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# Toxic Effects of Acute Exposure of Diazinon in turbot (*Psetta maxima*) Early Life Stage (ELS)

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**ABSTRACT:** In the present study, the toxic effects on the embryos and larvae of the turbot were used as a model to investigate the diazinon, which contaminates aquatic ecosystems. The number of dead embryos significantly increased in response to diazinon concentrations 0.2, 0.4, 0.8, 1.6, 3.2, and 7.4 mg/L. The 48h $LC_{50}$ value of diazinon for turbot embryos was estimated at 1.85 mg/L. Dose–response decreases in hatching success were recorded as 97, 92.4, 80.3, 60.3, 38.7 and 27.1%, respectively. The number of dead larvae significantly increased with increasing diazinon concentrations exposed for 24–96 h ($p < 0.05$). The 24, 48, 72 and 96h $LC_{50}$ values of diazinon for turbot larvae were estimated at 4.8 (6.4-5.12), 3.3 (4.73-2.52), 2.1 (3.29-1.65) and 1.23 (0.87–2.38) mg/L, respectively. There were significant differences in the $LC_{50}$ values obtained at different exposure times ($p < 0.05$). Diazinon caused lethal toxicity as well as nonlethal malformations during embryo-larvae development.

**Key words:** Turbot, Embryo-larvae, Acute toxicity, Sublethal effects, Diazinon

## INTRODUCTION

Many of the more than 1000 pesticides currently used in most of the countries of the world inadvertently reach aquatic ecosystems (Sucahyo *et al.,* 2008). pesticides are potentially toxic substances released into the environment in large amounts with the potential to cause adverse effects on human and wildlife populations (Galloway and Handy, 2003). Accordingly it may induce several behavioral and histopathological changes in fish and other aquatic species (Novak, 1992). Reduced biological phenomenon's like growth, reproduction, metabolic alterations as reflected by enzymatic stimulation or inhibition (Regoli and Principato, 1995). Diazinon belongs to the organophosphates, the most extensive and manifold group of insecticides. Because of its aquatic distribution, diazinon affects a wide range of non-target organisms, like invertebrates, mammals, birds and fishes, especially those inhabiting aquatic environment (Burkepile *et al.,* 2000). Diazinon toxicity vary widely and depend on organism age, weight, sex and climatic conditions. Sublethal doses may lead to reduced growth and reproduction in aquatic invertebrates, reduced emergence in stream insects, interference with algae-invertebrate interactions, reduced egg-production, decreased food-intake and body weight loss in birds, offspring with brain pathology, delayed sexual maturity and adverse behavioral modifications in mammals. In fishes, exposure to diazinon in sublethal doses is known to affect the nervous system, abnormalities in the gills, changes in the immune system and reproduction (Dutta and Meijer, 2003). Acute toxicity tests of adult fish using diazinon have shown that 96 h $LC_{50}$ values vary by several orders of magnitude between species (Ferrando *et al.,* 1991). Early ontogenetic stages of fish are generally regarded as the most sensitive life-history stages to toxic agents. This is important since during development sensitivity may change with some compounds showing higher sensitivity in embryos whereas others are more toxic to larvae. During early ontogenesis, critical development of tissues and organs takes place, a process which can easily be disrupted by unfavourable environmental conditions including exposure to toxic compounds (Foekema et al. 2008). In fish early life stage tests, toxicant effects can be examined through diverse endpoints, such as hatching success, embryo-

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larval morphology malformations and larval survival (Mhadhbi *et al.*, 2010). Little is known about the toxicity of diazinon to fish during these developmental stages.

Our objective was therefore to tests the toxicity of the diazinon environmentally most abundant pesticides on the early life stages (ELS) of turbot. The turbot was selected for the bioassay experiment because of it is widespread and presently cultured all over most parts of Europe.

## MATERIALS & METHODS

Turbot (*Psetta maxima*) eggs from a single stock of adults were obtained in kind from a fish hatchery (Insuiña S.L., Mougás, Spain). Eggs were transported to the laboratory in plastic bags inside portable ice-boxes, and maintained in aquaria with running natural seawater (salinity 34‰). Diazinon was purchased from (Sigma Chemical Co., St. Louis, MO). Stock solutions of Diazinon were dissolved in Dimethyl sulfoxide (DMSO, Sigma–Aldrich, Steinheim) and stored in amber-glass vials. Six concentrations in a 2x geometric scale, plus one control with no diazinon added were tested, using four replicates for each condition. Nominal concentrations 0.2, 0.4, 0.8, 1.6, 3.2 and 7.4 mg/L were used. All treatments, including controls, contained <0.01% (v/v) DMSO which was constant in all treatments for a given experiment. At the start of the first test, the stock solutions were prepared in sufficient volumes and stored in the dark at room temperature. Glass vials containing at least 500 ml of each exposure solution and 50 eggs per condition were used. Incubations were made in 1000 ml glass beakers, to avoid losses of the tested compounds from the solutions. The experimental design followed the recommendations from OECD guidelines [1] (OECD, 1998). The deviations from the guidelines are the higher number of eggs per experiment and the number of reported non-lethal endpoints. All of the experiments were performed using a semi-static test with water renewal every 48 h. Immediately after fish arrival at the laboratory, within 72 hours post-fertilization, the floating fertilised eggs were collected and the non-fertilised eggs at the bottom discarded. The eggs were examined under a dissecting microscope, and those embryos exhibiting normal development that had reached the blastula stage were selected for subsequent experiments. Briefly, 50 normal fertilized eggs were randomly selected and carefully distributed into exposure glass beakers containing 500 ml FSW. Treatments were incubated per quadruplicate in an isothermal room (18 ±1ºC), in dark. Neither food nor aeration was provided during the bioassays. Eggs were transferred into each beaker from the lowest to the highest concentration to minimize the risk of cross-contamination. The effects of the toxicants on turbot embryos and larvae were observed daily throughout the 6-day exposure period. Survival and malformation of larvae were observed and recorded every day after hatching. Mortality was identified by coagulation of the embryos, missing heartbeat, failure to develop somites and a non-detached tail en larva.

Differences between treatments were tested for significance by means of one-way analysis of variance (ANOVA). When differences among groups were significant, the Dunnett's test was employed to compare the control group to each of the experimental groups for calculation of No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC). Statistical significance was set at $p \leq 0.05$. The $LC_{10}$ and $LC_{50}$ and their 95% confidence intervals (95CI) were calculated according to the Probit method after normalizing data to the mean control response using Abbot's formula (Emmens 1948). For analysis, data were first arcsine-transformed to achieve normality (Hayes 1991). The results of the experiments conducted in the dark and under fluorescent light were analyzed using two-way ANOVA. Also, significant differences between parameters for pairs of curves were tested using an extra sum-of-squares F-test to determine if curves were statistically indistinguishable (Motulsky and Christopoulos 2004). Statistical analyses were conducted using the SPSS version 19.0 statistical software.

## RESULTS & DISCUSSION

The results show that diazinon is moderately toxic to turbot. The toxicity of diazinon on turbot fish increased with increasing concentration and exposure time. Diazinon exposures caused significant reduction of hatching success and larval survival according to monotonic dose: response patterns, which allowed the calculation of toxicity parameters, as well as identifying a number of body malformations (Fig. 1, Table 1 and 2). With increasing diazinon concentrations, the fish exposed duration 24 to 96 h had significantly increased number of dead turbot ($p < 0.05$).

In the larval stage, the number of dead larvae significantly increased with increasing concentrations exposed for 24–96 h ($p<0.05$).

There were significant differences in number of dead larvae between the exposure times 24–96 h in each concentration ($p<0.05$). The highest concentration of 7.2mg/L showed the highest larvae mortality. It has been observed that increasing diazinon concentrations had significant effects on hatching success. For example, when turbot embryos were exposed to 0.4 mg/L diazinon, 92.4% hatched whereas only 27.1% hatched when exposed to the 7.4 mg/L diazinon

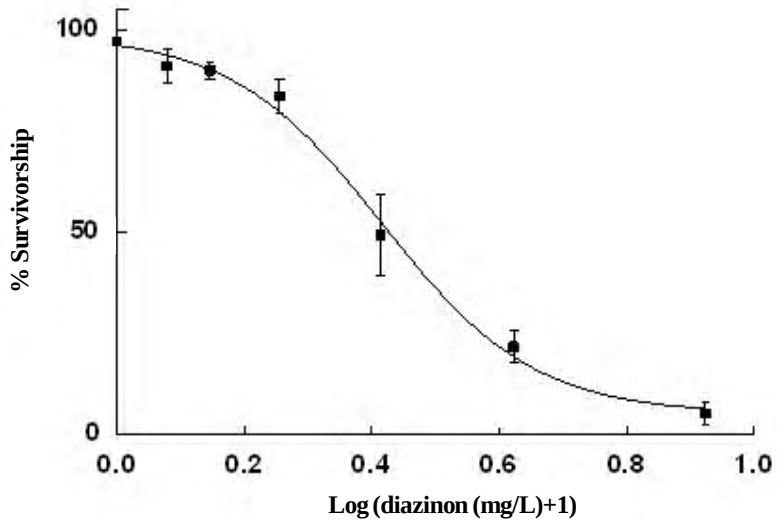


**Fig. 1. Dose–effect curves of lethal malformations of turbot embryos caused by diazinon at 96 h (mean value standard ± error)**

**Table 1. Turbot percentage hatching (48h) and larval survival (96h) exposed to diazinon**

| Diazinon | [µg/L] | Embryonal stage | | Larval stage | | | |
|---|---|---|---|---|---|---|---|
| | | Number of dead embryos | Hatching success (%) | Number of dead larvae | | | |
| | | | | 24 h | 48 h | 72 h | 96 h |
| | 0.2 | 4 | 97 | - | 3 | 5 | 8 |
| | 0.4 | 8 | 92.4 | 1 | 3 | 6 | 13 |
| | 0.8 | 23 | 80.3 | 4 | 11 | 17 | 34 |
| | 1.6 | 88 | 60.3 | 8 | 77 | 72 | 87 |
| | 3.2 | 77 | 38.7 | 10 | 73 | 123 | 114 |
| | 7.4 | 63 | 27.1 | 13 | 99 | 167 | 192 |
| Control | - | 5 | 96 | 3 | 4 | 6 | 5 |

**Table 2. Morphological abnormalities of turbot embryos larvae exposed to diazinon: (A) yolk sac alterations, (B) no rupture of the eggs membrane, (C) pericardial edema, (D) skeletal deformities, + indicate number of individuals affected(n=200 eggs)**

| Diazinon | [µg/L] | Embryonal stage | | | Larval stage | | | |
|---|---|---|---|---|---|---|---|---|
| | | A | B | C | A | B | C | D |
| | 0.2 | + | + | - | - | + | - | - |
| | 0.4 | - | + | + | + | - | - | - |
| | 0.8 | + | + | + | - | + | + | + |
| | 1.6 | + | - | ++ | - | - | + | + |
| | 3.2 | +++ | ++ | ++ | + | + | + | ++ |
| | 7.4 | +++ | ++ | +++ | + | + | +++ | +++ |
| Control | - | - | + | - | + | - | - | + |

concentration. In addition, the 48 h $LC_{50}$ value of diazinon for turbot embryos were found to be 1.85mg/L. This is demonstrate that turbot embryos were less sensitive to diazinon than common carp when the 48h $LC_{50}$ value founded by Aydin *et al.*, (2005) to be 0.999 mg/L (Table 3 ). Also, Hamm and Hinton, (2000) reported that early embryonic exposure in medaka was the greatest effect on hatching success. Embryo-larval toxicity assays with diazinon were performed on various fish species: rainbow trout, *Oncorhynchus mykiss* (Kikuchi *et al.*, 1996) and medaka, *O. latipes* Hamm and Hinton, (2000).

Our findings are similar to other studies which reported skeletal deformities as being the most frequent teratogenic effects in fish ELS exposed to diazinon (Fig. 2). In this finding, with increasing diazinon concentrations, the larvae exposed duration 24–96 h significantly increased the number of dead larvae. The differences in number of dead larvae between the durations 24 and 96 h in a concentrations up 0.8 mg/L

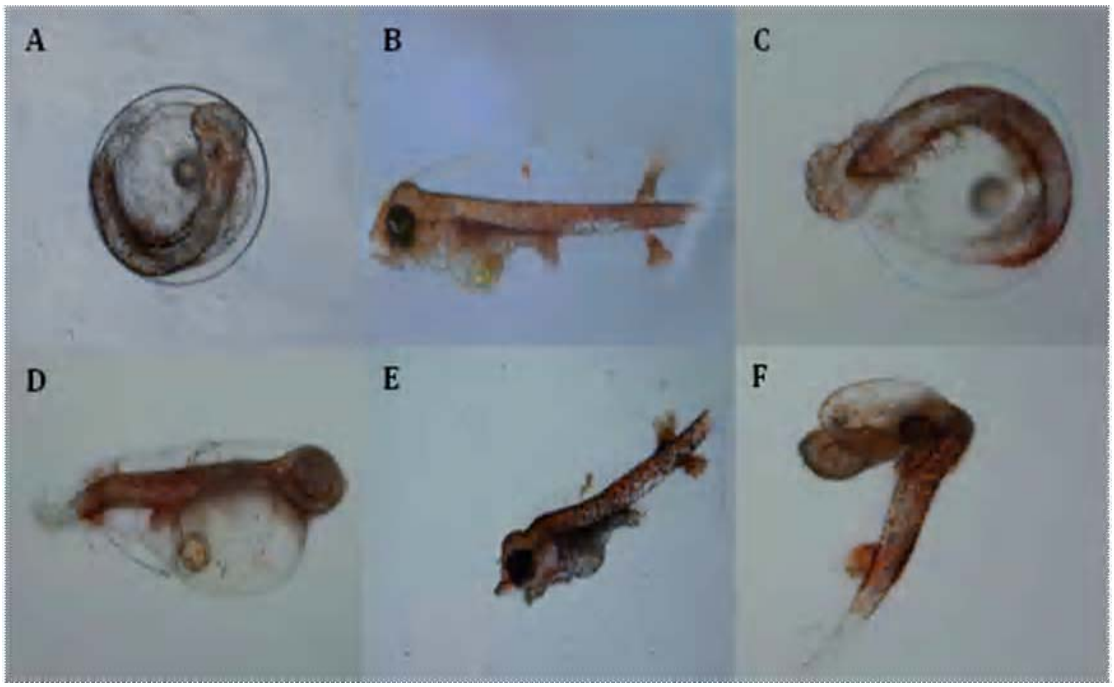


**Fig. 2. Morphological abnormalities of turbot embryos and larvae exposed to PBDE compounds: (A) Normal embryo, (B) Normal larvae, (C) no-rupture of the egg membrane, (D) yolk sac alterations, (E) pericardial edema, (F) skeletal deformities**

**Table 3. Toxicity of diazinon for turbot embryos larvae exposed as NOEC, LOEC, $LC_{10}$ and $LC_{50}$ [mg/L]**

| | (48h) hatching success | (96h) Larval survival | | | |
|---|---|---|---|---|---|
| | | 24h | 48h | 72h | 96h |
| NOEC | - | - | - | - | 0.2 |
| LOEC | 0.4 | - | - | - | 0.4 |
| $LC_{10}$ | 0.83 | 5.72 | 4.69 | 3.45 | 0.7 |
| 95% IC | 1.17-0.66 | 6.39-4.09 | 5.27-4.11 | 4.12-2.77 | 0.53-1.17 |
| $LC_{50}$ | 1.85 | 8 | 3.3 | 2.1 | 1.23 |
| 95% IC | 13.8-21.19 | 6.4-5.12 | 4.73-2.52 | 3.29-1.65 | 0.87-2.38 |

were found significat ($p$<0.05). In the present work, the 24, 48, 72, and 96 h $LC_{50}$ values of diazinon for turbot, larvae were found as 8, 3.3, 2.1, and 1.23mg/L, respectively and here we report diazinon to be highly toxic to turbot larvae. During development sensitivity may change with some compounds showing higher sensitivity in embryos whereas others are more toxic to larvae (Fent and Meier, 1994). Marty *et al.*, (1990) found that early life stage of *O. latipes* was the most sensitive to toxic effects. In fishes, there are differences in the acute toxicity of diazinon for various fish species. The 96 h $LC_{50}$ values range in tenths to several tens of mg/L (Giddings *et al.*, 1996). In European eel (*Anguilla anguilla*), the 96 h $LC_{50}$ values range even in hundredths of milligrams per liters (Sancho., 1993). The 96 h $LC_{50}$ values of diazinon was reported as 0.1-0.5mg/L for bluegill (*Lepomis macrochirus*), 0.88 mg/L for American oyster (*Crassostrea virginica*), 1.47mg/L for sheepshead minnow (*Cyprinodon variegates*), 1.65 mg/L for rainbow trout (*O. mykiss*), and 7.8 mg/L for fathead minnow, *Pimephales minnow* (U.S. EPA, 2000). The different toxicity of diazinon may be demonstrated on the example of two fish species used for ecotoxicological assessment of chemical substances. The 96 h $LC_{50}$ values of diazinon for guppy (*Poecilia reticulate*) was found to be 0.8mg/L but for zebrafish (*Brachydanio rerio*) it was found to be 8 mg/L (Keizer *et al.*, 1991).

## CONCLUSION

The results indicate that low levels of diazinon (0.8 mg/L) in the aquatic environment may have a significant effect on turbot populations. In addition, data presented herein on developmental sensitivity and toxicological endpoints may guide the development of experimental protocols employing species of concern.

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