

## **Measurement of lysosomal membrane destabilization in mussels impacted by *T/V Dubai Star Oil Spill***

Final Report submitted to  
Office of Spill Prevention and Responses  
California Department of Fish and Game

Submitted by  
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**Problem Statement:** A fuel oil spill from the *T/V Dubai Star* occurred in San Francisco Bay on October 30, 2009 and the spilled oil was transported to the Alameda County shoreline. Mussels and other living organisms in this area were exposed to and potentially injured by the oil and toxic chemical constituents such as PAHs (polycyclic aromatic hydrocarbons) contained in the spilled oil. It is important to assess the potential injuries to living organisms in a timely manner considering that evidence of potential injuries can diminish over time as natural weathering occurs and lowers the levels of various toxic chemicals in the water column. Therefore, the lysosomal membrane destabilization assay, which can be conducted fairly quickly, was used as a screening tool to document potential injuries and help guide further possible damage assessment studies. The lysosomal membrane destabilization work was conducted on behalf of DFG-OSPR as part of the natural resource trustees' natural resource damage assessment arising from the *Dubai Star Oil Spill*.

**Background:** Fuel oils that are being used for marine vessels contain many toxic chemicals, especially PAHs. When oil spills occur near estuarine and coastal shorelines, organisms such as bivalves along the shorelines are exposed to elevated levels of toxic chemicals that can accumulate in their tissues quickly. Lysosomal membrane destabilization assay can be used as an indicator for the assessment of potentially adverse impacts of recent or current exposure of bivalves to toxic chemicals (Hwang et al., 2008).

**Objectives:** The objective of this study is to assess possible impacts of the *Dubai Star Oil Spill* on the health of mussels by measuring lysosomal membrane destabilization in haemolymphs.

**Methods:** Mussels were collected from two spill-impacted sites, Crown Beach wall (Elsie Roemer groin wall, ALD03) and Harbor Bay (Bay Farm Island, ALD12) and one reference site, San Leandro Marina (ALE04) along the Alameda County shoreline in San Francisco Bay. Mussel collection was done by staff of the Office of Spill Prevention and Response, California Department of Fish and Game. Collected mussels were transported on ice immediately to the Environmental

Quality Laboratory in the Department of Civil and Environmental Engineering, University of California, Davis. Upon arrival at the laboratory, lysosomal destabilization was measured as soon as possible.

## **Technical Plan**

### **Task 1. Preparation for lysosomal membrane destabilization assay**

Supplies were purchased as needed to be able to measure lysosomal membrane destabilization in mussels that were expected to be delivered around November 30.

### **Task 2. Measurement of lysosomal membrane destabilization in mussels**

Heamolymphs (50-100 mL) collected from each of 10 mussels were mixed with saline solution. An aliquot of the solution was incubated in a light-proof chamber initially for 30 minutes and additional one hour with red dye solution. Percent of cells having destabilized lysosomal membranes were scored using a light microscope.

### **Task 3. Data submission**

The results of lysosomal membrane destabilization were submitted to the program manager. Results are presented in the table below.

**Table 1. Sample details and results of lysosomal membrane destabilization assay.**

Sample ID	ALE04-113009-R11-1-MU	ALD12-113009-R11-3-MU	ALD03-120109-R11-2-MU
Sample Collection Location	San Leandro Marina	Harbor Bay / Bay Farm Island	Crown Beach wall / Elsie Roemer groin wall
Sample type	Mussel (live)	Mussel (live)	Mussel (live)
Collection date	11/30/09	11/30/09	12/01/09
Delivery date	11/30/09	11/30/09	12/01/09
Delivery time	19:00	19:00	19:00
Transported on ice (Y/N)	Yes	Yes	Yes
Dead samples during transportation	None	None	None
Received by	Hyun-Min Hwang	Hyun-Min Hwang	Hyun-Min Hwang
Storage condition	Cold room	Cold room	Cold room
Storage temperature (Celcius)	4	4	4
Storage period (Hours)	42	42	42
Dead samples during storage	None	None	None
Measurement date	12/02/09	12/02/09	12/03/09
Measured by	H-M Hwang & J Lim	H-M Hwang & J Lim	H-M Hwang & J Lim
	<b>Lysosomal membrane destabilization</b>		
Sample number	(%)		
1	45	35	40
2	30	NA*	60
3	NA*	40	65
4	30	30	70
5	40	30	50***
6	45	NA*	70
7	30	Too small**	50***
8	Too small**	Too small**	80
9	Too small**	Too small**	NA*
10	Too small**	Too small**	NA*
<b>Average</b>	<b>36.7</b>	<b>33.8</b>	<b>64.2</b>
<b>Standard deviation</b>	<b>7.5</b>	<b>4.8</b>	<b>13.6</b>

**Legend:**

\*: Mussels were not too small but failed to extract enough cells

\*\*: Mussels were too small to extract enough haemolymph

\*\*\*: The total number of cells was much less than in others and thus have a greater uncertainty.

## Summary of findings

Average lysosomal membrane destabilization in the mussels (Figure 1) collected from San Leandro Marina and Harbor Bay/Bay Farm Island was less than 50% and similar to each other, exhibiting that they were relatively healthier than the mussels from Crown Beach Wall/Elsie Roemer Groin Wall that had significantly higher ( $p < 0.01$ ) lysosomal membrane destabilization (64.2%). In the case of mussels from Harbor Bay/Bay Farm Island, meaningful measurement was made in only 4 of 10 mussels so the observed lysosomal destabilization is statistically less meaningful than the measured values for the other two sites.

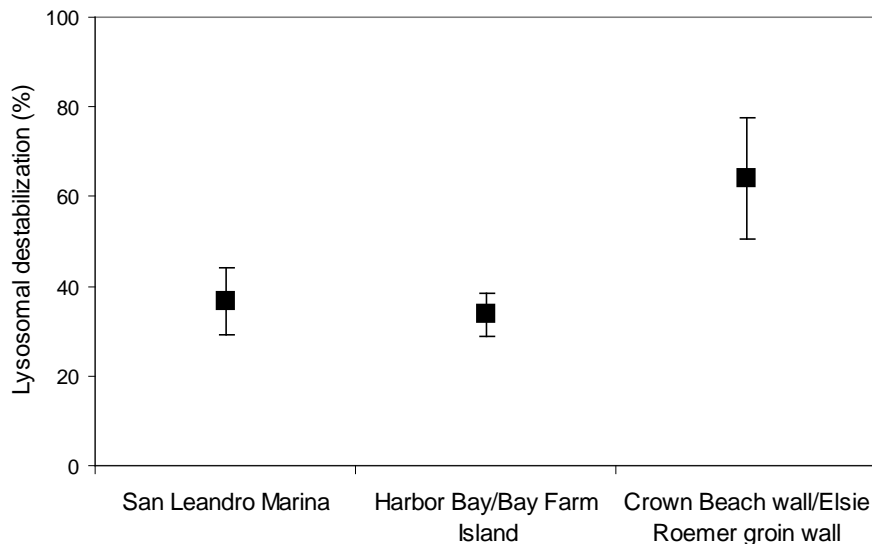


Figure 1. Lysosomal destabilization in mussels collected from the Alameda County shoreline in San Francisco Bay after the oil spill from a *T/V Dubai Star*.

Total PAH body burdens in mussels collected concurrently from San Leandro Marina and Crown Beach Wall/Elsie Roemer Groin Wall were 430 ng/g (dry wt.) and 19,600 ng/g (dry wt.), respectively (Entrix, 2010). PAH body burden was not measured for the mussels collected from Harbor Bay/Bay Farm Island on November 30, 2009, but was available for the mussels collected there on other dates. The PAH body burden for mussels collected at the same location on November 13, 2009 was 1,180 ng/g (dry wt.). Considering the fast decreasing patterns in PAHs over time observed in other locations, the PAH body burden in mussels collected on November 30 was likely much lower than this. For purposes of comparison, we assumed no change in concentration between November 13 and 30, 2009 and used 1,180 ng/g as the surrogate total PAH body burden in mussels collected from Harbor Bay/Bay Farm Island on November 30, 2009. The observed lysosomal membrane destabilization exhibited a positive correlation with total PAH body burdens (Figure 2). However, its statistical significance is weak because only three data points are available for the comparison. Hwang et al. (2008) reported that critical body residue (CBR) of PAHs was 2,100 ng/g (dry wt.) in terms of lysosomal membrane destabilization (at least 50% of destabilized cells) in eastern oysters

(*Crassostrea virginica*). Assuming this CBR in oysters is applicable to mussels without any conversion, total PAH body burdens in mussels from San Leandro Marina and Harbor Bay/Bay Farm Island were below the CBR and lysosomal destabilization was also less than 50%. PAH body residue in mussels collected from Crown Beach Wall/Elsie Roemer Groin Wall exceeded the CBR, suggesting that PAHs were likely responsible for the observed higher lysosomal membrane destabilization (64.2%). This study demonstrates that lysosomal destabilization could be used successfully as a rapid, cost-effective screening tool to identify exposure to and potential injury of bivalves from spilled oil as well as help guide further possible damage assessment studies.

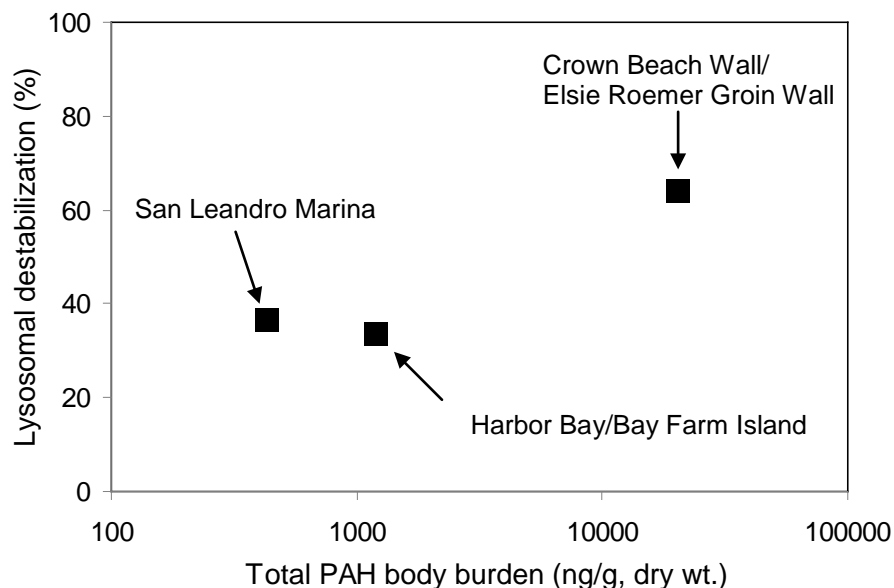


Figure 2. A plot of PAH body burdens versus lysosomal membrane destabilization, showing a positive residue-response relationship in mussels collected from the Alameda County shoreline in San Francisco Bay after the oil spill from a *T/V Dubai Star*.

## References

- Entrix. 2010. Dubai Star NRDA. Mussel sampling locations and results of 51 PAHs.
- Hwang, H-M., Wade, T.L., and Sericano, J.L. 2008. Residue-response relationship between PAH body burdens and lysosomal membrane destabilization in eastern oysters (*Crassostrea virginica*) and toxicokinetics of PAHs. *Journal of Environmental Science and Health, Part A*. 43, 1373-1380.

