Bioaccumulation and estrogenic effects of DDT, Arochlor 1254 and their 1:1 mixture on Zebrafish (*Brachydanio rerio*)

Abtract

The rate of accumulation, kinetic of elimination and the metabolism way of many chlorinated hydrocarbons (e.g. DDT, PCBs) have been in the last year sufficiently cleared up. Until today, their effects against the specific organisms were still a subject of controversial discussions. It was also the case for potential endocrine effects to influence the spermatogenesis correlated with possible changes of the population's vitality. Through a critical Literatur-Recherche, evidence is produced with special regard to substance mixture (e.g. PCBs) that as a matter of fact, the analysis of residues was not strongly discussed. However because of the lack of sufficient standard quality of laboratory safety mechanisms, the ecoeffects were scarcely taken into consideration

Substances with estrogenic effects are from special interest naturally ecosystematic. To clear this situation, three questions could be at the centre of attention:

1. Do the chemicals cause a special harmful effect of the male reproductive tract?
2. Could some particular chemical mixtures act to bind and activate the human estrogen receptor (hER)?
3. Are the life stages of an organism specially sensitive to the effects of chemicals and therefore be established as Screening- Test- System?

In the present study, the connected effects of DDT and Arochlor 1254 (A54) as single substance and in 1:1 mixture according to their estrogenic effectiveness on zebrafish (*Brachydanio rerio*) were therefore investigated.

For that, the short and long time exposure tests with the different tested concentrations and mixture of the chlorinated hydrocarbons were first of all checked.

The concentrations of the tested substances and their mixture ranged between 0.05 µg/l and 500 µg/l and separated by a factor of 10. It was turned out that the test concentrations of 500 µg/l were too toxic to zebrafish in all the cases. The LC50 values of DDT, A54 and the chemical mixtures were determined. The analysis of all the test concentrations showed that the rate of mortality was higher during the exposure to the chemical mixtures. Therefore the amount of chemicals absorbed by zebrafish was checked. For that the static fish test "305D" of the OECD guidelines for testing of chemicals was used (Bioaccumulation test). Afterwards according to the data established, the experiment was followed up with four concentrations of DDT, A54 as well as their 1:1 mixture anew each separated by a factor of 10 and ranging

between 0.05 µg/l and 50 µg/l. The Bioaccumulation test within 8 days showed that the zebrafish really picked up and accumulated the chemicals, but in all the cases no equilibrum was reached. The concentration of 0.05 µg/l was beared by the zebrafish before the end of the eighth day, later the chemicals were not more detectable (NOEC-values). At the other tested concentrations, the bioaccumulation factor within eigth days (BCF8days) increased with higher levels of the tested chemicals. The values of the BCF8days tests demonstrated that the zebrafish absorbed more DDT than A54.

Putting up on these analyses, the experiment was followed by the investigation of the life cycle (LC) of zebrafish. First of all, the eggs were exposed to the different tested concentrations of both chemicals and their mixture. It was turned out that the chemicals caused significant changes in the rate of hatchability and the reproduction and also influenced the length of juvenile fish. These three parameters were tightly correlated with the chemical content. In all the cases, no adult fish survived at the concentration 50 µg/l. Over and about that there was a deviation between the expected and the experimental durations of the life cycle stages (LCS), which were found delayed for few to many days according to the levels of the pesticides. The investigation of the life stages up to 6 weeks in the F-II generation demonstrated that the development of the life cycle stages lasted longer when the mixture of the chemicals was tested, than when the chemicals were tested alone and also that the fish seemed to tolerate more A54 than DDT.

During this investigation, the length of fish emerged were evaluated on fixed spcimens. By means of the Analysis of Variance (ANOVA) to compare the results, the F-ratio at degree of freedom (4,40) were 1.37, 1.65 and 0.88 for DDT, A54 and their mixture respectively. The length of fish was found more reduced with higher concentrations and more significant when the fish emerged in test chambers which were contaminated with the chemical mixture. The reduction in the rates of hatchability, adult survival and the population size, the retardation of the developmental stages of the LC and the inhibition of growth of test organisms by the chemicals and their mixture could have been due to the disorders that they caused in the male reproductive tract. That is why the quality, quantity and the duration of the activity of sperm released by the male zebrafish exposed to the different tested chemicals during mating events within a certain period were investigated using the methods of Leong (1988) and Shapiro et al (1994). The samples were collected gradually after 24 hours, 2 weeks, one and two months of exposure to the different tested concentrations of the chemicals. They showed that the decrease in sperm counts registered was closer to be significant with long time exposure of fish to the pesticides. The levels of the chemicals and the exposure time reduced thereafter the

number, activity and life span of sperm released by the treated male fish. These reductions were more significant when the test organisms were exposed to the pesticide mixture.

To know more about the causes of sperm degeneration, the examination of the spermatogenesis and the structure of the testes were carried up. For this, the testes were embedded in EPON 812 resin and cut in semi and ultrathin sections which were observed under light and electron microscope respectively.

Under light microscope, a reduction of the spermatids content in the lobule lumen of the testes was clearly demonstrated. This reduction in the number of the spermatids increased with higher tested concentrations and longer exposures of fish to the chemicals. As well, the efferent ducts were found compressed by the effects of chemicals leading to the enlargement of the interlobular space which become more delimited and bigger as the tested concentrations increased. At this level , the investigation was followed by seeking the causes of the reduction of the late stage of spermatogenesis with the electron microscope. It was observed that after 2 months of exposure to the chemicals, in the late stage of spermatogenesis were mostly the primary spermatids present, very few and sometimes no secondary (SpII) and tertiary spermatids (SpIII) which consequently reduced also the number of mitochondria. Thereafter, it was found out that there was no heterophagic vacuoles whose presence in the cytoplasm is a sign of phagocytic activity for germinal components.

In general, the absence of phagocytizing activities which play an important role in the spermatid maturation could be the reason of the scarcity of spermatids in the lumen. Finally the inhibition of the development of cytoplasmic organelles such as mitochondria could be an explanation to the reduction of sperm activity and life span released during mating events.

In the present study, it could be shown that, DDT and A54 could act synergically and cause disorders of the male zebrafish reproductive tract, reduction of the growth and changes in the spermiogenesis.