

## ANNEX 7

### Using the micronucleus test and the single cell gel (comet) assay to study clastogenic effects of PCB77 in fish

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PCBs (polychlorinated biphenyls) are stable pollutants found in almost every compartment of terrestrial and aquatic ecosystems. They are highly lipophilic and therefore have the potential of accumulating in the fat stores of animals. Aquatic species (mammals and fishes) in particular undergo health problems caused probably by the PCBs. The mechanisms by which PCBs exert their adverse effects are still unclear. It is known that PCBs induce some important biotransformation enzymes, but their mutagenic properties remain controversial.

Our aim was to determine the DNA breakage and clastogenic potential of a planar PCB (PCB 77) *in vivo* in fishes, using the single cell gel electrophoresis (SCGE) and the micronucleus (MN) assay on erythrocytes of brown trout exposed for 3, 9, and 14 days to 4 ng/ml PCB 77 in water. Blood was taken by a caudal puncture and the erythrocytes were either deposited in the agarose gel (0.6%) for the SCGE or smeared directly on slides for the MN assay. Five fishes were studied per treatment. Fifty and 2000 erythrocytes per concentration were analysed for SCGE and MN, respectively. EMS (ethylmethanesulphonate) at a concentration of 25 mg/l was used as positive results for PCBs were found.

In parallel PCB 77 was also tested on human lymphocytes. The lymphocytes were exposed for 30 minutes, 1 hour, 3 hours, and 48 hours to PCB 77 (0.01, 0.1, 10, 25, 100 µg/ml). In contradiction of a previous finding (Sargent *et al.*, 1989), no increase of either DNA SS breaks or MN was observed. The different results may be related to the fact that, in our experiment, the PCBs were tested at a final DMSO concentration of 0.5%, instead of 2% as for Sargent.

The data indicate that PCB 77 does not have clastogenic properties in human lymphocytes or fish erythrocytes.