DEVELOPMENT OF SUBLETHAL ASSAYS OF TOXICITY: PCB METABOLITE CYTOSOLIC DISTRIBUTION AND EFFECTS

SCCWRP researchers are endeavoring to analyze the accumulation of toxicants by marine organisms and the partitioning of these toxicants into toxic and detoxicated pools within the tissues. Additionally, they are attempting to detect biochemical effects attributable to the non-detoxicated portion of the substance. In this preliminary report, David A. Brown and several associates* discuss their progress to date in investigating the distribution of oxygenated PCB metabolites (PCBols) in liver tissue of the kelp bass.

Just as certain heavy metals can be detoxicated by binding to metallothionein, PCBs and some other organic contaminants can be detoxicated by binding to glutathione, glucuronic acid, or sulfates. This has been shown for both mammals and fish. The detoxicating substances occur in a low molecular weight fraction of the cytosol that can be separated from the enzyme-containing fraction, and also the metallothionein fraction, by chromatography.

After exposing kelp bass in the laboratory to 0.9 mg PCB or PCBol/kg fish/day in food for 4 and 28 days, the researchers measured the effects in a number of ways. They examined the fish liver tissue, which has been shown to be the most important site of detoxication, for PCB and PCBol levels. Also, they examined liver tissue for partitioning of PCBol into glutathione, enzyme, and metallothionine pools; and for effects on various enzyme systems, metal levels, blood chemistry parameters and histological effects.

They were unable to observe marked effects of these levels of PCB and PCBol on liver tissue of fish exposed for four days. This was somewhat surprising, because these levels of PCBs are considered high; researchers working with flatfish have demonstrated reproductive abnormalities at levels lower than these.

It was also surprising since preliminary analysis indicated that exposure to PCBs or PCBols resulted in increases of PCBols in the enzyme pool (Figure 1), a site of toxic action for these metabolites.

The researchers found that PCBols increased in the enzyme pool even though glutathione was still available for detoxication. They speculate that other substances such as glucuronides or sulfates may play a more important role in detoxication than glutathione in these fish. Alternatively, they suggest that detoxication processes may be inefficient following acute exposure.

Besides occurring at measurable levels in the liver tissue, few effects of the PCBs or PCBols could be observed. Some severe histological effects were related to laboratory holding of the kelp bass, because both controls and exposed fish were affected while sea-caught kelp bass did not show such effects. In general, these laboratory-induced effects may be assumed to have overwhelmed any biological changes, such as those in blood chemistry, that may have occurred as a result of the PCB and PCBol exposures. The following results need to be considered in light of the laboratory effects:

1. Glutathione did not appear to be depleted in either PCB- or PCBol-exposed fish (Table 1).
2. The activities of several enzymes measured in this study were not altered by exposure to PCBs or PCBols for four or 28 days (Table 1).

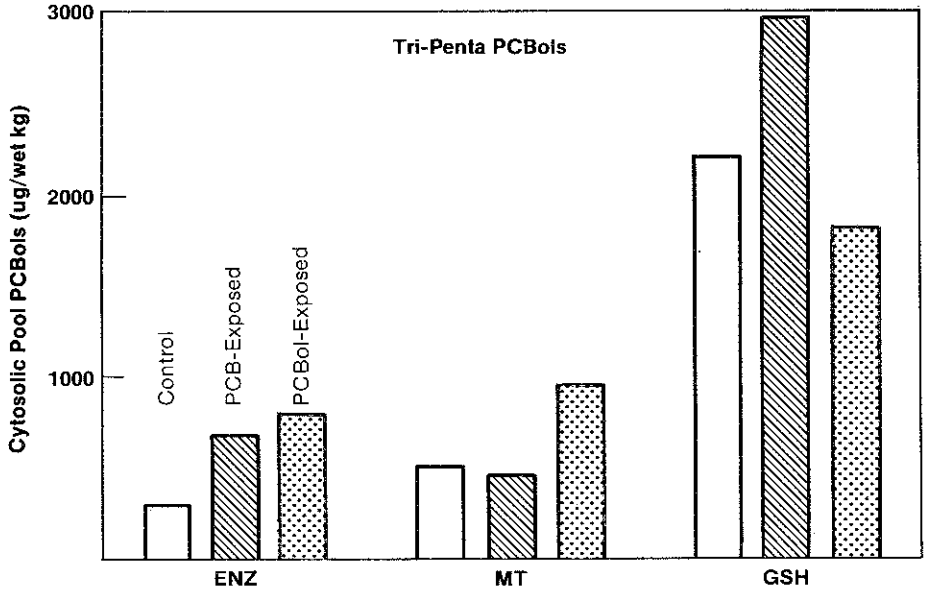

Figure 1. Concentrations of PCBol and PCBol-like compounds in each of the enzyme-containing (ENZ), metallothionein-containing (MT) and glutathione-containing (GSH) pools of kelp bass exposed to 0.9 mg PCB or PCBol/kg fish/day for 4 days.

Exposure		Homogenate Protein (ug/0.1 ml)	Cytosolic Glutathione (umol/g prot)	Catalase (K/g prot)
Control	4 Day	1636 ± 124	40.6 ± 2.4	2685 ± 748
PCB	4 Day	1864 ± 223	31.5 ± 7.8	2787 ± 572
PCBol	4 Day	1800 ± 264	38.1 ± 6.4	2977 ± 112
Control	28 Day	2013 ± 138	24.9 ± 2.4	3223 ± 138
PCB	28 Day	2065 ± 122	27.8 ± 0.4	2723 ± 518
PCBol	28 Day	1717 ± 74	26.9 ± 1.7	2928 ± 276

		Superoxide Dismutase (U/g prot)	Se-GSH-Peroxidase (U/g prot)	Glyceraldehyde Phosphate Dehyd (U/g prot)
Control	4 Day	25700 ± 6310	27.5 ± 5.9	3524 ± 190
PCB	4 Day	22230 ± 2810	28.6 ± 4.3	3880 ± 415
PCBol	4 Day	29720 ± 3190	31.5 ± 1.5	3805 ± 359
Control	28 Day	37880 ± 1730	13.1 ± 4.4	3940 ± 201
PCB	28 Day	41270 ± 3150	13.6 ± 2.1	3708 ± 37
PCBol	28 Day	36000 ± 1490	11.3 ± 2.1	3873 ± 122

Table 1. Glutathione concentrations and enzyme activities in livers of kelp bass exposed to 0.9 mg PCB or PCBol/kg fish/day for 4 or 28 days. Mean ± standard error. N=2 to 3 tanks/exposure with the value for each tank calculated from the mean of 2 fish per tank.

3. Body weight, liver weight, and liver somatic index were unchanged by the exposures. Some blood chemistry parameters including glucose, urea nitrogen, sodium, chloride, cholesterol and alkaline phosphatase were altered following 4-day exposures to PCB and PCBol but not following 28-day exposures. Concentrations of cadmium, copper and zinc in various subcellular pools were not altered by four or 28 days exposure to PCBs or PCBols. However, metal levels in these kelp bass were low to begin with; perhaps further reductions could not be expected. The measurements referred to here are reported in Brown et al. (1986).
4. Histological examination of livers, gills, kidneys, and intestines from both 4- and 28-day exposures showed that the only lesion attributable to PCB or PCBol exposure was the occurrence of certain inclusions in hepatocytes (liver cells), containing phospholipids and some protein (Perkins, 1986). Otherwise, the pathology occurring in liver, kidney and gill tissues of laboratory-held fish was much more severe than in fish sampled directly from the sea.

Brown and his co-workers suggest that the observed increase of phospholipid-containing inclusions in PCB- and PCBol-exposed fish may merit further investigation. They note that recent studies have shown that xenobiotic promoters such as DDTs and PCBs exert much of their effects by acting on membrane receptors, thereby altering phospholipid metabolism. This important cell function involves a number of families of enzymes and ultimately affects cellular growth and regulation (Hokin, 1985).

Further work needs to be done to complete the investigation. Besides the liver, other tissues need to be tested for sensitivity to PCBs and PCBols. And because of the sensitivity of kelp bass to laboratory holding conditions, better seawater facilities will be required in order to conduct the exposures properly.

* S. M. Bay, D. J. Greenstein, P. Szalay, R. W. Gossett, C. F. Ward, A. M. Westcott, K. D. Rosenthal and E. M. Perkins

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