Bull. Environ. Contam. Toxicol. (1997) 59:802-806

Bulletin of Environmental Contamination and Toxicology

Increase of Plasma Vitellogenin Concentration in Rainbow Trout (*Oncorhynchus mykiss*) Exposed to Effluents from Oil Refinery Treatment Works and Municipal Sewage

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Received: 16 March 1997/Accepted: 20 August 1997

The aim of the present study was to examine whether effluents from an oil refinery with new "best available technology" for the effluent treatment may contain estrogenic substances. Standard municipal sewage has been shown to contain such compounds (Purdom *et al.* 1994), but few, if any studies have been carried out on highly purified refinery effluent. For comparison, and as a positive control, studies of municipal sewage were also done. Vitellogenin is produced in the liver of female fish and is the precursor of egg proteins. Its production is induced by estrogens (Tata and Smith 1979) and is normally not synthesised by immature fish or males. As an indicator of estrogenic compounds in these effluents, we used the increase in plasma vitellogenin concentrations in male rainbow trout (*Oncorhyncus mykiss*) after and during exposure (Sumpter and Jobling 1995, Purdom *et al.* 1994)

MATERIALS AND METHODS

The effluent from the ESSO - Slagentangen Oil Refinery was chosen because the toxicity of process chemicals and effluents from this refinery has been studied (Riisberg *et al* 1996; Roseth *et al* 1996). This refinery is located at the outlet of the Oslofjord where the hydrodynamic conditions seem to be satisfactory, and no serious impact on water quality has been recorded (Jensen *et al. 1992).* The treatment works receive waste water from the oil refining process, drainage and ballast water. The three streams are mixed and stored in a large tank in order to smooth out variation in salinity. Thereafter the effluent is purified by skimming and sedimentation before being fed into the biological treatment system. This last section is a BIOX™ plant and has an aerobic and an anaerobic compartment. It was introduced in 1993 in order to meet the legal specifications for phenolics and "oil in water". According to typical routine analysis carried out at the refinery, 105 m^3/hour- of effluent are produced, containing < 1 mg/L dissolved oil, and *ca.* 0.2 mg/L phenolics. The total organic carbon (TOC) is 16 mg/L, whereas NH_3 plus NH_4' is < 0.1 mg/L. The municipal sewage plant receives domestic waste water from Oslo. It is located at Bekkelaget at the inner part of the Oslofjord. TOC was 23.2 mg/L and NH_3 plus NH_4' , 24.9 mg/L. Rainbow trout (mean weight 350 g) of both sexes purchased from a commercial fish farm were used in the experiments. Both immature and mature fish were employed. The fish were anaesthetized with

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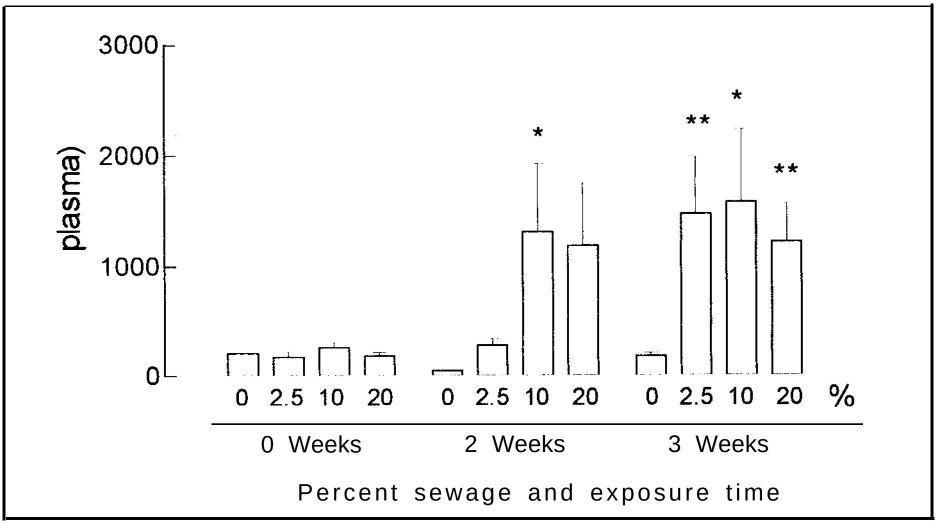

Figure I. Vitellogenin in male rainbow trout plasma after exposure to mixtures of pure water and sewage for the times indicated. Each value is the mean from 5 to 9 fish, with SEM given as bars. Kruskal-Wallis' one-way ANOVA shows extreme significance when the controls were pooled. Dunn's post test for comparism of groups showed significant difference from the controls as indicated (**, $P < 0.01$; *, $P < 0.0.5$).

3% benzocaine, weighed and measured. Blood was sampled, and each fish was individually marked with a numbered tag attached to its first dorsal fin. Sixteen fish were released into each of the four 500 L tanks at each locality.

The tanks received high quality drinking water mixed with 0% (controls), 2.5%, 10% or 20% effluent or sewage at a rate of 330 L per hour. The mixing was mainly passive, but enhanced by the movements of the fish. The fish were fed every second day. Throughout the experiments the pH, the water temperature and oxygen saturation were recorded (pH=5.5-7; T=9-11°C; O_2> 70% saturation). Blood samples were taken from the fish which were weighed and measured after one (oil refinery treatment works experiments only) and after two weeks. At the end of the exposure period (three weeks) all fish were killed and blood was sampled. Fish weight and length, the sex, the degree of sexual maturation, and the liver and gonad weight were each noted in order to calculate the gonadosomatic- (GSI) and the hepatosmatic- (HSI). These indices are the gonadal wet weight and the hepatic wet weight divided by the total fish weight respectively. The condition index (CI) was calculated as (fish weight)/(fish length)^3

Vitellogenin analyses of the samples from the male fish were performed by homologous radioimmunoassay as described by Sumpter (1985). Cortisol was also measured in all blood samples to evaluate a possible effect of experimental stress (Carrangher *et al.* 1989; Pottinger and Pockering 1990). Analysis was performed

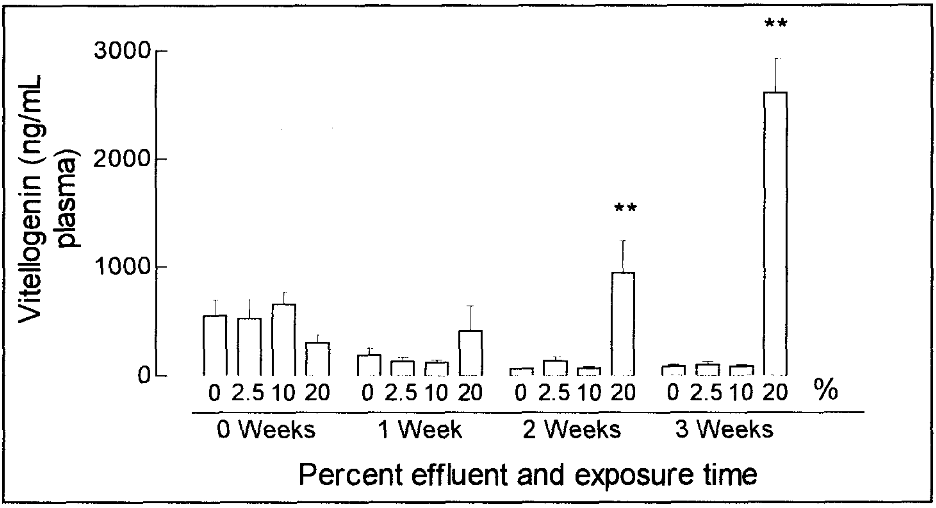

Figure 2. Vitellogenin in male rainbow trout plasma after exposure to mixtures of pure water and oil refinery effluent for the times indicated. Each value is the mean from 5 to 9 fish, with SEM. Statistical analysis was carried out as in 1ig. I.

using radioimmunoassay as described by Olse en *et al.* (1992).

RESULTS AND DISCUSSION

In the control group at the sewage works there was no significant change in plasma vitellogenin concentration during the experimental period (Fig 1). The values were similar to those previously reported for unexposed rainbow trout (Sumpter and Jobling 1995; Joblin g*et al.* 1996) In all exposed groups, plasma vitellogenin concentration increased during the experimental period (Fig. 1). However, no changes in GSI, HSI or CI were found in any group.

At the oil refinery (Fig. 2) a marked increase in vitellogenin was seen at the 20% effluent level, in all but one fish (which died after two weeks). As shown in Fig. 2 the plasma concentration of vitellogenin in the fish used at the oil refinery was slightly elevated at the start of the exposure period. The rainbow trout used at the oil refinery were taken directly from a farm with a high density of fish at different stages of sexual maturation. It could therefore be that excreted estradiol from females elicited a low vitellogenin increase in males kept in close confinement. Interactions between sexes due to steroids released from the fish into the water are reported (Van Weerd and Richter1 I991).

Stress is known to reduce plasma vitellogenin levels in fish (Carranghe *et al.* 1989; Pottinger and Pickering 1990), but no significant differences were found in plasma cortisol concentrations between the fish groups (results not shown). Stress is therefore not likely to have caused the observed vitellogenin difference.

The oil refinery has an efficient mechanical and blological effluent treatment system, and the toxicity of the effluent is low with an LC_{50} for rainbow trout > 20% after three weeks exposure. There has been no response in the *Microtox*™ test system (unpublished data from routine testing). The vitellogenin response obtained in this study was moderate, and a rather high concentration of effluent had to be used in order to produce an effect.

The results clearly indicate that oil refineries, even with the best available effluent treatment technology may contain bioactive substances. These substances must be rather recalcitrant to biodegradation and may therefore represent an environmental hazard. “Upstream” efforts, for instance removal of certain process chemicals, may be more cost effective than a further purification of the effluent (Siljeholm 1996). Chemical and biological studies are now being carried out in order to identify the active substances.

Acknowledgments. We thank the personnel at Bekkelaget sewage treatment works and ESSO-Slagentangen for practical help during the field experiments. This work was financed by the Norwegian Research Council and the ESSO-Slagentangen refinery.

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