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**Immuno-localisations (GSSP) of subcellular accumulation sites of
Phenanthrene, Aroclor 1254 and Lead (Pb) in relation to
cytopathologies in the gills and digestive gland of the mussel**

Mytilus edulis

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Abstract

Cell and tissue pathology of both, gill and digestive tissue, has been the subject of many studies for the elucidation of contaminant-induced biological effects. In the present study, cellular pathological alterations were linked to subcellular sites of chemical accumulation in gills and digestive gland tissues. For this purpose, mussels (*Mytilus edulis*) were exposed to the organic contaminants aroclor 1254 (PCB) (20µg/L), phenanthrene (PAH) (150µg/L) or the metal lead (Pb) (2.5mg/L). The localization of chemicals at the subcellular level was analysed by an antibody-based detection system (GSSP) by the use of commercially available antibodies specifically directed against the chemicals. Pathological changes were analysed in parallel in identical samples by transmission electron microscopy. After exposure to the different contaminants, cell organelles such as mitochondria, the endo-lysosomal system as well as endoplasmic reticulum showed clear evidence of chemically-induced

alterations. Large numbers of crystalloid inclusions were found in mitochondria and in autophagic lysosomes as well as multilamellated whorls after PAH and aroclor exposure. Immunocytochemical detection of the chemicals showed their accumulation inside of various cell organelles such as lysosomes, mitochondria, and nuclei. Additionally, chemicals were localized in association to membranes, cilia and microvilli of gill and digestive gland cells. Furthermore, the chitinous rod and mucus secretions of gill epithelial cells were positively labelled for contaminants indicating their role in protection. Localization of contaminants by immuno-detection in combination with pathological diagnosis gives insights into the cellular targets of chemical attack.

Keywords: Immuno-Localisations; GSSP; Subcellular Accumulation; Phenanthrene; Aroclor 1254; Lead; Cytopathologies; Gills; Digestive Gland; Mussel; *Mytilus edulis*

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Marine invertebrates and bivalve molluscs, in particular, accumulate high amounts of various environmental chemicals in specific cellular compartments for further metabolism and elimination. Histo- and cytopathology of both gills and digestive gland tissue of marine molluscs reflect the hazardous potential of environmental contaminants (Pipe & Moore, 1986; Sunila, 1988; Domouhtsidou & Dimitriadis, 2000; Livingstone et al., 2000). In the present work, we used both the pathological diagnosis and the ultrastructural immuno-localization of chemicals simultaneously to study subcellular contaminant-induced effects. Therefore, an immunocytochemical approach to localize different environmental chemicals was applied in parallel to histopathological diagnosis of subcellular changes.

Mussels were exposed via the seawater (one mussel per liter) to 150 µg/l phenanthrene (dissolved in acetone), 20 µg/l aroclor 1254 (dissolved in ethanol) or 2.5 mg lead nitrate for up to 10 days at 14 ±1 °C. Control series without the contaminants were carried out in seawater and with the solvent (acetone or ethanol). Seawater was changed every 24 hours and contaminants at the respective concentration or vehicle concentration for the controls were added.

For ultrastructural localisation of the organic contaminants and the metal lead we applied a pre-embedding technique and a signal amplification system based on Gold-Substituted Silver-intensified Peroxidase (GSSP) according to Van den Pol and Gorcs (1986) (Einsporn and Koehler 2007, in preparation). Monoclonal mouse anti-phenanthrene and mouse anti-PCB antibodies were purchased from Research Diagnostics Inc. (Flanders, NJ, USA) and mouse anti-lead from Biodesign International (Maine, USA). Visualization of chemicals and ultrastructural examinations were performed with a Zeiss EM109 electron microscope and digital image analysis system (Soft Imaging System's iTEM, Muenster, Germany).

Accumulation of contaminants was detected in association with various subcellular components of gills and digestive tissue. The endo-lysosomal system of the digestive cells is the site where the lipophilic chemical phenanthrene was detected. Reaction product of the PCB mixture aroclor 1254 was observed inside mitochondria of digestive duct cells and associated with mucus secretions and the chitinous rod of gill filaments. Lead accumulation was mainly detected in granules inside of lysosomes of both gill and digestive epithelial cells (Fig. 1), but also in the cell nucleus. Controls incubated without the anti-PCB antibody or DAB did not show any GSSP labelling. Immuno-labelling of non-exposed organisms for aroclor 1254, phenanthrene and lead did not result in the appearance of any GSSP reaction product.

In general, bioaccumulation and storage of organic contaminants is higher in tissues which are lipid-rich and contain high amounts of lysosomes such as the digestive gland. The lysosomal compartments accumulate diverse chemicals and pharmaceuticals such as metal ions, transuranics, asbestos, polycyclic aromatic hydrocarbons (PAHs), heterocyclics, antipsychotic drugs, and nanoparticles (Moore et al., 2006). In the present study, uptake of contaminants was expected to occur from the water column mainly in the dissolved form. However, it can not be ruled out that amounts of the highly lipophilic chemicals are taken up in the microcrystalline form and transported via the gut system to the digestive gland.

In parallel to the localization of chemicals by immuno-cytochemistry, various cytopathological changes were diagnosed after exposure to the different toxicants in gills and digestive tissue (Table 1). In the present study, reported subcellular alterations in the digestive gland were similar to those found in the gills concerning mitochondrial changes. However, the lysosomal elements of the digestive gland showed specific alterations such as aggregation of residual bodies, proliferation of autophagosomes and an increase in the numbers of multi-lamellated aggregates. Accumulation of chemicals inside the lysosomes may lead to severe alterations of these cell organelles with impairment of lysosomal functions.

An important finding of the present study is the detection of aroclor 1254 at the subcellular level inside of mitochondria. The observed localization of aroclor 1254 corresponds with several reported effects of aroclor 1254 on signal transduction systems such as calcium homeostasis, phosphoinositol hydrolysis, and protein kinase C in mitochondria (Tilson et al., 1998).

Mucus granules play an important role in the detoxification process by absorption of contaminants as shown in our study for aroclor 1254 and thereby prevention of entering of chemicals inside of other cells. Dimitriadis et al. (2003) localized Pb in mucus granules of the gill epithelium and Marigomez (2002) also detected the metal Zinc in association to extracellular mucus on the branchial lamellae. PCBs in association to the chitinous structures

might be explained by interactions of the chemicals with the positively charged glucosamide residues.

In other studies, various cellular and subcellular pathological alterations in Mytilidae have been observed after laboratory exposure to Pb, PCBs and PAHs (Sunila, 1988; Lowe and Clarke, 1989; Dimitriadis et al., 2003). In the present study, cytopathological alterations after Pb, PCBs and PAHs exposure in cell organelles and compartments suggest a close cause-effect relationship to the subcellular localization of the contaminants.

Our results show that the combined use of cytopathology and tracing of contaminants by immuno-detection are successful tools for the subcellular localization of contaminants and description of potential pathological effects at their target sites. Nevertheless, more studies in this field of research are needed to clarify the hazardous potential of different contaminants inside organisms and to understand their detailed mechanisms of toxicity.

References

- Dimitriadis, V.K., Domouhtsidou, G.P., and Raftopoulou, E. (2003). *Environmental Pollution*, 125, 345-353.
- Domouhtsidou, G.P., and Dimitriadis, V.K. (2000). *Archives of Environmental Contamination and Toxicology*, 38, 472-478.
- Livingstone, D.R., Chipman, J.K., Lowe, D.M., Minier, C., and Pipe, R.K. (2000). *International Journal of Environment and Pollution*, 13, 56-91.
- Lowe, D. M., and Clarke, K.R. (1989). *Aquatic Toxicology*, 15, 345-358.
- Marigomez, I., Soto, M., Cajaraville, M., and Angulo, E. (2002). *Microscopy Research and Technique*, 56, 358-392.

Moore, M.N., Icarus, A.J., and McVeigh, A. (2006). *Marine Environmental Research*, 61, 278-304.

Pipe, R.K., and Moore, M.N. (1986). *Histochemical Journal*, 18, 557-564.

Sunila, I. (1988). *Journal of Invertebrate Pathology*, 52, 137-141.

Tilson, H.A., Kodavanti, P.R.S., Mundy, W.R., and Bushnell, P.J. (1998). *Toxicology Letters*, 102-103, 631-635.

Van den Pol, A.N., and Gorcs, T. (1986). *Journal of Comparative Neurology*, 252, 507-521.

Figure caption

Fig. 1. Gill epithelium of a Pb-exposed mussel after incubation with the anti-lead antibody. Pb accumulation appears at granular inclusions inside of lysosomes (arrows). CM (cell membrane), LY (lysosome), MV (microvilli), N (nucleus)

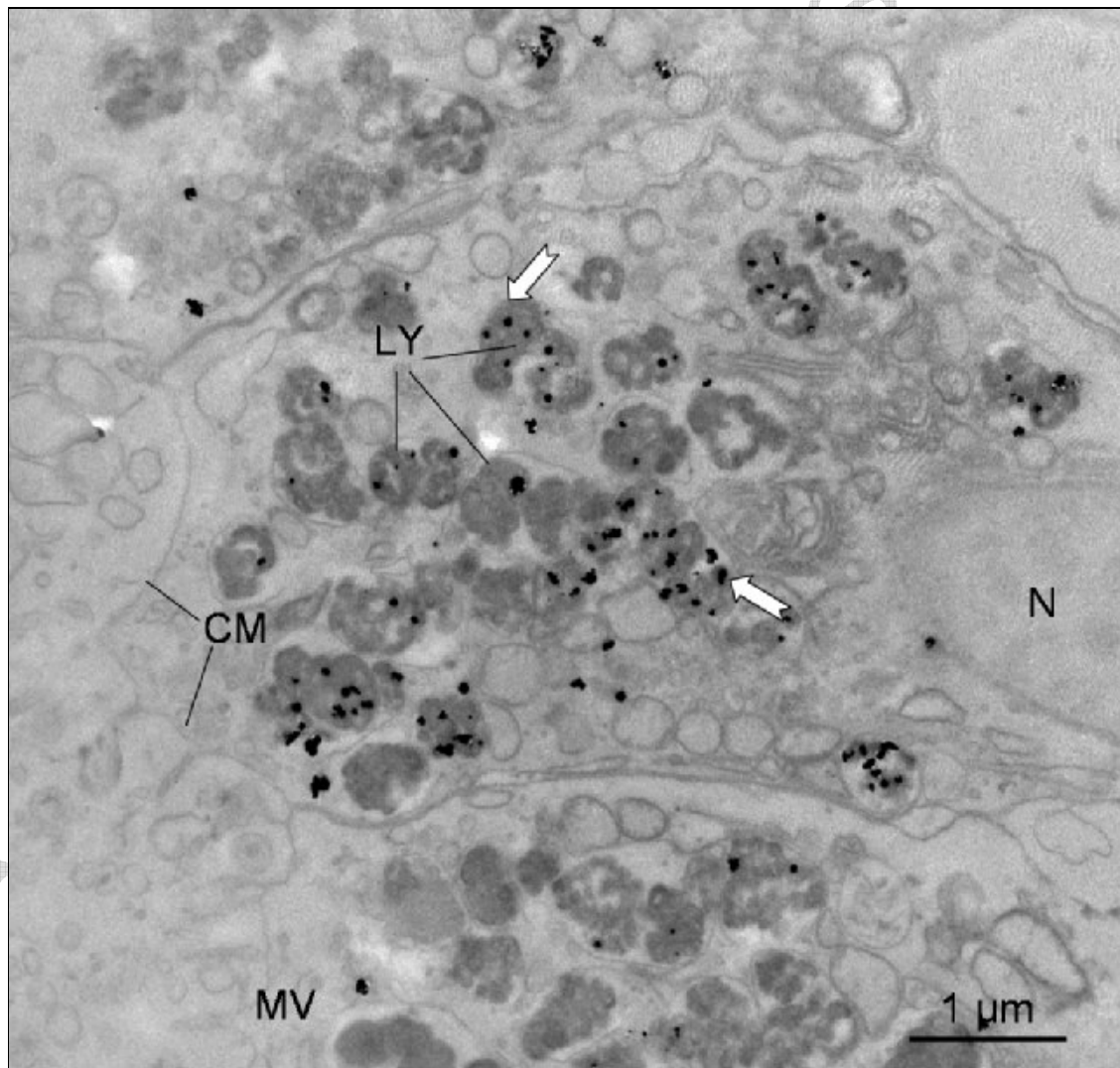


Table 1.

Ultrastructural changes after laboratory exposure to aroclor 1254 (PCBs), phenanthrene (PAH) and the metal lead (Pb) in gills and digestive gland of *Mytilus edulis*

Contaminant	Tissue	Lysosomes			Mitochondria		Golgi apparatus	ER	Nucleus	
		Proliferation of auto-phagosomes	Aggregations of residual bodies	Crystalline inclusions in lysosomes	Reduction of mitochondrial cristae	Crystalline inclusions in mitochondria	Augmentation of Golgi complexes	Formation of ER whorls	Deformation of nuclear envelope	Augmentation of nucleolus
PCB	Gills	-	-	-	++	-	-	+++	-	-
	Digestive epithelium	++	+++	-	++	+++	++	+	-	++
PAH	Gills	-	-	-	++	-	-	-	+	-
	Digestive epithelium	+++	+++	+++	++	+++	++	+++	+	-
Pb	Gills	-	-	-	+	-	-	-	++	-
	Digestive epithelium	++	+++	-	++	-	-	++	++	-