Evaluation of 2001 Mussel tissue contaminant threshold exceedance

Massachusetts Water Resources Authority

Environmental Quality Department
Report ENQUAD 2002-05

Citation:

Hunt C.D., S. Abramson, L.F. Lefkovitz, J. Neff, G. Durell, K.E. Keay, M.P. Hall. 2002 **Evaluation of 2001 Mussel tissue contaminant threshold exceedance.** Boston: Massachusetts Water Resources Authority. Report 2002-05. 48 p.

EVALUATION OF 2001 MUSSEL TISSUE CONTAMINANT THRESHOLD EXCEEDANCE

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Contract No. S274
Task Order No. 55 (Amendment 1)

April 16, 2002

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

The Massachusetts Water Resources Authority's (MWRA) long-term Harbor and Outfall Monitoring (HOM) Program (MWRA 1997) for Massachusetts and Cape Cod Bays includes long-term biomonitoring for fish and shellfish. The goal of the biomonitoring is to provide data to assess potential environmental impacts of effluent discharge into Massachusetts Bay. These data are used to ensure that discharge from the new outfall does not result in adverse impacts to fish and shellfish by comparing values with established thresholds (MWRA 2001a). One of the indicator species used in the fish and shellfish monitoring program is the blue mussel *(Mytilus edulis*). To determine the biological condition and short-term accumulation of anthropogenic contaminants in mussel tissue and to evaluate NPDES permit conditions for bioaccumulatable compounds, arrays of mussels are deployed in and recovered from Boston Harbor and the Bays. In 2001, blue mussels *(Mytilus edulis)* were collected from a reference location (Rockport, MA) and deployed in suspended cages at four sites in Boston Harbor and the Bays (Table 1, Figure 1):

- Off Deer Island Light (DI) (~2 m above bottom)
- Outfall Site (OS) – three locations (depth of 10-15 m above bottom, water depth ~ 30 m (MLW)) at varying distances from outfall:
  - Outfall Site "B" Buoy (LNB) – 1000 m from outfall
  - Outfall Site Array 1 (M4- threshold array) – 60 m from outfall
  - Outfall Site Array 3 (4R) – 15 m from outfall
- Boston Inner Harbor (BIH) (1.5 – 4.5m above bottom - Rise and fall with tide, so that it is at a constant depth below the water surface)
- Cape Cod Bay (CCB) (10-15 m above bottom)

Table 1. Planned and Actual Sampling and Locations for Mussel Surveys.

Station #	Station Abbrev.	Sampling Site	Planned Location		Actual Location	
			N Latitude	W Longitude	N Latitude	W Longitude
1M	DI	Deer Island Light	42º20.4'	70º57.2'	42º20.400'	70º57.198'
M4[a]	OS	Outfall Site - Mussel Array 1	42º23.1'	70º49.3'	42º23.209'	70º47.262'
4R	OS	Outfall Site - Mussel Array 3	42º23.1'	70º49.3'	42º23.166'	70º47.680'
LNB	OS	Boston "B" Buoy	42º23.1'	70º49.3'	42º22.674'	70º47.130'
6	BIH	Boston Inner Harbor	42º21.5'	71º02.9'	42º21.500'	71º62.898'
9	CCB	Cape Cod Bay	41E55.5'	70E20.0'	41º54.703'	70º20.139'
RP	Rockport	Rockport – Pre-deployment	42º39.6'	70º35.7'	42º39.660'	70º35.736'

[a]Station M4 is used for compliance with thresholds.

The MWRA Contingency Plan (MWRA 2001a) specifies numerical or quantitative thresholds that may suggest that environmental conditions in the Bay may be changing or might be likely to change. The Plan provides a mechanism to confirm that a threshold has been exceeded, to determine the causes and significance of the event, and to identify the action necessary to return the trigger parameter to a level below the threshold (if the change resulted from effluent discharge). Fish and shellfish thresholds have been established for tissue contaminant concentrations (organic and inorganic) and liver disease incidence (MWRA 2001a, MWRA 2001b).

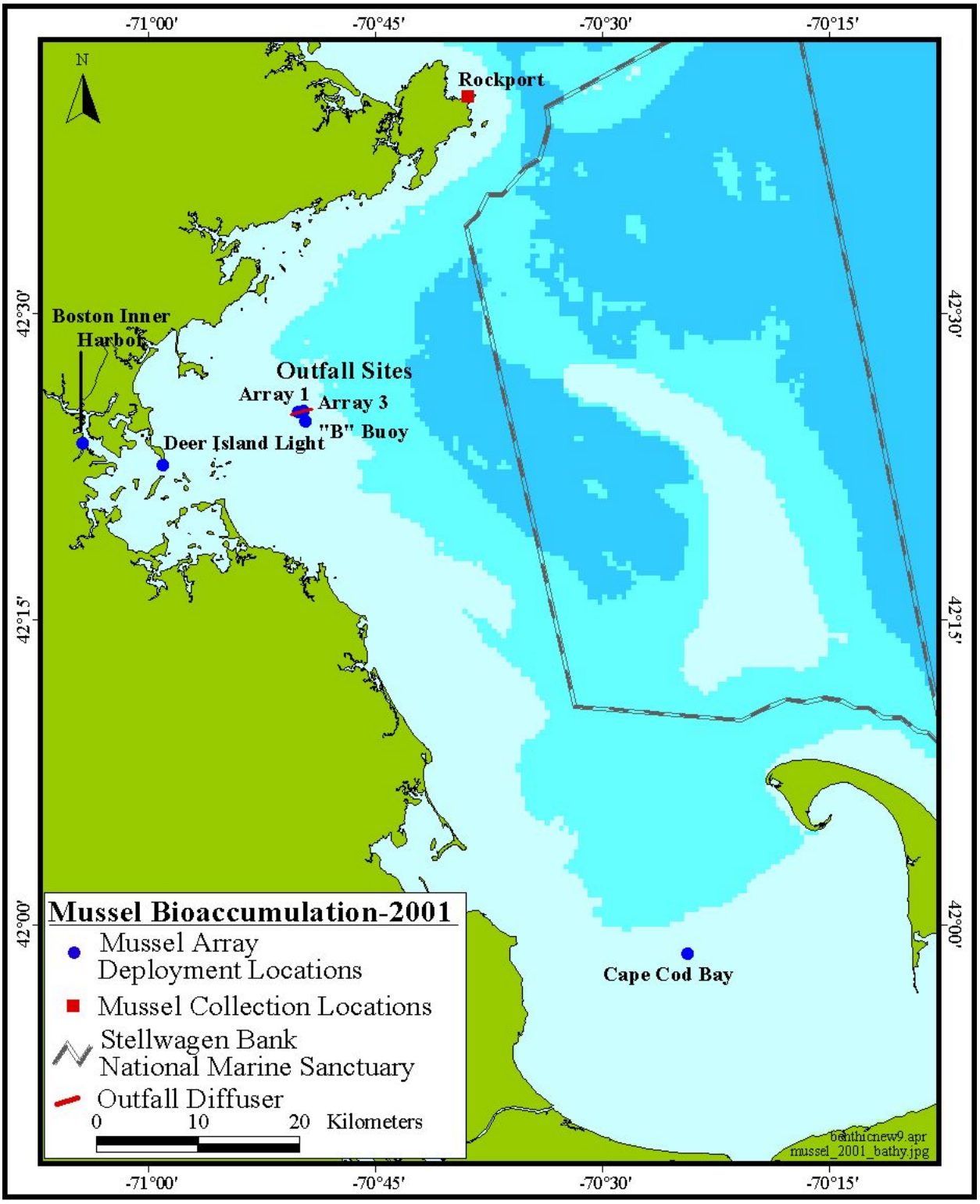

Figure 1. Mussel Collection and Deployment Locations

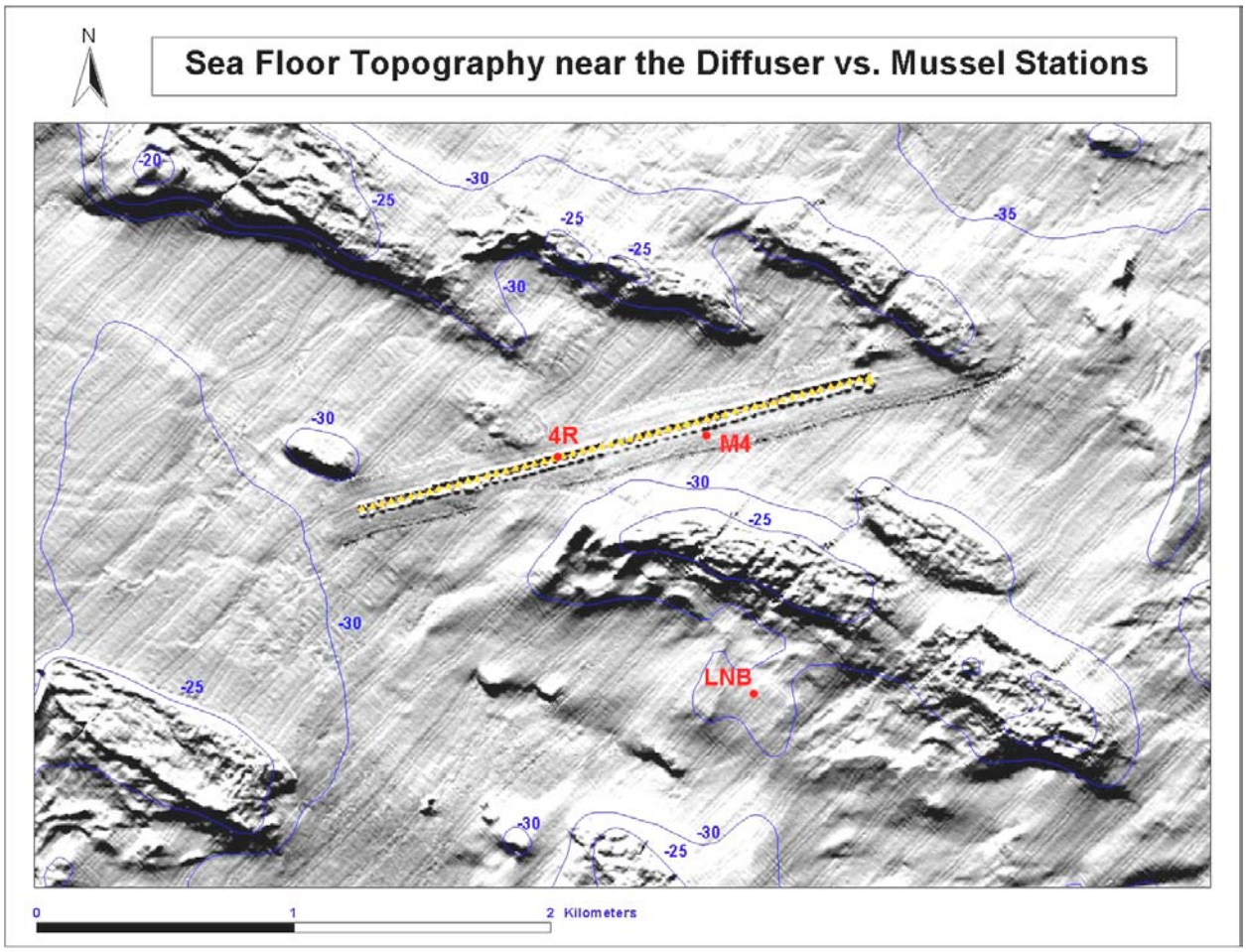

Figure 2. Location of Three Arrays Deployed in the Vicinity of the Outfall

In 2001, the mussels deployed at the Outfall Site exceeded thresholds set for Total PAH (NOAA 24 PAHs) and Total Chlordane (MWRA 2002). These exceedances were unexpected, since the thresholds had been set at a level above any expected change from baseline (Table 2). As a result, an investigative study was conducted to understand why the levels were greater than expected and evaluate possible factors affecting measured contaminant levels in caged mussels. The approach taken was to conduct calculations of expected bioaccumulation using recent science and data from other parts of the MWRA monitoring program and also compare calculated water column PAH and chlordane concentrations (from the measured mussel concentrations) to recent low level organic contaminant analyses (Shea 1997). This report summarizes the methods and approach used to estimate contaminant concentrations in mussel and water and the results of the comparisons. Recommendations for further understanding these factors are also included.

Table 2. Baseline, Caution and Warning Levels and 2001 Results for MWRA Mussel Bioaccumulation Tests

Parameter	Baseline	Caution Level	Warning Level	Outfall Site 2001	Exceedance
PCB[b] (ppm wet weight)	0.0110	1	1.6	0.0096	No
Lead[b] (ppm wet weight)	0.415	2	3	0.240	No
Mercury[b] (ppm wet weight)	0.019	0.5	0.8	0.018	No
Chlordane[a] (ppb lipid)	102	205	None	250	Yes, Caution Level
Dieldrin[a] (ppb lipid)	25	50	None	25	No
DDT[a] (ppb lipid)	241	483	None	205	No
PAH[a] (ppb lipid)	1,080	2,160	None	3,024	Yes, Caution Level

[a]Since organic pollutants concentrate more readily in the lipids of animal tissue, the Outfall Monitoring Task Force (OMTF) agreed that organic compounds should be normalized to lipid content. This is not the same as FDA limits, which are in terms of wet weight; the lipid-normalized chlordane value of 250 ppb translates to 2.2 ppb wet weight. The FDA limit is 100 ppb wet weight.

[b]Thresholds were based on FDA limits for PCBs, mercury, and lead. The Caution Levels are 50% of the FDA limit and the Warning Levels are 80% of the FDA limit. For other constituents, the OMTF established Caution Level thresholds at twice baseline average for total chlordanes, total DDTs, total PAHs, and dieldrin. Threshold levels for PAHs were determined using the 24 PAH compounds that have been measured in the Outfall Monitoring Program since 1992. MWRA currently measures a total of 48 PAH compounds. Complete results for all constituents are reported in MWRA's Annual Fish and Shellfish Report. Baseline data from the outfall site were collected from 1992-2000 (except 1995). Measurements in 2001 of other contaminants not part of the Contingency Plan—lindane, hexachlorobenzene, aldrin, endrin, and mirex—were at very low levels at all locations sampled, similar to levels found in previous years.

2. METHODS/DATA TREATMENT

To evaluate the potential cause(s) of the PAH and chlordane exceedances in mussels deployed at the Massachusetts Bay Outfall Site, theoretical bioaccumulation equations (Pruell *et al.* 1986, Bergen *et al.* 1993, Neff and Burns, 1996) were used 1) to estimate the bioaccumulation of these contaminants in mussels based on the dilution of measured effluent concentrations and 2) to estimate the water concentrations that these mussels were exposed to while deployed in Massachusetts Bay using measured mussel contaminant data. Calculations were performed using individual compounds (Table 3) and then the calculated values were summed as defined for the threshold for 24 NOAA PAHs and total chlordane. Calculations were also performed for two PCB congeners, PCB 138 and PCB 153. It was assumed that the uptake of contaminants by mussels was from the dissolved phase only, based on work by Geyer *et al.* (1982) and Pruell *et al.* (1986). Recent data and information on the contaminants were used, including MWRA effluent concentrations, unpublished organic compound phase partitioning data in effluents from treatment plants similar to MWRA's, and K_{ow} values. Finally, the offshore effluent dilution and low offshore background concentrations were factored into the calculations performed. The background concentration was assumed to be that of Cape Cod Bay.

Table 3. Chemical Analytes Evaluated During Mussel Threshold Exceedance Investigation.

Chemical Analytes	
Chlordane	**Polynuclear Aromatic Hydrocarbons (PAHs)**
Heptachlor	***High Molecular Weight***
Heptachlor epoxide	Benzo[*a*]anthracene
cis-chlordane	Benzo[*a*]pyrene
trans-nonachlor	Benzo[*b*]fluoranthene
Polynuclear Aromatic Hydrocarbons (PAHs)	Benzo[*e*]pyrene
Low Molecular Weight	Benzo[g,h,i]perylene
1-methylnaphthalenes	Benzo[*k*]fluoranthene
1-methylphenanthrene	Chrysene
2,3,5-methylnaphthalenes	Dibenzo[*a,h*]anthracene
2,6-methylnaphthalenes	Fluoranthene
2-methylnaphthalenes	Indeno[1,2,3-*c,d*]pyrene
Acenaphthene	Perylene
Acenaphthylene	Pyrene
Anthracene	**Polychlorinated biphenyls (PCBs)**
Biphenyl	2,2′,3,4,4′,5′-Cl_6(138)
Fluorene	2,2′,4,4′,5,5′-Cl_6(153)
Naphthalene	
Phenanthrene	

Several steps were performed in the investigation of the threshold exceedances. They included:

1. Calculation of Bioconcentration Factors (BCFs) for each compound based on equations from Pruell *et al.* (1986) and Bergen *et al.* (1993),
2. Calculation of contaminant concentrations in mussels from effluent data and dilution,
3. Calculation of contaminant concentrations in water based on measured mussel concentrations,
4. Comparison of measured and calculated concentrations in mussels,
5. Evaluation of uncertainty in calculations.

2.1 Bioconcentration Factors

The log of bioconcentration of PAHs from solution in water by marine organisms is directly proportional to their K_{ow}s (Bierman 1990). Bioaccumulation of PAHs from suspended particles, sediments, and food is thought to involve an intermediate step in which the PAHs desorb or are released into solution within the mussels' gut from the solid matrix and then partition into lipid-rich tissues of the marine organisms. Thus, bioavailability of PAHs from sediments and suspended particles is less than that from solution in water (Pruell *et al*. 1987). Pruell *et al*. (1986) and others have developed regressions between log K_{ow} and log BCF for PAHs, PCBs, and marine animals, including mussels (Equation 1).

$$\log BCF = A \log Kow + B \qquad \text{(Equation 1)}$$

where:

$$BCF = \frac{[\text{contaminant}]_{\text{organism}}\ (\text{wet weight})}{[\text{contaminant}]_{\text{water}}\ (\text{dissolved})}$$

K_{ow} = octanol/water partition coefficient
Alog and B = the slope and intercept of the regression equation

These regressions can be used to estimate the concentration of individual PAHs and PCBs in tissues of mussels, based on mean concentrations in solution in the ambient water, or to estimate concentrations in water, based on concentrations in mussel tissues (Neff and Burns, 1996).

The slope and intercept values from Pruell *et al.* (1986) were used to calculate the log BCFs for each of the PAH compounds[a]:

$$\log BCF = 0.965 \log Kow - 1.40$$

The slope and intercept values from Bergen *et al.* (1993) were used to calculate the log BCFs for the four chlordane compounds and each of the PCB congeners:

$$\log BCF = 0.82 \log Kow - 0.52$$

The resulting bioconcentration factors were then used to calculate the predicted concentrations in mussels and water, depending on the starting measured concentration, i.e. diluted effluent or mussels, respectively.

2.2 Predicted Contaminant Concentrations in Mussels

Using Equation 2, contaminant concentrations in mussels were predicted over a range of dilutions (70:1 to 400:1) to which the mussels were likely to be exposed in Massachusetts Bay:

[a] Axelman *et al.* (1999) have suggested that these factors are site specific for PAHs.

$$Cm = \frac{Water(Ca)}{(LipidFraction * (10\text{^}-[A\log(Kow) + B]) * 1000g / L)} \qquad \text{(Equation 2)}$$

where:

Cm = estimated tissue concentration in ng/g, wet weight
Water (Ca) = estimated contaminant concentration in the ambient water in ng/L
Assumed to be composed of background water and effluent at deployment location
Lipid fraction = 0.37 / percent lipid of the mussels on wet weight basis (Neff and Burns 1996)
K_{ow} = octanol/water partition coefficient
Alog and B = the slope and intercept of the regression equation relating bioconcentration factors and K_{ow}

Because direct measurements of ambient water concentrations were not made during the 2001 deployment, Measured effluent concentrations of contaminants and the measured dilution of effluent were used to estimate the concentrations in ambient water. This included PAH data from eight Deer Island effluent samples analyzed by Battelle and chlordane and PCBs effluent data provided to Battelle by MWRA. The proportion of contaminants in the dissolved phase was estimated from an unpublished study of treatment plants similar to the Deer Island plant (Table 4).

First, the concentrations of contaminants in effluent were corrected to reflect only the dissolved fraction, using the equation:

$$Ced = Ce * \%\ dissolved \qquad \text{(Equation 3)}$$

where:

Ced = concentration in the dissolved fraction of the effluent (ng/L)
Ce = measured effluent concentration (ng/L)
% dissolved = the average percent dissolved fraction in effluent from a series of treatment plants[b]

The concentration of the diluted effluent in the environment was then corrected for background concentrations of contaminants that may have been present in the waters of Massachusetts Bay. Estimated values for Cape Cod Bay were used as the background concentrations (see Section 2.3). The background concentration was calculated using the following equation:

$$Water(Ca) = \frac{Ced}{Dil} + Cb \qquad \text{(Equation 4)}$$

where:

Water (Ca) = the estimated (ambient) water concentration (ng/L)
Ced = concentration in the dissolved fraction of the effluent (ng/L)
Dil = the dilution of the effluent at the point of exposure
Cb = estimated background water concentration (ng/L)

[b] Average percent dissolved determined as the fraction passing a 0.7 μm GFF filter.

Table 4. Percent Dissolved Fraction of PAHs, PCBs, and Chlordane in Effluent from Six Treatment Plants (Unpublished Data)

Contaminant Group	% Dissolved (Plant Average) n = 6	% CV
NOAA PAHs		
Low Molecular Weight		
1-Methylphenanthrene	44.0	21.3
2,3,5-Trimethylnaphthalene	73.8	10.8
2,6-Dimethylnaphthalene	85.3	4.1
2-Methylnaphthalene	94.0	2.0
Acenaphthene	84.6	6.4
Acenaphthylene	77.1	8.0
Anthracene	46.1	20.6
Biphenyl	89.1	2.5
Fluorene	78.2	8.9
Naphthalene	93.5	5.0
Phenanthrene	58.8	18.5
High Molecular Weight		
Benz(a)anthracene	15.4	49.7
Benzo(a)pyrene	10.7	16.5
Benzo(b)fluoranthene	18.6[a]	57.1
Benzo(e)pyrene	13.3	39.4
Benzo(g,h,i)perylene	12.4	34.8
Benzo(k)fluoranthene	11.6	38.5
Chrysene	18.0	33.3
Dibenzo(a,h)anthracene	18.6[a]	57.1
Fluoranthene	35.9	35.0
Indeno(1,2,3-c,d)pyrene	12.4	24.7
Perylene	18.6[a]	57.1
Pyrene	38.0	28.6
PCBs		
153/168	57.3	20.5
129/138/160/163	56.6	23.2
Chlordane		
Heptachlor	48.7	36.2
Heptachlor epoxide	45.7	57.4
Alpha chlordane	17.7	26.8
Trans-nonachlor	11.8	25.8

[a]Percent dissolved values were not available for this compound. An average percent dissolved value of all the measured HMW PAHs was used.

There were several data requirements for the calculation of bioaccumulation using the above equations. As a result, several sources of information were used to meet these requirements (Table 5).

Table 5. Data Requirements for Bioaccumulation Calculations

Data Requirement	Data Source
Octanol/water partitioning coefficients (K_{ow})	Literature
Bioconcentration factors	Literature
Lipid content of the organisms	Measured values
Wet/dry weight ratio of the organisms	Measured values
Concentration of contaminants in the water at the point of exposure	Estimated from other data
Estimate of dilution	From plume tracking study, July 2001
Contaminant concentration in effluent	Measured values
Contaminant concentration in background water	Estimated from other data

Where data were not available, values were estimated using the available data. For example, to predict the contaminant concentration in background water using Equation 4, the following data sources were used:

- Ced = Data from 8 MWRA effluent samples from June – August 2001 (Measured using low level MDL methods and corrected for dissolved fraction using Equation 3),
- Dil = 90 (from July plume tracking survey conducted about mid-way through the mussel deployment),
- Cb = Estimated from 2001 Cape Cod Bay mussel data (See Section 2.3),
- Water (Ca) = calculated and entered into Equation 2.

2.3 Predicted Contaminant Concentrations in Water

The second set of calculations that were performed estimated the water contaminant concentrations based on the measured mussel contaminant concentrations. The equation used to calculate the water concentration was the equation used to calculate bioaccumulation in mussels, rearranged with the water concentration term on the left of the equation:

$$Water(Ca) = Cm * Lipid\ \ Fraction * (10\text{^}{-}[A\log(Kow) + B]) * 1000\ g/L \quad \text{(Equation 5)}$$

where:

Water(Ca) = estimated contaminant concentration in the ambient water in ng/L based on mussel concentrations

Cm = estimated tissue concentration in ng/g, wet weight

Lipid fraction = 0.37 / percent lipid of the mussels on wet weight basis

K_{ow} = octanol/water partition coefficient

Alog and B = the slope and intercept of the regression equation relating bioconcentration factors and K_{ow}

3. RESULTS

3.1 Predicted Contaminant Concentrations in Mussels vs. Measured Values

Table 6 presents the contaminant concentrations measured in mussels deployed during the 2001 fish and shellfish monitoring program and reported in wet weight units. The 60-m array (Outfall Site M4) is the data set used for threshold comparisons.

As expected, mussels deployed closest to the outfall diffusers (i.e. 15-m array) had higher concentrations than mussels deployed further from the outfall. The exception to this trend was the Deer Island deployment, which may be influenced by contaminant sources other than the outfall.

Table 6. Measured Concentrations of Contaminants in Mussels

Contaminant Group	15-m Array	60-m Array	LNB	DI	CCB	Rockport
24 NOAA PAHs (ng/g wet)	33.6	26.4	16.3	34.3	15.7	5.6
NOAA LMW PAHs (ng/g wet)	4.6	3.0	3.0	5.3	4.4	2.1
NOAA HMW PAHs (ng/g wet)	29.1	23.4	13.3	29.0	11.3	3.4
Total Chlordane (ng/g wet)	2.7	2.2	1.7	1.2	0.9	0.3
PCB 138 (ng/g wet)	2.3	1.7	1.4	6.1	2.5	0.4
PCB 153 (ng/g wet)	3.2	2.5	2.0	7.3	3.0	0.4
Lipid Content (% wet wt.)	1.25	0.89	0.83	0.99	1.73	0.49

[1]Threshold listed is for Total PCBs.
NA = Not applicable

The estimated mussel concentrations calculated using the methods described in Section 2.1 are presented in Table 7. Estimates were made for a range of dilutions from 70:1 to 400:1. The 90:1 dilution is the initial dilution[c] measured at the outfall on July 19, 2001 during the MWRA plume tracking study (Hunt *et al.* in preparation). Concentrations measured in mussels from the 60-m array are shown for comparison. The measured concentrations of organic contaminants in mussels from the 60-meter array (threshold array) fall within a factor of about 2 – 3 of the predicted concentrations estimated for the measured dilution of 90:1. For total chlordane, the measured concentrations exceed predictions. For the two PCB congeners, the predicted values exceed the measured ones by 2-3 fold.

The disparity between the measured and predicted chlordane concentrations may be due to the fact that chlordane tissue data are susceptible to analytical interferences, which may cause the measured tissue concentrations to be over estimated. The tissue sample extracts could be analyzed by GC/MS to confirm the concentrations of chlordane in mussel tissue. The measured tissue concentrations of PAHs may be lower than predicted because the PAHs measured in mussel tissue were >80% HMW PAHs. These are slowest to come to equilibrium in tissue. It may be that the deployment time was too short for these compounds to reach equilibrium, and therefore were measured at levels lower than predicted.

[c] Dilution achieved at the point the hydraulic mixing of effluent into the ocean is complete.

Table 7. Predicted Mussel Concentrations Over a Range of Dilutions Compared to Measured Concentrations in the 60-m Array Mussels.

Contaminant Group	Predicted Mussel Concentrations at Various Dilutions						Measured Mussel Conc.
	70:1	*90:1*	*120:1*	*150:1*	*200:1*	*400:1*	60-m Array Conc.
24 NOAA PAHs (ng/g wet)	71.9	58.0	45.8	38.4	31.1	20.1	26.4
NOAA LMW PAHs (ng/g wet)	6.4	5.6	4.8	4.4	3.9	3.2	3.0
NOAA HMW PAHs (ng/g wet)	65.5	52.4	40.9	34.1	27.2	16.9	23.4
Total Chlordane (ng/g wet)	0.83	0.76	0.70	0.67	0.63	0.57	2.2
PCB 138 (ng/g wet)	4.10	3.52	3.00	2.69	2.39	1.92	1.7
PCB 153 (ng/g wet)	7.15	5.95	4.90	4.27	3.65	2.70	2.5

The predicted values of contaminants may have been overestimated or underestimated compared to the measured values due to several sources of error or uncertainty in the calculations used to derive these estimates and in the data itself. The potential sources of uncertainty are discussed in the next section and quantified where possible.

3.2 Uncertainty Evaluation

Given the complexity of the bioaccumulation equations and the number of terms involved in predicting mussel contaminant equations, there exist several sources of error (Table 8), which create uncertainty in the calculations used to derive these estimates and in the data itself. To determine the overall error of the predicted mussel values, each term in the bioaccumulation equations was evaluated for its level of uncertainty.

Table 8. Sources of Error or Uncertainty Associated with Bioaccumulation Calculations

Sources of Error (Uncertainty)	Level of Uncertainty
Octanol/water partitioning coefficients (K_{ow})	30 – 50%
Lipid content of the organisms	13 – 45%
Wet/dry weight ratio of the organisms	6 – 12%
Concentration of contaminants in the water at the point of exposure	---
Estimate of dilution	~15%
Exposure levels and duration	Factor of 2 – 4
Contaminant concentration in effluent	50 – 100%
Contaminant concentration in background water	50 – 100%
Measured results in mussels	0 – 283% for individual compounds 6 – 22% for totals

3.2.1 Octanol/water partitioning coefficients (K_{ow})

Estimated K_{ow}s for different PAHs vary widely, depending on the method used to estimate them. Güsten *et al.* (1991) reported that estimated and measured log K_{ow}s for individual PAHs vary over a range of about 0.3 log units or more: variability increases with molecular weight. This variability introduces a potential error into estimates of the relationship between PAH concentrations in water and mussel tissues. In addition, octanol is not a perfect surrogate for tissue lipids (Connell 1993). The solubility of PAHs, particularly high molecular weight ones, may be quite different in octanol and in the tissue lipids of different species of marine animals, increasing the potential error in the estimation of the water/tissue concentration relationship for PAHs.

3.2.2 Lipid content of the organisms

It often is informative to normalize tissue concentrations of nonpolar organic chemicals to tissue lipid concentration. This normalization is based on the premise that most of the nonpolar organic chemical accumulated from water or food by a marine animal partitions into the tissue lipids. Normalization allows for a more accurate estimate of the relationship between the concentration of a chemical in the ambient water and its concentration in the tissues of a marine animal in equilibrium with the chemical in the water, the bioconcentration factor (BCF). However, a wide range of different methods has been used to measure the lipid fraction in the tissues of freshwater and marine animals (Randall *et al*. 1991). Variability in tissue lipid concentrations determined by different analytical methods introduces a significant source of error in estimates of the relationship between concentrations of nonpolar organic contaminants in water and tissues of marine organisms (Randall *et al.* 1991). Randall estimated that for the same tissue different methods for determining tissue lipids give results that vary by a factor of approximately 3.5. This variability is different for different species of marine animals, because differences in the chemical composition of the tissue lipids in different species. Randall *et al*. (1991) reported that the ratio of maximum to minimum lipid concentration in mussels analyzed by four analytical methods was 2.11. Chloroform/methanol consistently gave the highest estimate of lipid concentrations in fish liver and muscle, polychaetes, and mussels. This variability must be kept in mind when using published regressions to relate concentrations of nonpolar organic chemicals in water and tissues of marine animals.

The methods used to collect mussel lipid data for the MWRA HOM program has been consistent within the program itself, but these methods differ from those used by Pruell *et al.* (1986) in the development of the bioconcentration regressions used to predict mussel contaminant concentrations. An adjustment was made to account for the difference between lipid content used to develop the equation (0.37%) and the lipid content of the mussels in this study (0.49 – 1.73%).

3.2.3 Wet/dry weight ratio of the organisms

As part of the MWRA HOM program, measured contaminant and lipid data for mussel tissue are reported on a dry weight basis. The predicted mussel concentrations produced using Equation 2 were on a wet weight basis. Therefore, the measured mussel contaminant and lipid values needed to be converted to wet weight for purposes of comparison and also before calculating the predicted water concentrations. The variability in tissue dry weight for the 8 composite samples of 60-m array mussels was 15.1 ± 1.2 % dry weight, 8.0 % coefficient of variance. The range of variability for the other mussel sites was fairly low (5.7 – 12.1 %CV).

3.2.4 Exposure duration, frequency, and dilution

The mussels near the diffuser are exposed to both background levels of contaminants and different levels of dilute effluent, while those at Rockport are not exposed to the MWRA effluent (net water circulation is

to the south which does not bring dilute effluent to the north). The transport of the effluent from the diffuser throughout Massachusetts Bay has been modeled by Signell *et al* (1996). Predicted dilutions reach 200 to 400 fold within less than 10 km of the diffuser (Figure 3). On average the plume is centered around the outfall with net transport to the south.

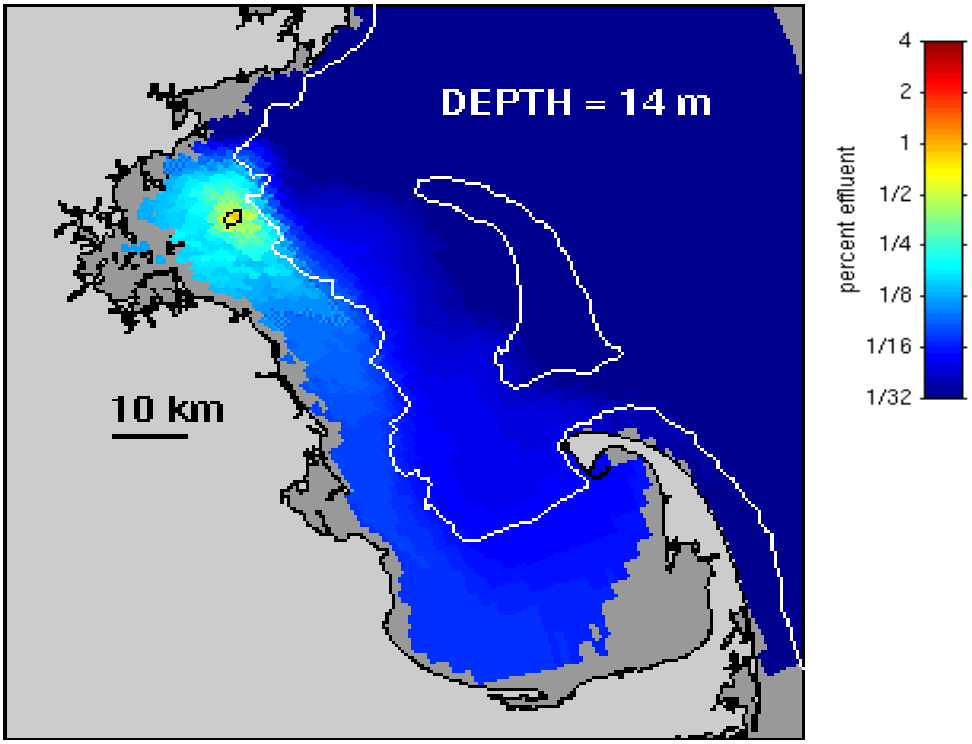

Figure 3. Modeled dilution at 14 m depth during typical summer stratified conditions (from Signell *et al*. 1996).

Water quality monitoring since the outfall began discharging effluent in September 2000 has consistently shown that ammonia is a good short-term tracer of the diluted effluent near the outfall. Contoured ammonia data from mid-depth for the 2001 summer surveys conducted while the caged mussel moorings were deployed is shown in Figure 4. Dilute effluent concentrations are variable in the vicinity of the outfall.

Figure 4 shows that the effluent plume was in different locations over the course of the mussel deployment. The mussels are exposed to a background level of any contaminant as well as to the levels emanating from our effluent. The amount of MWRA effluent to which the mussels are exposed is determined by four main factors:

1. Effluent flow
2. Current speed and direction.
3. Rise height of effluent, which is determined by the depth and strength of stratification.
4. Background build-up of effluent (i.e. far-field dilution).

All of these vary over the course of 60-days, resulting in uncertainty.

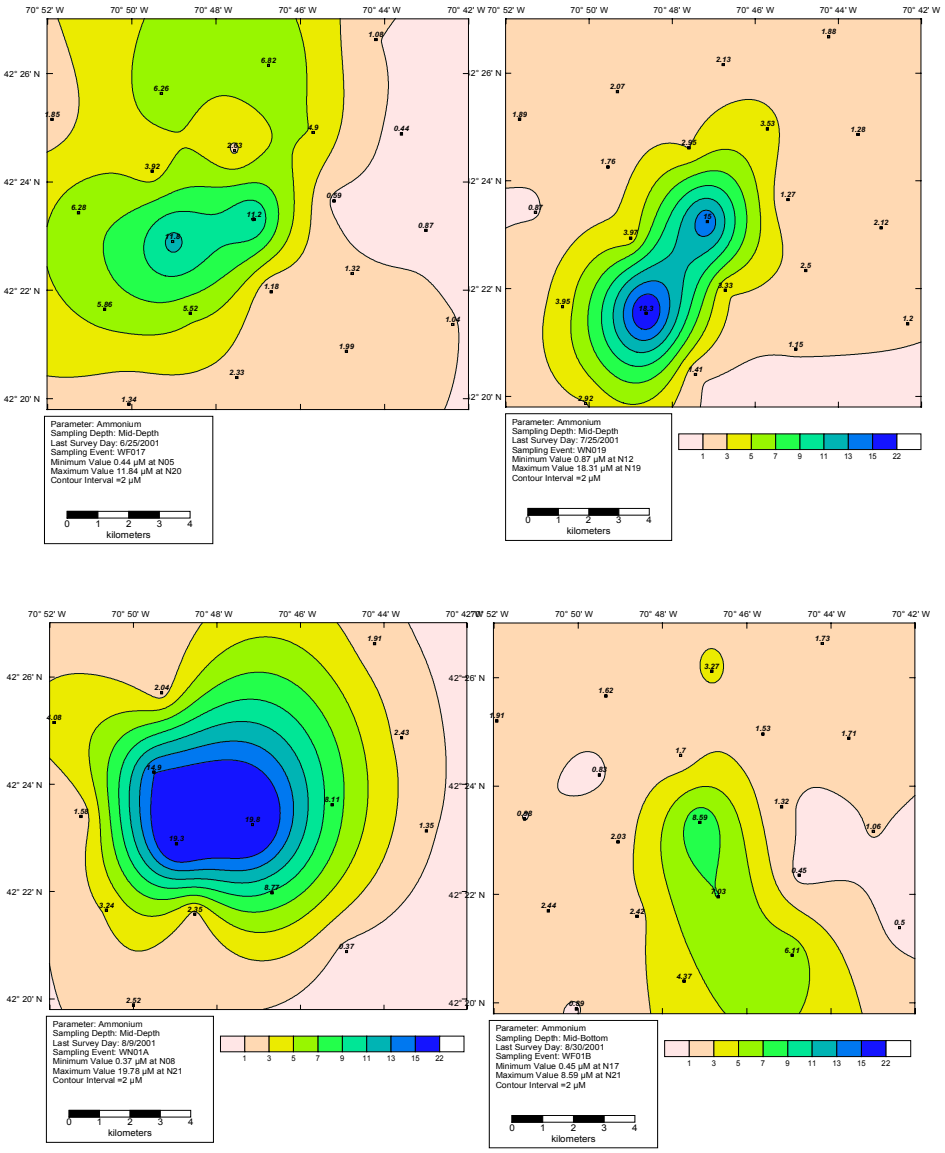

Figure 4. MWRA effluent plume signature at mid depth (~15 m) in Massachusetts Bay derived from measured ammonia concentrations.

The data in Figure 4 show that mussels near the outfall were exposed to higher effluent concentrations. The actual duration that the various moorings were bathed in most concentrated parts of the effluent plume is not clear. Because tidal current, net water column transport, and turbulence interact, the mussels at each location near the outfall may have been exposed for short periods to initial dilution levels, and later by more dilute effluent that passed over the mooring location. Therefore, the initial dilution of 90:1 that was measured in the July 2001 dye dilution study is used as the background dilution for the calculations in this report.

3.2.5 Contaminant concentration in effluent

The concentration of organic contaminants in the effluent during the deployment period was measured in eight effluent samples. These sample data showed very high variability in the concentrations of NOAA PAHs measured (Table 9). This variability could be the result of several factors, including treatment plant flow, rainfall events, analytical precision, etc.

Table 9. Means, Standard Deviations, and Coefficients of Variance of NOAA PAHs Measured in Deer Island Treatment Plant Effluent

Compound Set	Mean (ng/L)	SD (ng/L)	CV%
Total NOAA PAHS	584	496	85.0
NOAA LMW PAHs	252	240	95.1
NOAA HMW PAHs	332	259	78.0

Also, the effluent samples were not filtered before analysis and therefore represent the total concentrations of contaminants in the effluent (extracted using SW846 solid phase methods (EPA 1996)). Particle-bound contaminants, however, may not have been completely extracted with the approach used.

The calculations used to predict mussel tissue concentrations of contaminants assume exposure and uptake of only the dissolved contaminants, which are more bioavailable. As described above, we used the average dissolved/particulate fractionation in data from a group of treatment plants similar to the Deer Island treatment plant (Table 4) to estimate the dissolved concentrations of contaminants in MWRA effluent. Use of these data adds an unknown potential error to the calculation of predicted bioaccumulation by mussels.

3.2.6 Contaminant concentration in background water

Ambient water contaminant concentrations were not directly measured as a part of this study. Water concentrations were modeled using measured tissue concentrations from mussels deployed in reference locations (Table 10). The concentrations listed are assumed to represent only the bioavailable (primarily dissolved) fraction in the water column. Concentrations measured in Massachusetts Bay in 1996 by Shea (1997) are listed on the right side of the table for comparison.

The total threshold PAH, total chlordane and two PCB concentrations in water predicted from the mussel tissue concentrations were within a factor of 2 to 3 across the five deployment locations in Massachusetts Bay. The total PAH concentrations ranged from 5.9 (CCB) to 8.2 ng/L (mooring closest to the diffuser). Deer Island and Rockport (source of the mussels) deployment locations had the highest estimated water column PAH concentrations at 9.3 and 9.9 ng/L, respectively. Predicted Total Chlordane was extremely low at 0.001 to 0.003 ng/L. The higher values were for moorings located within 1 km of the diffuser. The predicted PCB congeners concentrations ranged from 0.002 (Rockport) to 0.007 ng/L (closest to the outfall). The PAH estimates suggest composition LMW concentrations are relative constant while the HMW PAHs decrease with distance from the outfall.

The predicted concentrations were a factor of 2 – 4 higher than the dissolved concentrations measured by Shea in the mid 1990s. HMW PAHs were predicted within a factor of 2.3 of the measured dissolved fraction. Predicted Total chlordane was approximately a factor of 2 higher than measured concentrations within Massachusetts Bay. Concentrations of the two PCB congeners, 138 and 153, were under-predicted by a factor of 6 and 20.

The disparity between the predicted and measured ambient water concentrations could be attributed to 1) error associated with the measurement of the lipid fraction used to predict water concentrations from measured mussel concentrations, 2) K_{ow} values used to back calculate predicted water concentrations, 3) analytical constraints in measuring very low concentrations of contaminants in the water column, and 4) time. When the measurements of ambient water concentrations were measured in 1995, the Massachusetts Bay outfall was not yet operational.

After a brief evaluation of the estimated water concentrations and comparing them to measured values from 1996, it was determined that the Cape Cod Bay mussel-derived water estimates were the best background concentrations to use for the purposes of this study, given the information available. Actual measurements, however, are necessary to achieve a better understanding of actual background concentrations.

Table 10. Comparison of Predicted Water Contaminant Concentrations with Measured Historical Values in Massachusetts Bay

	Predicted Water Concentrations from Mussel Data (ng/L)						Measured Water Concentrations (Shea 1997) (ng/L)														
Station	**15 m (Array #3**	**60 m (Array #1)**	**LNB**	**DI**	**CCB**	**Rockport**	**Massachusetts Bay "B" Buoy (LNB)**			**Mass Bay-Stellwagen**			**Cape Ann**			**Cape Cod Bay-West**			**Georges Bank**		
Compound Set	(Calculated as dissolved fraction only)						D	P	T	D	P	T	D	P	T	D	P	T	D	P	T
Total NOAA PAHS	8.22	7.51	7.62	9.30	5.91	9.90	2.73	3.27	6.00	1.30	2.01	3.31	2.47	2.99	5.45	3.18	3.69	6.86	0.72	1.63	2.36
NOAA LMW PAH	7.21	6.37	6.98	8.05	5.62	9.57	2.45	2.01	4.46	1.08	0.93	2.00	2.22	1.84	4.06	2.86	2.25	5.11	0.63	0.87	1.50
NOAA HMW PAH	1.02	1.14	0.64	1.25	0.29	0.32	0.28	1.26	1.54	0.22	1.08	1.31	0.24	1.15	1.39	0.32	1.44	1.76	0.09	0.76	0.87
PCB (138)	0.006	0.006	0.005	0.019	0.004	0.002	0.031	0.008	0.039	0.026	0.014	0.040	0.046	0.012	0.058	0.074	0.013	0.087	0.004	0.002	0.006
PCB (153)	0.007	0.007	0.006	0.019	0.005	0.002	0.024	0.005	0.029	0.019	0.003	0.022	0.025	0.006	0.031	0.062	0.009	0.071	0.004	0.001	0.005
Total Chlordane	0.03	0.03	0.03	0.01	0.01	0.01	0.01	0.00	0.01	0.00	0.00	0.01	0.01	0.00	0.02	0.01	0.00	0.01	0.00	0.00	0.00

D = dissolved; P = particulate; T = total

3.2.7 Measured results in mussels

The measured PAH and chlordane concentrations in mussel tissue in 2001 were somewhat variable. This variability differed for each analyte measured (Table 11). Some of the PAH compounds seemed to be highly variable between mussel replicate samples (i.e. Dibenzo[*a,h*]anthracene and fluorene), whereas the PCB congeners and chlordane compounds seemed to be less variable (6 – 31% and 5 – 23%, respectively). It is interesting to note that once the individual PAH compounds were summed into Total PAHs, the coefficient of variance dropped to about 7 – 22%. Note: the variability (%CV) of individual PAHs was most likely related to the concentration. For example, the concentrations of some of the HMW PAHs were very low, which may account for the high % CV.

Table 11. Coefficients of Variance (%) for Measured Mussel Contaminant Concentrations

Compound Set	Analyte	CV% for 60-m Array (n = 8)	Range of CV% for All Sites (n = 5 or 8)
NOAA PAHs	Acenaphthene	0	0 – 186.8
	Acenaphthylene	0	0 – 188.2
	Anthracene	50.3	14.6 – 102.4
	Benzo[*a*]anthracene	10.1	10.1 – 30.3
	Benzo[*a*]pyrene	11.1	0 – 45.8
	Benzo[*b*]fluoranthene	11.6	7.8 – 32.1
	Benzo[*e*]pyrene	9.3	9.3 – 16.8
	Benzo[*g,h,i*]perylene	12.1	10.1 – 28.3
	Benzo[*k*]fluoranthene	9.0	8.9 – 61.5
	Biphenyl	18.5	18.5 – 107.8
	Chrysene	8.9	8.9 – 19.1
	Dibenzo[*a,h*]anthracene	282.8[a]	0 – 282.8
	Fluoranthene	8.0	8.0 – 21.7
	Fluorene	282.8[a]	10.5 – 282.8
	Indeno[*1,2,3-c,d*]pyrene	14.3	11.6 – 137.9
	Naphthalene	8.2	8.2 – 42.5
	Perylene	48.6	0 – 114.9
	Phenanthrene	9.8	9.8 – 23.3
	Pyrene	9.3	9.3 – 72.7
	1-Methylnaphthalene	8.2	8.2 – 15.2
	1-Methylphenanthrene	7.5	7.5 – 62.5
	2,3,5-Trimethylnaphthalene	0	0 – 223.6
	2,6-Dimethylnaphthalene	0	0 – 186.2
	2-Methylnaphthalene	11.8	7.8 – 19.2
PCBs	PCB (138)	10.2	6.3 – 22.5
	PCB (153)	11.3	7.4 – 30.8
Chlordanes	Cis-Chlordane	11.9	7.3 – 19.6
	Heptachlor	0	0 – 0
	Heptachlor epoxide	0	0 – 0
	Trans nonachlor	9.6	4.9 – 22.8
Totals	Total NOAA PAHs	7.0	7.0 – 19.8
	Total NOAA LMW PAHs	8.5	8.5 – 21.7
	Total NOAA HMW PAHs	7.2	7.0 – 21.6
	Total Chlordane	11.1	6.0 – 20.4

[a]Concentrations of these compounds were very low (mostly non-detects) which accounts for some of the variability seen.

4. DISCUSSION AND RECOMMENDATIONS

Section 4.1 of this report discusses the critical factors in predicting mussel contaminant concentrations identified during the study, and Section 4.2 discusses whether the contaminant levels that exceeded mussel threshold values in 2001 are of ecological concern.

4.1 Critical factors

During the course of this investigative study, several factors were identified as being critical to the prediction of levels of contaminants in caged mussels. These factors include:

- Duration and frequency of exposure to the diluted effluent plume,
- Ambient water contaminant concentrations,
- Effluent contaminant concentrations,
- Measurement errors and estimates of K_{ow}.

Given the errors or uncertainty associated with each of these factors, a best estimate of mussel contaminant concentrations would be within a factor of 1.5 of the measured mussel concentrations. At worst, there would be a difference of a factor of 3 between predicted and measured mussel tissue concentrations. Not considering additive error, the mussel contaminant data collected in 2001 suggest that the best prediction that can be made at this time with the information available is within a factor of ± 3.

The PAH and chlordane concentrations measured in the mussels deployed in the vicinity of the outfall in 2001 appear to be consistent with predictions based on recent theory of bioaccumulation in mussels and using measured concentrations in the effluent, assumed partitioning between dissolved and particulate phases and the likely water column concentrations the mussels were exposed to at the deployment locations. Additional investigations in the summer of 2002 could reduce the uncertainties associated with this assessment.

4.2 Toxic Effects of Threshold Exceedances

Even though thresholds were exceeded, there is no indication that there is any environmental impact from these exceedances. Water quality criteria for PAHs and chlordanes were met before dilution, and the mussels deployed at different MWRA monitoring stations contained concentrations of polycyclic aromatic hydrocarbons (PAHs) and chlordane well below the applicable FDA limits (Table 12). However the concentrations exceeded screening values, based on estimated concentrations in the water in equilibrium with the mussels. Concentrations of all other contaminants in the mussels were below screening values. Concentrations of total PAHs[a] were highest in mussels deployed 15 m from the diffuser; lowest concentrations were in mussels from Rockport. A majority of the PAH burden in mussels near the diffuser were low molecular weight PAHs, probably derived from petroleum sources. The concentration of total, low, and high molecular weight PAHs and chlordane in the mussels decreased with distance from the diffuser, indicating that at least part of the body burden was coming from the treated wastewater effluent. The concentration ratio of low to high molecular weight PAHs in the mussels also decreased with distance from the diffuser, suggesting that the fraction of the total PAHs in the mussels from combustion sources increased with distance from the diffuser.

[a] Total PAHs (46 parent and alkyl congeners) were used for this evaluation, since this group of compounds better represents the total effects being investigated.

Table 12. Concentrations of total PAHs (46 parent and alkyl congeners)[a], total low molecular weight PAHs (2-3 ring PAH), high molecular weight PAHs (>3 ring PAHs), and chlordane in whole soft tissues of mussels deployed at MWRA monitoring sites. Significant decrease in scope for growth is observed at total low molecular weight tissue residues greater than about 100 ng/g wet wt (Widdows *et al*., 1995).

Contaminant Group	Measured Contaminant Concentrations in Mussel Tissue (ng/g wet wt.)					
	15 m	60 m	LNB	DI	CCB	Rockport
Total PAHs	117.29	88.84	39.14	57.97	20.77	5.92
Total LMW PAHs	75.13	55.74	20.93	23.19	7.67	2.50
Total HMW PAHs	42.16	33.10	18.21	34.79	13.11	3.42
Total Chlordane	2.73	2.19	1.67	1.20	0.89	0.26

[a]All PAHs measured are used in this comparison of LMW to HMW PAHs, whereas only the threshold PAHs are used in previous discussions.

Nonpolar organic chemicals, such as PAHs and pesticides, are thought to exert their toxic effects by narcotic action; they accumulate in and on cell membranes to a critical concentration that causes membrane swelling and loss of transport functions, leading to narcosis and finally death (Abernethy *et al*. 1988, McCarty and Mackay 1993). Chemicals, such as pesticides, often have a specific mode of toxicity that occurs at tissue concentrations below those that cause narcosis. The toxicity of nonpolar narcotic toxins is additive. That is, toxic effects are observed when the molar concentration of the sum of all the toxic chemicals reaches a critical value in the tissues. This critical concentration is different for different species of animals. Donkin *et al*. (1991) reported that the critical tissue effects concentration (TEC: the concentration in tissues causing a 50% reduction in filtration rate) of individual low molecular weight PAHs (log K_{ow} <5.0) in mussels ranges from 0.08 to 0.24 mmol/kg wet wt. Higher molecular weight PAHs have much higher TECs in mussels; pyrene has a TEC of >0.94 mmol/kg and fluoranthene has a TEC of 3.10 mmol/kg. Therefore, HMW PAHs are less toxic (higher TEC values) than the LMW PAHs.

Widdows *et al*. (1995) determined concentrations of total and individual PAHs, several pesticides, and tributyltin in soft tissues of mussels collected at a large number of coastal sites around Great Britain. They also measured the scope for growth of the mussels. Scope for growth is the amount of total caloric energy consumption that is available for growth and reproduction. Stress from exposure to and bioaccumulation of toxic chemicals or adverse environmental conditions cause a progressive, dose-related decrease in the scope for growth of mussels. Donkin *et al*. (1989) and Widdows *et al*. (1995) showed a strong negative correlation between tissue concentrations of total low molecular weight PAHs and scope for growth. A significant decrease in scope for growth was observed at a concentration of total low molecular weight PAHs in the mussel tissues of about 100 ng/g wet wt. This concentration can be used as an index of the incipient effects concentration for total low molecular weight PAHs in mussels. Examination of Table 12 reveals that none of the mussels contained 100 ng/g wet or more of total low molecular weight PAHs. Mussels from the 15-m station have a tissue residue of total low molecular weight PAHs that is 75% of the potentially toxic concentration. By this criterion, none of the mussels are suffering adverse effects from PAHs accumulated from the treated wastewater discharge.

The 100 ng/g index concentration for total low molecular weight PAHs in tissues of mussels is a conservative value; mussels containing this concentration of total PAHs survive well, grow, and reproduce (Donkin *et al*. 1989, Widdows *et al*. 1995). Many mussels collected along the British coast, as well as many collected in the US National Status and Trends Mussel Watch Program, contain more than 100 ng/g wet wt total low molecular weight PAHs. Many of these populations seem to be doing well. Thus, the index value should be used as a conservative estimate of body burden of low molecular weight PAHs above which long-term biological effects could occur.

Donkin *et al*. (1989) did not analyze the same suite of PAHs as were analyzed in this investigation. Therefore, we took an additional approach to evaluate whether the mussels might be experiencing toxic effects from the PAHs in the effluents. An "average" TEC of 0.2 mmol/kg wet wt was chosen, based on the empirical determinations of Donkin *et al*. (1991). The hazard quotient (HQ) for each low molecular weight PAH in the mussel tissue samples was estimated as the ratio of the observed concentration in the tissues divided by the TEC value converted to ng/g wet wt.

The mussels deployed at the different locations contained low concentrations of low molecular weight PAHs in their tissues (Table 13). Alkyl naphthalenes, fluorenes, and phenanthrenes were the most abundant PAHs in the mussels deployed near the diffuser. Naphthalene and phenanthrene were the PAHs present at highest concentrations in the Rockport mussels. Naphthalene, however, is a common contaminant. Hazard quotients for the individual low molecular weight PAHs in the mussel tissues were all well below 1, the value indicating the potential for toxic effects (Table 14). Highest HQ values were for alkyl fluorenes and phenanthrenes in mussels from the 15-m site. The HQs for mussels from each site were summed to produce a hazard index (HI), indicating the potential additive toxicity of all the low molecular weight PAHs in the mussel tissues. HIs ranged from 0.0018 to 0.00008, indicating a very low order of potential risk of toxic effects to the mussels. Thus, by two measures of ecological risk, the mussels deployed near the wastewater diffuser were at low risk of harm from PAHs in the effluent.

The mussels also contained 0.26 to 2.73 ng/g wet wt total chlordane in their whole soft tissues (Table 12). An approach similar to that for PAHs can be used to predict the health risks to the mussels from tissue chlordane. There is no mussel-specific TEC for chlordane. However, Shephard (1995) developed an approach for estimating the tissue screening concentration (TSC) for toxic chemicals in tissues of freshwater and marine animals. The TSC for a chemical is estimated with the simple relationship:

$$TSC = AWQC \times BCF$$

Where AWQC is the lowest available water quality criterion for protection of aquatic life and BCF is the wet weight bioconcentration factor, the ratio at equilibrium of the concentration of the chemical in the tissues of the organism to the concentration of the chemical in the ambient water. The AWQC for chlordane is 0.004 μg/L and the measured BCF is approximately 14,100 L/kg, giving a TSC of 0.056 μg/g wet wt (56 ng/g). Thus, the HQ for chlordane in the most contaminated mussels in this investigation (15-m site) is 0.05. Thus, the total HI for low molecular weight PAHs and chlordane in tissues of mussels from the 15-m site is 0.052, assuming that the toxicity of the two groups of compounds are additive. Thus, the mussels are not at risk from exposure to PAHs and chlordane from the wastewater effluent.

A similar exercise could be performed for other nonpolar organic contaminants in the tissues of mussels from the various deployment sites. However, concentrations of other nonpolar organic contaminants in the effluent, receiving waters, and mussel tissues were low. It is doubtful that chemicals present in mussel tissues at a concentration that is a small fraction (less than about 0.1 %) of the critical body residue concentration contribute significantly to the total toxic stress of the host animal (McCarty and Mackay 1993). In addition, many of the nonpolar organic contaminants of concern are high molecular weight, high log K_{ow} compounds that behave like high molecular weight PAHs and do not contribute to neutral narcosis in proportion to their molar concentrations. These compounds probably are too large to enter and swell cell membranes. Some of these chemicals, such as pesticides, may exert their toxic effects by a different mechanism than neutral narcosis; their toxicity would not be additive and would have to be modeled separately.

Table 13. Concentrations of individual and total low molecular weight PAHs in whole soft tissues of mussels that had been deployed at locations near the wastewater diffuser, in Boston Harbor, and at reference sites in Cape Cod Bay and Off Rockport.

PAH (ng/g wet wt.)	15 m	60 m	LNB	DI	CCB	Rockport
Naphthalene	1.30	0.75	0.74	0.84	1.41	0.60
C1-Naphthalenes	0.42	0.30	0.28	0.61	0.33	0.46
C2-Naphthalenes	0.66	0.00	0.00	1.21	0.15	0.00
C3-Naphthalenes	1.50	1.41	0.00	1.36	0.00	0.00
C4-Naphthalenes	6.54	4.23	0.00	0.36	0.00	0.00
Acenaphthene	0.08	0.00	0.05	0.14	0.08	0.00
Acenaphthylene	0.06	0.00	0.06	0.14	0.09	0.00
Biphenyl	0.29	0.41	0.38	0.18	0.37	0.11
Fluorene	0.08	0.03	0.14	0.27	0.24	0.07
C1-Fluorenes	1.08	0.00	0.00	0.00	0.00	0.00
C2-Fluorenes	3.79	2.82	0.00	0.00	0.00	0.00
C3-Fluorenes	11.63	7.83	0.00	0.00	0.00	0.00
Dibenzofuran	0.24	0.48	0.43	0.35	0.46	0.09
Anthracene	0.28	0.15	0.13	0.36	0.23	0.07
Phenanthrene	1.01	0.89	0.84	1.63	1.30	0.52
C1-Phenanthrenes/Anthracenes	2.24	1.95	0.93	1.94	0.74	0.42
C2-Phenanthrenes/Anthracenes	11.15	9.37	5.18	3.78	1.49	0.16
C3-Phenanthrenes/Anthracenes	11.45	8.45	5.90	4.72	0.19	0.00
C4-Phenanthrenes/Anthracenes	5.83	4.46	2.33	1.73	0.00	0.00
Dibenzothiophene	0.20	0.18	0.02	0.05	0.00	0.00
C1-Dibenzothiophenes	0.99	0.81	0.01	0.00	0.00	0.00
C2-Dibenzothiophenes	5.40	4.46	1.20	1.35	0.00	0.00
C3-Dibenzothiophenes	8.92	6.79	2.32	2.16	0.62	0.00
Total LMW PAHs	75.13	55.74	20.93	23.19	7.67	2.50

Table 14. Hazard Quotients (measured concentration/threshold effects concentration) and hazard indices (sum of HQs) for low molecular weight PAHs in the tissues of mussels that had been deployed at MWRA monitoring sites. Effects concentration was set at 0.2 mmol/kg wet wt, determined by Donkin *et al*. (1991) to be the concentration in tissues required to decrease feeding rate by 50%.

PAH	15 m	60 m	LNB	DI	CCB	Rockport
Naphthalene	0.000051	0.000029	0.000029	0.000033	0.000055	0.000023
C1-Naphthalenes	0.000015	0.000011	0.000010	0.000021	0.000011	0.000016
C2-Naphthalenes	0.000021	0.000000	0.000000	0.000039	0.000005	0.000000
C3-Naphthalenes	0.000044	0.000042	0.000000	0.000040	0.000000	0.000000
C4-Naphthalenes	0.000178	0.000115	0.000000	0.000010	0.000000	0.000000
Acenaphthene	0.000003	0.000000	0.000002	0.000004	0.000003	0.000000
Acenaphthylene	0.000002	0.000000	0.000002	0.000004	0.000003	0.000000
Biphenyl	0.000010	0.000013	0.000012	0.000006	0.000012	0.000004
Fluorene	0.000002	0.000001	0.000004	0.000008	0.000007	0.000002
C1-Fluorenes	0.000030	0.000000	0.000000	0.000000	0.000000	0.000000
C2-Fluorenes	0.000098	0.000073	0.000000	0.000000	0.000000	0.000000
C3-Fluorenes	0.000279	0.000188	0.000000	0.000000	0.000000	0.000000
Dibenzofuran	0.000007	0.000014	0.000013	0.000010	0.000014	0.000003
Anthracene	0.000008	0.000004	0.000004	0.000010	0.000006	0.000002
Phenanthrene	0.000028	0.000025	0.000023	0.000046	0.000036	0.000015
C1-Phenanthrenes	0.000058	0.000051	0.000024	0.000050	0.000019	0.000011
C2-Phenanthrenes	0.000270	0.000227	0.000125	0.000092	0.000036	0.000004
C3-Phenanthrenes	0.000260	0.000192	0.000134	0.000107	0.000004	0.000000
C4-Phenanthrenes	0.000124	0.000095	0.000050	0.000037	0.000000	0.000000
Dibenzothiophene	0.000005	0.000005	0.000001	0.000001	0.000000	0.000000
C1-Dibenzothiophenes	0.000025	0.000020	0.000000	0.000000	0.000000	0.000000
C2-Dibenzothiophenes	0.000127	0.000105	0.000028	0.000032	0.000000	0.000000
C3-Dibenzothiophenes	0.000197	0.000150	0.000051	0.000048	0.000014	0.000000
Hazard Index	**0.001842**	**0.001359**	**0.000512**	**0.000599**	**0.000225**	**0.000079**

4.3 Open questions

This study has lead to a further understanding of the factors that controlled the exceedances of PAHs and chlordane in deployed mussels in 2001, but several questions remain to be answered. These include:

- What is the actual average concentration of contaminants to which the mussels are exposed at the caged mooring locations?
- What fraction of the Deer Island effluent, as well as the diluted effluent at the exposure locations, is dissolved and therefore most bioavailable to the deployed mussels?
- What are the average exposure duration, frequency, and concentration of the effluent plume experienced by the mussels?
- Are the present threshold values or deployment locations appropriate for the intended purpose of the fish and shellfish monitoring program?

4.4 Recommendations

As described in Section 4.3 and earlier, one source of uncertainty in these evaluations is the concentrations of contaminants to which the deployed mussels are exposed. Three approaches were investigated to help address this uncertainty during 2002 mussel deployments :field measurements of PAHs and pesticides in the offshore water column, better characterizing effluent concentrations of these compounds, and analyzing mussels from more than one deployment at a constant distance (60m) from the outfall.

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4.4.1 Offshore water column measurements

The challenge with offshore water measurements is that the contaminant concentrations are very low, and specialized, very sensitive, techniques are required for accurate quantification. This is exactly why caged mussel bioaccumulation deployments are routine monitoring tools. Mussels concentrate contaminants over a long period of time and even in clean water reach contaminant body burdens easily quantified with standard techniques. In addition, water samples are most often collected as grab samples, and different, more complex, sampling techniques are needed to determine average water column concentrations over the 60-day mussel deployment period. Mussel tissue concentrations, on the other hand, represent an integrated contaminant exposure (e.g., an average water column concentration).

The offshore analytical challenges were addressed through an evaluation of the analytical detection levels and sample volumes necessary to quantify dissolved and particulate PAHs and pesticides at the concentrations determined by Shea (1997) and estimated from the mussel tissue data for 2002. This approach was considered feasible, although expensive, as long as a small number of samples were required. However, initial estimates showed that adequate definition of the magnitude of spatial and temporal variability in the concentrations required collection of several dozen samples, spaced throughout the deployment. Moreover, development of techniques and equipment for the ultra-clean collection and processing of 24-hour composites in the field is required. The preliminary cost estimate for an adequate number of samples dwarfed that of the entire mussel study. Therefore , the concept was not pursued further, nor is it recommended.

4.4.2 Effluent Measurements

Contaminant concentrations are typically high enough in secondary effluent that standard trace-level analytical techniques can achieve the needed detection limits at reasonable costs. Moreover, understanding the distribution of organic contaminants between the dissolved and suspended phases can substantially further understanding of offshore exposure. Therefore, an expanded effluent measurement program was explored and the following recommendations made.

4.4.2.1 Technical Requirements

Effluent discharges are typically characterized by collecting and analyzing a 24-hour composite sample during typical flow conditions. Storm (high flow) conditions are often also sampled in this manner, to understand concentration changes during such atypical conditions. Collection of such composite samples are recommended for further evaluation of the offshore exposure concentrations

Collection of 2.5-L effluent samples for each organic contaminant analysis of interest (e.g., PAH, PCB, and pesticides) is sufficient for high sensitivity, compound-targeted, sample preparation and analysis methods. Partitioning of the dissolved and particulate phases by filtration through 0.7 µm glass fiber filters, with both the filter and filtrate extracted and analyzed separately is recommended. Standard gas chromatography/low-resolution mass spectrometry (GC/LRMS) of the extract following cleanup for the

PAH analyses and gas chromatography/electron capture detection (GC/ECD) for the PCB and pesticide analyses can achieve adequate quantification of individual compounds.

However, improved accuracy, at more expense, can be achieved using an isotope-dilution GC/LRMS method for the PAH analysis. Improved sensitivity and overall data quality can be obtained for the PCB and pesticide analyses using gas chromatography with high-resolution mass spectrometry (GC/HRMS). These methods are currently being successfully used at Battelle for characterizing POTW effluent in New Jersey to assess the contaminant loadings to the New York/New Jersey Harbor Estuary. The HRMS technique is particularly suitable for the chlorinated pesticide compounds (e.g., chlordane), which are susceptible to interferences and compromised data quality due to the complex nature of the effluent sample matrix, while comparable data quality (and sufficient sensitivity) can be obtained using LRMS for the PCB analysis. Evaluation of whether this improved accuracy is required for the effluent sampling program suggested below is ongoing.

4.4.2.2 2002 Sampling at Deer Island

The most effective way to supplement MWRA's ongoing weekly collection and analysis of organic contaminant samples in Deer Island effluent during the bioaccumulation deployments is under development. Some, if not all, of the samples collected for organic contaminants are recommended for both dissolved and particulate phase analysis as this will substantially improve predictions of contaminant bioaccumulation in the mussels deployed near the outfall.

Increasing the frequency of sample collection is also recommended although the specific requirements are under investigation. One approach is to continue to collect 24-h composite effluent samples on a weekly basis for the first month of the deployment, then to increase the frequency to 2 to 3 times per week over the last 30 days. This will allowdocument short-term variability in the effluent concentrations (e.g. "contaminant spikes") and provide more finely resolved data as the mussel come to final equilibration with the contaminants in the water phase. Other scenarios under consideration include collection of one or more long term composite sample (e.g. collected over the course of a week or a month), and the best sampling technique to capture storm related changes in effluent contaminant concentrations. Replicate effluent samples (i.e., paired samples from the east and west disinfection basin) are under considerations to ensure variability in the measurement program is well constrained.

4.4.3 Modifications to 2002 mussel deployments

To support further evaluation of the 2001 threshold exceedance, deployments of mussel arrays in 2002 will be similar with the exception of the 15 meter deployment, which will not be repeated. This means the outfall site deployment would consist of three mussel arrays 60 m from the diffuser line and one array at LNB). Arrays would also be deployed at other locations as in the past.

The multiple arrays located 60m from the outfall are intended to provide redundancy in case of vandalism or other damage to one or more array. If the arrays are successfully retrieved at each location in 2002, analysis of 4 samples from each of two widely separated 60 m arrays is recommended. This differs from the previous surveys in which all samples analyzed came from a single array. This recommendation will enable examination of variability in the bioaccumulation at this distance from the outfall without increasing the analytical cost to the program. One of the arrays included under this scenario will be from the same location as the threshold relevant mussels obtained in 2001. Finally, to retain comparability for comparison to the thresholds, all eight composite samples from the two 60 m arrays would be used in threshold testing.

4.4.4 Other Recommendations

Even with improved effluent data under the recommendations above, the analytical data must be coupled with a better understanding of the factors that influence the contaminant transport, fate, and exposure to ensure reliable understanding of the contaminant response in the mussel. These factors include the dilution, and how it changes with distance from the discharge, and effects of variable tidal currents on the plume and contaminant dilution. In addition, the amount of particle-bound, and relatively unavailable, organic contaminants in the gut of the mussels, and the degree to which mussels take up contaminants from filtered particles in addition to dissolved phase contaminants should receive research attention. Last, the impact of using different lipid content determinations in different data sources should be better understood, such that the bioaccumulation models can compensate for such differences.

Resolution of these latter uncertainties are beyond the MWRA monitoring program. Thus, MWRA's continuing evaluation of the 2001 mussel threshold exceedance should be on the areas that MWRA can most directly develop data, the effluent and 2002 mussel deployments. The other uncertainties provide both relevant and substantial topics for evaluation by the research community.

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