USING QUANTITATIVE ULTRASTRUCTURE TO MEASURE THE EFFECTS OF CONTAMINANTS ON THE FISH GILL

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The epithelial lining of the gill lamellae of fish is a good model for use in toxicological studies because its cells can be exposed to toxicant directly (in the ventilatory water), and because these gill cells are known to be highly sensitive to toxicants.

This study used a technique that will greatly aid the field of fish toxicology, a technique that can also revolutionize the discipline of histopathology in general. This technique uses **stereology**. (Stereology is the discipline that measures three-dimensional structures from two-dimensional sections through the structures.)

The technique measures the absolute volumes and surface areas of cells and subcellular structures from sections of gill tissue viewed by electron microscopy, so that treated fish (e.g., toxicant-exposed) can be compared to untreated, control fish.

The technique starts with sections of plastic-embedded gill tissue, cut strictly perpendicular to the lamellae. (Stereological techniques often require random sections from many planes, but they can be modified for use on single-plane sections.) The first value of interest is volume density (Vv), the ratio of the volume of a subcellular organelle to the volume of the cell that contains it (Bolender 1992). Volume densities are obtained simply by placing a grid over micrographs of the sectioned cells, and counting grid points: Vv = points on the organelle divided by points on the entire cell.

Volume densities are merely **relative** volumes, but absolute volumes are required (that is, the absolute volume of an organelle per cell). To get these absolute volumes, it first is necessary to find the absolute volume of an average cell, and then multiply that by Vv (that is: avg. cell vol. X vol. organelle / vol. of cell = vol. of the organelle in an avg. cell). Finding the key value, average cell volume, is not trivial, and has been a difficult problem in the past. It is determined as D/Na, where Na is the number of cell profiles per area in the sections and D is the absolute average diameter of the cell nuclei. D is the difficult parameter to measure. For the present study, I calculated D indirectly from the profile diameters and shapes of the sectioned nuclei (B-bar method: Gittes and Bolender, 1987), then confirmed my findings with modern, unbiased methods (Mayhew and Gundersen, 1996). Such modern methods now allow D to be measured simply by focussing through the nuclei within the block of gill tissue with a special microscope (optical dissector technique: Bolender and Charleston, 1993).

Stereological techniques measure surface areas as well as volumes. Surface density (Sv), the ratio of the area of a cellular membrane to the volume of the cell type containing that membrane, is easily measured from photographed sections (Bolender, 1992). Then, the absolute area of membrane per cell is obtained by multiplying Sv by average cell volume.

In the present study, I tested whether the stereological techniques were sensitive enough to detect subtle and specific effects of stressors in gill epithelial cells. I exposed fish (larval sea lampreys, *Petromyzon marinus*) to three very different types of waterborne stressors: Methylmercury, the insecticide Kepone, and heat shock. A range of lethal and sublethal, and long- and short-term exposures was used. Previous studies of the effects of toxicants on gill structure had concluded that such effects are generalized and nonspecific responses to stress, but past studies did not have

enough resolution to recognize any specific responses. I found specific responses: Methylmercury increased the volumes of the gill cells and most organelles, whereas heat shock, by contrast, decreased the sizes of most cell structures. These findings are consistent with the known biochemical effects of mercury and heat. (Mercury causes membranes to leak so that cells enlarge by taking in water, whereas heat causes cells to shed chunks of their cytoplasm.) Exposure to Kepone produced very few effects (some mitochondrial swelling, for example), but these effects were consistent with the known toxicological actions of Kepone.

Overall, my findings indicate that, with stereological techniques, fish gills can be used to identify the specific actions of different toxicants, an important new development in fish toxicology. With such specificity, it should be possible to better document the effects of waterborne contaminants on fishes.

References

Bolender, R 1992 Biological stereology: History, present state, future directions. Microscopy Research and Technique 21: 255-261.

Bolender, R, and J Charleston 1993 Software for counting cells and estimating structural volumes with the optical disector and fractionator. Microscopy Research and Technique 25: 314-324.

Gittes, F, and R Bolender 1987 Counting cell nuclei with random sections: The effect of shape and size. Micron Microscopica Acta 18: 59-70.

Mallatt, J, J Bailey, S Lampa, M Evans, and W Tate 1995 Quantitative ultrastructure of gill epithelial cells in the larval lamprey *Petromyzon marinus*. Canadian Journal of Fisheries and Aquatic Sciences 52: 1150-1164.

Mallatt, J, J Bailey, S Lampa, M Evans, and S Brumbaugh 1995 A fish gill system for quantifying the ultrastructural effects of environmental stressors: methylmercury, Kepone, and heat shock. Canadian Journal of Fisheries and Aquatic Sciences 52: 1165-1182.

Mayhew, T, and H Gundersen 1996 'If you assume, you can make an ass out of u and me': a decade of the disector for stereological counting of particles in 3D space. Journal of Anatomy 188: 1-15.