman 1998). A comparison of TEM methods with newer techniques using epifluorescence microscopy showed that the TEM methods provided significantly lower precision than epifluorescence techniques (Bettarel et al. 2000).

Techniques for viral enumeration using epifluorescence microscopy include the use of various nucleic acid staining methods. Commonly used fluorochromes include DAPI, YOPRO-1, and SYBR Green I (Noble and Fuhrman 1998; Bettarel et al. 2000). A newer SYBR stain termed SYBR Gold has been developed, which yields a much brighter longer-lasting fluorescence signal (Chen et al. 2001). It is possible that epifluorescence microscopy using the SYBR Gold staining technique is ideal for enumerating viruses in the marine environment. The SYBR Gold stain has been observed to stain both RNA and DNA viruses, but not detritus (Noble 2001). For these reasons the MPIA can be developed using epifluorescence microscopy and SYBR Gold staining to directly enumerate the number of virus-like particles (VLPs) per milliliter.

The first goal of this study was to develop a protocol for performing the assay on natural samples and to calibrate the assay results using standard toxicological testing and a known contaminated sampling site. The second goal of this study was to use the field trial and laboratory data to determine whether or not the MPIA would be a useful screening tool for environmental water column and sediment samples in an estuarine environment.

Materials and Methods

STUDY AREA AND SAMPLING SITES

Rookery Bay National Estuarine Research Reserve is an 110,000-acre marine wetland reserve on the southern edge of the growing metropolis of Naples, Florida, and adjacent to the Florida Everglades. In addition to providing nearby Naples residents with opportunities for boating and fishing, the reserve functions as a much-needed haven of undisturbed estuarine habitat on the densely populated west coast of Florida. The reserve managers are actively pursuing restoration of altered ecosystems and are attempting to restore the natural sheet water flow to the estuary. A main concern of reserve managers continues to be potential inputs of anthropogenic compounds from multiple nonpoint sources including roads, river and canal drainage, and nearby

urban development. A method of screening reserve samples for potentially bioactive contaminants was considered a high priority.

This study was performed from August 2002 through April 2004. Three sampling sites were selected: Rookery Bay (lower Henderson Creek) at 26°01'04" N, 81°44' W, lower Blackwater River at 25°56'01" N, 81°35'07" W, and Fakahatchee Bay at 25°53'05" N, 81°28'06" W.

Each of the three sites was selected based on amount of nearby development and the presence of existing water quality monitoring stations. These water quality stations had continuous water quality data monitors that were used in determining if existing water quality conditions had any effect on assay results. The Fakahatchee Bay site was the least affected by ongoing development and was considered a reference site. The Blackwater River site was considered mildly to moderately influenced by development and nearby agriculture. The Rookery Bay site was considered most likely to be contaminated due to runoff from nearby roads and construction sites. The Naples Bay site, which is located next to a busy marina was also considered a potentially contaminated site and was sampled once at the conclusion of the study.

Environmental positive control sites, Sweetwater River (27°57' N, 82°32' W) and Bullfrog Creek (27°50' N, 82°32' W), were selected based on known heavy contamination from the previously conducted Tampa Bay Healthy Beaches study (Rose et al. 2000). Both sites were found to have heavy levels of bacterial contamination from nearby wastewater treatment facilities and agriculture. Potential inputs of mutagenic compounds were also suspected.

MPIA PROCEDURE

The MPIA was performed using a technique similar to one previously described for the Microscreen Assay with some modifications (Houk and DeMarini 1988; DeMarini et al. 1990). The *Pseudomonas aeruginosa* strain and the protocol used for the assay have been previously described (McDaniel et al. 2001). In preparation for performing the assay, one colony of the bacteria was inoculated into 1 ml of growth media in triplicate and allowed to incubate overnight on a rotary shaker at 28°C. This culture was used to inoculate 30 ml of growth media and returned to the shaker incubator. Growth of the cultures was monitored by absorbance at 600 nm.

A 1:2 dilution series using 100 µl of the water sample and 100 µl of growth media was prepared in a 96-well microtiter plate for each sample to be tested as well as a positive and negative control plate followed by the addition of 75 µl of the bacterial isolate in the logarithmic phase of growth. The

positive control plate contained a dilution series of the potent mutagenic compound Mitomycin C from 0.0625 to 0.5 µg ml⁻¹. The negative controls consisted of a similarly prepared plate using sterile artificial seawater instead of sample. The growth medium was sterile ASWJP (artificial saltwater supplemented with peptone and yeast extracts).

If the assay was performed on a known compound for verification of an experimental result, the plates were prepared similarly using an appropriate non-toxic solvent (i.e., acetone or methanol). The solvents were obtained from Fisher (Pittsburgh, Pennsylvania) and were guaranteed to be 99.5% pure solvent (contaminant free). In this case a negative control plate was also prepared similarly using only diluted solvent. All comparisons between treatment and controls were made between plates with the corresponding negative or solvent negative control with the same dilution factor or percentage of solvent (Houk and DeMarini 1987). No positive induction responses were obtained from solvent controls (data not shown).

ENUMERATION OF VIRUSES

The VLPs were enumerated using SYBR Gold nucleic acid stain (Molecular Probes, Eugene, Oregon), with epifluorescence microscopy using an Olympus BX-60 epifluorescence microscope with a 100× objective and blue excitation essentially as described by Noble and Fuhrman (1998) with the substitution of SYBR Gold stain for SYBR Green.

PROCEDURES FOR ENVIRONMENTAL SAMPLES

The Rookery Bay Reserve has widely varying levels of salinity depending on the amount of freshwater input to differing sites. The assay was performed as described above on artificial seawater control samples with three differing salinities ranging from normal seawater salinity decreasing to freshwater level (35, 26, and 0 psu). The level of VLPs was compared between samples using SYBR Gold staining as above.

Adapting the assay protocol to the environment began with samples from the water column. It has previously been observed with environmental samples that microbial contamination would need to be removed by filtration (Houk and DeMarini 1988; McDaniel et al. 2001). The assay was initially performed using the Rookery Bay Reserve seawater samples, which were prefiltered either to remove natural bacteria (0.2 µm filtration) or to remove both bacteria and viruses (0.02 µm filtration) before being used in the assay. This step was completed to determine which method would give adequate sensitivity without interference from natural bacteria and viruses. The virus-free (0.02 µm filtration)

technique was selected for use in all further MPIA testing.

Duplicate water samples were obtained simultaneously and sent on ice to the Florida Department of Environmental Protection (DEP) Central Laboratory for toxicological screening (2600 Blair Stone Road, Tallahassee, Florida 32399 -2400). The first three field samplings included testing for pesticides and heavy metals. Due to positive MPIA results without possible pollutant detection, the subsequent sample testing was expanded to assay for priority organic pollutants, including PCBs and PAHs. Subsequent pesticide analysis included more comprehensive assays that included organochlorine, organonitrogen, and phosphorus pesticides, as well as testing for herbicides. This expanded range was used for all subsequent water column and sediment samples.

The assay was adapted for use with sediment samples using a protocol similar to the previously performed study of river sediment samples using the Microscreen Assay (Vargas et al. 2001). This prior study compared various solvent extraction methods on the sediments and determined that the samples were best left untreated to prevent any loss of genotoxic compounds (Vargas et al. 1995).

Sediment samples were obtained at each site using a sediment push corer. The first few centimeters of sediment were used to sample only the most recently deposited contaminants. The samples were transported on ice to the laboratory and processed within 24 h. Identical samples for toxicological screening were also placed on ice and sent with the corresponding water column samples to the DEP laboratory for processing as previously described. The MPIA sediment samples were thoroughly mixed to homogenize the sediments and then centrifuged at 10,000 g for 10 min to extract the pore water. The pore water was 0.02 μm filtered and used in the MPIA as above.

Environmental contaminants that were detected by toxicological screening were verified as probable causes of a positive MPIA response by being tested individually in the assay using an appropriate dilution solvent (i.e., acetone or methanol), which had previously demonstrated to be nontoxic to the bacterial isolate used for the assay.

STATISTICAL ANALYSES

Two general types of statistical analysis were conducted. Control viral counts and MPIA sample counts were evaluated by paired *t*-test between samples, and replicate Naples Bay samples were compared by analysis of variance using Minitab statistical software (Minitab, Inc., State College, Pennsylvania). An induction was considered statistically significant when $p < 0.05$. In addition to

standard statistical testing, multivariate analysis of MPIA induction parameters and measured environmental parameters were conducted using Primer v.5.2.9 software (Primer-E Ltd., Plymouth Marine Laboratory, United Kingdom). The similarity matrices of both the induction parameters and water quality parameters were constructed using normalized Euclidian distances. These matrices were compared to determine if a statistically significant relationship existed between them using the RE-LATE test. This nonparametric method uses the sample statistic ρ , where a perfect correlation would equal one and values may range from zero to one. This protocol also generates a *p* value for determining statistical significance of the correlation.

WATER QUALITY DATA

General water quality indicators, including temperature, salinity, dissolved oxygen, depth, pH, and turbidity, were obtained from YSI model 6600 extended deployment system data sondes permanently moored at the reserve's water quality stations. The data was collected continuously and directly downloaded from the data sonde to a personal computer for outlier analysis by the reserve staff. Data from the sites that corresponded to the time points for the MPIA sampling were used in the statistical analysis.

Results and Discussion

The reserve was relatively free of mutagenic contaminants. The most commonly observed pollutants were the pesticide Hexazinone in water column samples and arsenic in sediment samples. Toxicological testing in the reserve did not reveal any contamination by herbicides and minimal contamination by pesticides. There were no incidents of detected PAHs or PCBs, which were major compounds of concern to reserve managers. The analysis determined a relatively low level of pollution throughout the reserve; some incidences of contamination were observed at all of the field sites, including the supposedly pristine Fakahatchee Bay reference site.

All MPIA samplings were compared to water quality data obtained at the same times and sampling sites. Multivariate analysis of all of the MPIA assay parameters, including percentage change in VLP abundance, control and treatment VLP counts, and whether the MPIA testing was positive or negative, was compared to all water quality parameters to determine if the general water quality condition had any effect on the assay. No correlation was observed between the data sets, indicating that the general water quality did not interfere with assay results.

TABLE 1. Environmental positive control sampling, September 17, 2003. A positive MPIA result is indicated by a statistically significant increase in VLPs in comparison to a negative control ($p < 0.05$). Statistically significant induction response was observed in the Mitomycin C positive controls for all experiments performed. NS = not significant, P = pesticides, M = metals, and Org = priority organic pollutants (PAHs, PCBs).

Sampling Site	Sample Type	Percent Change in VLPs	Significance of MPIA (p value)	Chemical Analyses Performed	Compounds Detected
Bullfrog Creek	Water column	74	NS	P, M, Org.	None
	Sediment	179	0.037 (+)	P, M, Org.	Arsenic, Chlordane
Sweetwater Creek	Water column	592	0.009 (+)	P, M, Org.	Atrazine
	Sediment	502	0.058 (+)	P, M, Org.	Benzoanthracene, Benzopyrene, Benzofluoranthene, Benzoperylene, Chrysene, Chlordane

ENVIRONMENTAL POSITIVE CONTROL SAMPLING

Positive control testing was performed after the protocol was adapted for environmental samples. The sites for sampling were selected due to known high contamination, which was based on an extensive survey of Tampa Bay area watersheds (Rose et al. 2000). The two sites selected were designated as Bullfrog Creek and Sweetwater River. Water column and sediment samples were collected from both sites and tested with the MPIA. Samples for toxicological testing were obtained from the same water and sediments.

At the positive control site excellent correspondence was observed between positive MPIA results and laboratory detection of contaminants (Table 1). Several highly toxic compounds were detected at both sites, including some PAHs, which are known to result in a positive MPIA response (McDaniel et al. 2001).

RESERVE SAMPLING

Testing of the assay was performed to determine the potential effect of varying salinity on assay results. The MPIA was performed with a Mitomycin C positive control and negative controls with the range of salinities as described above. The positive control demonstrated significant induction when compared to the negative controls, regardless of salinity. All of the negative controls were nearly identical, demonstrating no response to varying salinities (data not shown). This insensitivity of the assay to salinity variation was important due to the wide ranges in salinity observed routinely in reserve water samples.

In the first field sampling there was no significant prophage induction response in the samples from the Fakahatchee Bay or Blackwater River sites. A statistically significant positive induction response was detected from the Rookery Bay water column sample. In the second sampling, significant positive MPIA responses were observed at both the Blackwater River and Fakahatchee Bay sites. In both cases the DEP laboratory detected no contaminants. As previously stated, tests conducted on the first

sampling included only a limited range of pesticides plus heavy metals. Because of these initial positive MPIA responses the range of laboratory testing was expanded to include a wider range of pesticides, herbicides, and priority organic pollutants (PAHs, PCBs). The MPIA was not performed on the third reserve sample because the MPIA calibration samples sent to the DEP laboratory for toxicological analysis were lost during shipping.

Beginning in March 2003, with the fifth field trial, sediment samples were also analyzed. It was observed that the sediment samples throughout the reserve were fine grain, rich in organic matter, and generally anaerobic. Anaerobic sediments were easily detected by the odor of hydrogen sulfide (Madigan et al. 1997).

The results of the field testing at Fakahatchee Bay are listed in Table 2. Of the 16 samples analyzed, 4 (25%) positive MPIA responses and 3 (19%) laboratory detections were observed. In the water column, 2 positive MPIA responses were observed, which were not accompanied by a laboratory detection of a contaminant. At the Fakahatchee Bay site the only laboratory-detected contaminant was arsenic, and it was only found in the sediment samples. The MPIA response to the arsenic in the sediments was inconsistent. In 2 of the samples the arsenic was accompanied by a positive MPIA response, and in 1 case by a negative MPIA response. Under reducing conditions such as within anaerobic sediments, arsenic would have been in the reduced form arsenite, which spontaneously combines with hydrogen sulfide to form a nontoxic precipitate (Madigan et al. 1997). The intermittent positive responses of the MPIA to arsenic in the sediments indicate that disturbances of the sediment can sometimes lead to oxidation of deposited, reduced arsenic contaminants. This has implications for reserve managers because perturbations of the sediments due to dredging or restoration activities could lead to release of bioactive (oxidized) arsenic.

Independent testing of the MPIA verified that the MPIA isolate is highly sensitive to the oxidized form

TABLE 2. MPIA results from Fakahatchee Bay. Statistically significant induction response was observed in the Mitomycin C positive controls for all experiments performed. NS = not significant, P = pesticides, M = metals, Org = priority organic pollutants (PAHs, PCBs), and Herb = herbicides.

Sample Number	Date	Sample Type	Percentage Change in VLPs	Significance of MPIA (p value)	Chemical Analyses Performed	Compounds Detected
1	August 5, 2002	Water column	165	NS	P, M	None
2	October 25, 2002	Water column	2,944	0.03 (+)	P, M	None
3	December 19, 2002	Water column	N/A	Not Done	M (other samples lost)	No metals detected
4	January 23, 2003	Water column	123	NS	P, M, Org, Herb	None
5	March 20, 2003	Water column	-42	NS	P, M, Org, Herb	None
		Sediment	3	NS	P, M, Org, Herb	Arsenic
6	May 6, 2003	Water column	-75	NS	P, M, Org, Herb	None
		Sediment	-63	NS	P, M, Org, Herb	None
7	June 24, 2003	Water column	-17	NS	P, M, Org, Herb	None
		Sediment	-50	NS	P, M, Org, Herb	None
8	August 11, 2003	Water column	148	0.049 (+)	P, M, Org, Herb	None
		Sediment	230	0.024 (+)	P, M, Org, Herb	Arsenic
9	October 13, 2003	Water column	68	NS	P, M, Org, Herb	None
		Sediment	1,849	0.027 (+)	P, M, Org, Herb	Arsenic
10	November 10, 2003	Water column	104	0.035	P, M, Org, Herb	None
		Sediment	60	NS	P, M, Org, Herb	None

of arsenic (Fig. 1). The response of the assay organism to 1 mg l^{-1} of arsenate (KH_2AsO_4) was higher than to the positive control Mitomycin C ($0.5 \mu\text{g ml}^{-1}$). The level of arsenate used for comparison was roughly comparable to arsenic levels found in the reserve ($3.1\text{--}6.7 \text{ mg kg}^{-1}$). It was also observed that the level of induction decreased with increasing concentration of arsenic, which indicated increasing toxicity. The MPIA test organism was also observed to be nonturbid (killed) in the well of the microtiter plate at these higher

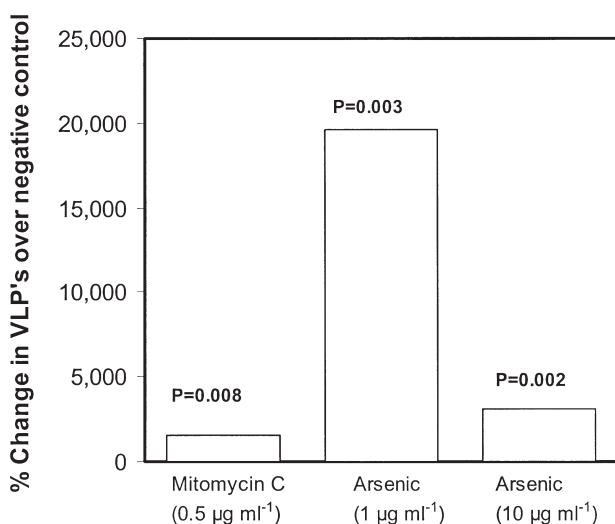


Fig. 1. The response of the MPIA to arsenate. Note that the response is much higher than the positive control Mitomycin C. At higher doses of arsenic the induction response decreases due to toxicity.

concentrations, which provided additional confirmation of the toxicity of arsenate. A similar phenomenon has been observed with higher concentrations of Mitomycin C. The fact that the response to the sediment samples containing arsenic was mild to negative supports the contention that the arsenic in these samples was in its reduced or partially oxidized form.

The results of the Blackwater River sampling are presented in Table 3. At this site the pesticide Hexazinone was observed in water column samples on two occasions, both in conjunction with a positive MPIA response. The occurrence of a pesticide at this site is consistent with the close proximity of agricultural areas. The presence of a pesticide in conjunction with a positive MPIA response indicates that agricultural runoff at this site has the potential to be harmful to the local biota.

The compound Bis-phthalate was also observed at this site, accompanied by a negative MPIA response, which is consistent with the low toxicity of this chemical. Phthalates are common plasticizing agents widely used in many industries and are commonly observed environmental contaminants (USEPA 1987). These compounds are known to be irritants but are not toxins or mutagens, and they can be isolated from naturally occurring fungi (Windholz et al. 1976).

Similarly to the Fakahatchee Bay site, arsenic was detected in the sediments on 3 occasions. The inconsistent response of the MPIA to arsenic at this site was also similar, as previously discussed. The MPIA showed a positive response to the arsenic contaminants on 2 of the 3 sediment samples. There were also 2 positive MPIA results that were

TABLE 3. MPIA results from Blackwater River. Statistically significant induction response was observed in the Mitomycin C positive controls for all experiments performed. NS = not significant, P = pesticides, M = metals, Org = priority organic pollutants (PAHs, PCBs), and Herb = herbicides.

Sample Number	Date	Sample Type	Percentage Change in VLPs	Significance of MPIA (p value)	Chemical Analyses Performed	Compounds Detected
1	August 5, 2002	Water column	184	NS	P, M	None
2	October 25, 2002	Water column	192	0.007 (+)	P, M	None
3	December 19, 2002	Water column	N/A	Not Done	M (other samples lost)	No metals detected
4	January 23, 2003	Water column	109	0.017 (+)	P, M, Org, Herb	None
5	March 20, 2003	Water column	-36	NS	P, M, Org, Herb	None
		Sediment	998	0.078 (+)	P, M, Org, Herb	Arsenic
6	May 6, 2003	Water column	-39	NS	P, M, Org, Herb	None
		Sediment	-76	NS	P, M, Org, Herb	Bis(e-ethylhexyl) phthalate
7	June 24, 2003	Water column	159	NS	P, M, Org, Herb	None
		Sediment	130	NS	P, M, Org, Herb	None
8	August 11, 2003	Water column	297	0.024 (+)	P, M, Org, Herb	Hexazinone
		Sediment	5	NS	P, M, Org, Herb	Arsenic
9	October 13, 2003	Water column	130	NS	P, M, Org, Herb	None
		Sediment	-0.4	NS	P, M, Org, Herb	Arsenic
10	November 10, 2003	Water column	1,851	0.004 (+)	P, M, Org, Herb	Hexazinone
		Sediment	82	NS	P, M, Org, Herb	None

not associated with a laboratory compound detection at this site.

The results of the Rookery Bay sampling are shown in Table 4. The results at this site are generally similar to the other sites. There was one laboratory detection of Hexazinone in the water column that did not result in a statistically significant positive MPIA (9th sampling). The percentage increase in VLPs in this sample was relatively high at 237%. The MPIA response was not statistically significant because of high variability in the viral counts. This result indicates that when performing the MPIA, a large increase in viruses in the environmental sample should be investigated further as a potential positive response.

The MPIA demonstrated the same variable response to arsenic in the sediments at the Rookery Bay site. Two of the sediment samples at this site contained arsenic, and 1 of these had a positive MPIA response. Similarly to the Blackwater River site, a phthalate compound was detected on one occasion, in conjunction with a negative MPIA result. At this site, 4 positive MPIA results were not associated with a laboratory detection of any compound.

At all 3 reserve sites 9 positive induction responses have occurred without concurrent toxic contaminant detection. This is likely due to the limited range of laboratory testing that is possible to perform, although false positive MPIA results

TABLE 4. MPIA results from Rookery Bay. Statistically significant induction response was observed in the Mitomycin C positive controls for all experiments performed. NS = not significant, P = pesticides, M = metals, Org = priority organic pollutants (PAHs, PCBs), and Herb = herbicides.

Sample Number	Date	Sample Type	Percentage Change in VLPs	Significance of MPIA (p value)	Chemical Analyses Performed	Compounds Detected
1	August 5, 2002	Water column	274	0.003 (+)	P, M	None
2	October 25, 2002	Water column	207	NS	P, M	None
3	December 19, 2002	Water column	N/A	Not Done	M (other samples lost)	No metals detected
4	January 23, 2003	Water column	117	0.024 (+)	P, M, Org, Herb	None
5	March 20, 2003	Water column	-50	NS	P, M, Org, Herb	None
		Sediment	132	NS	P, M, Org, Herb	None
6	May 6, 2003	Water column	-21	NS	P, M, Org, Herb	None
		Sediment	-55	NS	P, M, Org, Herb	None
7	June 23, 2003	Water column	-6	NS	P, M, Org, Herb	None
		Sediment	139	NS	P, M, Org, Herb	Diethyl phthalate
8	July 22, 2003	Water column	65	NS	P, M, Org, Herb	None
		Sediment	11	NS	P, M, Org, Herb	Arsenic
9	October 13, 2003	Water column	237	NS	P, M, Org, Herb	Hexazinone
		Sediment	2,920	0.001 (+)	P, M, Org, Herb	Arsenic
10	November 10, 2003	Water column	678	0.028 (+)	P, M, Org, Herb	None
		Sediment	119	0.018 (+)	P, M, Org, Herb	None

cannot be ruled out. In contrast, false negative results were rare. As mentioned earlier the only occasion a potentially bioactive toxin was not detected by the MPIA was 1 sample containing Hexazinone.

One potentially contaminated Naples Bay site outside the reserve boundary was evaluated using MPIA alone. This site was located at a water quality station on a busy waterway in Naples Bay adjacent to Naples City Dock (Naples Bay water quality station). The area is characterized by heavy boat traffic and potential contamination from fuels, oils, and boat toilet discharges.

At the Naples Bay site, all of the water column samples demonstrated a statistically significant positive MPIA response with an average percentage change in VLP abundance of 227%. Two of the three sediment samples were also positive with an average percentage change in VLP abundance of 244%. These results suggest that it is likely some type of bioactive contaminant is present at this site. It was also observed that there was a statistically significant level of variability within this single set of samples ($p < 0.05$). Water column samples were less variable than the sediment samples from the single sampling. This was expected, due to the limited diffusion of compounds within sediments. Because of the variability within samples, multiple samples should be screened from a single site, similarly to the sampling regime used for the reserve sites in order to obtain a more consistent picture of the level of contamination.

LABORATORY VERIFICATION OF POSITIVE MPIA RESULTS

Field samples tested by the MPIA demonstrated positive results in conjunction with DEP laboratory detection of arsenate and PAHs, to which the assay has a known sensitivity. Positive MPIA field samples were also found to contain the compounds Fenthion, Endosulfan, Atrazine, Chrysene, Chlordane, and Hexazinone. Subsequent MPIA testing with these individual compounds has verified that the MPIA is sensitive to Fenthion, Endosulfan, Atrazine, and Chrysene at levels similar to those detected by the DEP laboratory (data not shown). Testing with Chlordane and Hexazinone are pending.

The MPIA appears to be a good screening tool for detection of bioactive contamination in marine water column and sediment samples. The assay appeared to perform particularly well with heavily contaminated samples. Over a 2-yr period of reserve sampling, the assay demonstrated a positive prophage induction response in conjunction with toxic compounds that were also detected by toxicological testing. The assay failed to show a prophage induction response to samples with detected com-

pounds that were not highly toxic or mutagenic. No interference was observed due to varying salinity or general water quality parameters.

The number of positive MPIA results that were not associated with a detected toxic compound is one potential drawback of this method. It is possible the assay is detecting mutagens and antimetabolites that are not detected by the standard range of toxicological testing. Alternatively, the assay may be susceptible to false positive results, but no false positives have been observed in response to any control or solvent control compounds used in performing the assay. Despite the potential for false positive results, the low cost of the assay in comparison to toxicological testing, makes the MPIA a useful screening tool for determining areas that may contain potential contamination and target them for more detailed examination.

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