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Acute Toxicity of the Water-Soluble Fraction of Diesel Oil (WSD) on Beluga (*Huso Huso*)

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Abstract

Among the different types of pollutants, oil production is one of the most significant contaminants of aquatic ecosystems. Acute toxicity tests (96 h) were conducted to determine the lethal toxicity of the water-soluble fraction of diesel oil (WSD) to beluga (Huso huso), a unique and reliable model of commercial fish species in the Caspian Sea. The acute toxicity test was performed in a static renewal system based on the standard method proposed by the OECD over 96 h, during which physicochemical parameters of the test water, including pH, dissolved oxygen, and temperature, were monitored. To determine the lethal range of diesel oil for fish, a range-finding test was carried out in fiberglass tanks containing 200 L of municipal water and 7 fish. The acute toxicity test was then performed in 5 treatments in triplicate. The obtained data were analyzed using probit analysis with a 5% confidence limit. According to the results, the 24 h LC50, 48 h LC50, 72 h LC50, and 96 h LC50 diesel oil on beluga were 36.96%, 33.67%, 23.85%, and 19.42% WSD, respectively. The Maximum Acceptable Toxicant Concentration (MATC) of diesel oil on beluga was 1.94%, and the Lowest Observed Effect Concentration, equal to the 96 h LC50, was 9.554%. The safe level was calculated based on several standard methods. The results indicated that beluga, unlike other aquatic species, is less resistant to diesel oil.

Keywords: Acute Toxicity; Lethal Concentration (LC50); Water-soluble fraction of Diesel oil (WSD); Beluga (Huso huso).

1. Introduction

Among the different types of pollutants, petroleum products are among the most relevant to aquatic ecotoxicology (Pacheco and Santos, 2001a). Accidents involving petroleum hydrocarbon spills occur frequently around the world, and the annual worldwide estimate of petroleum input to these areas exceeds 1,300,000 metric tons (NAP, 2003). An accident or oil spill would severely harm the economic resources of coastal communities, and as an inland sea, the Caspian Sea is more vulnerable to oil spills and pollution. The Caspian Sea contains about 100,000 million barrels of oil. Daily extraction of crude oil and its transportation are the main sources of pollution in the Caspian Sea. Many cities and industries surround the Caspian Sea. Pollution from these cities and industries enters the Caspian Sea either directly or through rivers. Several rivers that carry wastewater from cities and industries first enter the Anzali Marsh (wetland), which is located in the western part of the Caspian Sea and are considered point and nonpoint sources along the seaboard. In addition, many ships navigating around the coastal area emit pollutants into the sea.

Effluents from large oil spills and industrial processes contain highly toxic chemicals like petroleum and its derivatives, which lead to the pollution of aquatic environments, including rivers, ponds, and lakes. The accumulation and persistence of petroleum products in the aquatic environment pose a threat to biological life, as evidenced by the chronic and acute poisoning of fish and other aquatic organisms (Langston, 1990; George, 1990). Although these large oil spills are widely covered in the media, it is believed that the principal source of inland water contamination from petroleum and its derivatives is small and continuous leakages from underground bulk storage tanks, which reach groundwater and later rivers (Tiburtius et al., 2005). Contamination of water bodies by hydrocarbons has been shown to produce subtle changes in fish that are both chronically and briefly exposed (Sabo and Stegeman, 1977). The toxic effects on fish depend on the amount of oil spilled, the area covered by the oil, and the chemical characteristics of the oil (Kiihnhold, 1980); the fish species and the duration of the oil's influence on the fish are also important (Onuoha and Nwachukwe, 1990). There are only a few reports concerning the LC50 of diesel oil exposure on sturgeon fish (Simonato et al., 2006).

The beluga (Huso huso) is a very important commercial fish due to its expensive caviar. The beluga produces the most expensive caviar and has been cultured in Iran since 1991 (Yousof-pour, 1992). Considering the increasing cases of environmental accidents involving spills of petroleum distillate products into continental waters in recent years in Iran and other littoral countries of the Caspian Sea, the present study aimed to investigate the biochemical, physiological, and histopathological parameters of Huso huso exposed to diesel oil as potential biomarkers to assess pollution by these petroleum products and, accordingly, to obtain information about the threat posed by these spills to this neotropical fish species.

2. Material and Methods

2.1. Animals

The beluga Huso huso belongs to the family Acipenseridae (Brandt, 1869; Linnaeus, 1758), weighing 200 ± 20 g (mean ± SD) and was supplied by the Institute of Aquaculture of the Marjani for Sturgeon, Golestan, Iran. Only healthy fish, as indicated by their activity and external appearance, were maintained alive on board in a fiberglass tank. Samples were transferred to an aerated tank with 200 L of dechlorinated water for one week (20 °C; pH 7.6; OD 7.2 mg O2; total hardness 180 mg CaCO3) under a constant photoperiod of 12:12 L: D. Fish were kept under natural photoperiodic conditions, fed 2% of their body weight by hand with formulated pellets, and fasted for 24 hours before each experiment. No mortality occurred during this period (Gooley et al., 2000).

2.2. Preparation of WSD

To obtain the WSD, one part of commercial diesel oil was added to four parts of water in a glass container. The mixture was then exposed to intense sunlight for 6 hours, simulating a diesel spill in tropical conditions (Nicodem et al., 1998).

2.3. Tests for acute toxicity

The exploratory range of concentration of test chemicals was determined through a series of range-finding experiments (APHA/AWWA/WPCF, 1998). The fish were exposed to WSD, and mortality values were measured at 0, 24, 48, 72, and 96 hours. They were subjected to acute static toxicity tests performed in aerated tanks, each containing seven fish. The WSD was prepared in five concentrations: 4%, 12%, 20%, 28%, and 36%. One control group was exposed only to water (the same as that used for acclimation). Seven fish in three replicates were used for each concentration and the negative control under the same conditions. Fish specimens were exposed randomly to a particular dose in each static unit containing different concentrations of test chemicals. No food was provided to the animals during the test, and no fish was used more than once. A fish was considered dead when it did not respond after prodding with a wooden rod; dead fish were immediately removed with special plastic forceps to avoid possible deterioration of water quality. Every effort was made to provide optimal conditions (dissolved oxygen, pH, and conductivity) for the fish during the test.

2.3. LC50 calculation

LC50 values and their confidence limits (95%) were calculated by Boudou and Ribeyre (1997). The percentages of fish mortality were determined for each WSD concentration at 24, 48, 72, and 96 hours of exposure.

The number of dead fish per group was recorded against the time of their death in a tabular form as specified by Sprague (1972). The data obtained were used to calculate the median lethal concentration (LC50) of the WSD on

Huso huso using the arithmetical method of Karber and Dede (1997) and based on Hotos and Vlahos (1998). The LC1-99 values are derived using simple substitution probit of 1, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 99, respectively, for probit of mortality in the regression equations of probit of mortality for diesel oil (Hotos and Vlahos, 1998), and LC50 values were calculated from the data obtained in acute toxicity bioassays by Finney's (1971) method of "probit analysis." In Finney's method, the LC50 value is derived by fitting a regression equation arithmetically and also by graphical interpolation, taking logarithms of the test chemical concentration on the X-axis and the probit value of percentage mortality on the Y-axis (Finney, 1971).

A concentration of the determined LC50 value was prepared by diluting the stock concentration of the WSD, and seven fish were exposed to it.

At the end of the acute test, the LOEC and NOEC are determined for each endpoint measured. In addition, the maximum acceptable toxicant concentration (MATC) is estimated for the endpoint with the lowest NOEC and LOEC. The MATC can also be used to generate application factors (AFs) for chemicals. The AF is derived as the numerical value of the ratio of the MATC to the LC50. It is assumed that the AF for the acute-to-chronic toxicity ratio (ACR), which is a variant of the AF, is the inverse of the AF and is also used to estimate an MATC for species when only acute toxicity data are available. Within 48 hours, the death of fish was observed based on the available data.

Results

The mortality of Beluga for WSD concentrations of 4%, 12%, 20%, 28%, and 36% was examined during exposure times of 24, 48, 72, and 96 hours (see Tables 1-4). Fish exposed during 24-96 hours had a significantly increased number of dead Beluga with increasing concentration. Toxicity testing of the water-soluble fraction of diesel oil (WSD) on Huso huso was conducted at 24, 48, 72, and 96 hours, respectively. Within 24 hours, fish deaths were observed at 28% (one death) and at 36% (two deaths) (see Table 1). Within 48 hours, fish deaths were observed at 20% (one death), 28% (two deaths), and 36% (three deaths) (see Table 2). Within 72 hours, fish deaths were observed at 12% (one death), 20% (two deaths), 28% (four deaths), and 36% (seven deaths) (see Table 3). Within 96 hours, fish deaths were observed at 12% (two deaths), 20% (three deaths), 28% (six deaths), and 36% (seven deaths) (see Table 4).

Table 1: Toxicity testing at 24 hours

CONC(%WSD)	No. surviving	% alive	% dead
0	7	100	0
4	7	100	0
12	7	100	0
20	7	100	0
28	6	86	14
36	4	57	43

Table 2: Toxicity testing at 48 hours

CONC(%WSD)	No. surviving	% alive	% dead
0	7	100	0
4	7	100	0
12	7	100	0
20	6	86	14
28	5	71	29
36	3	43	57

Table 3: Toxicity testing at 72 hours

CONC(%WSD)	No. surviving	% alive	% dead
0	7	100	0

4	7	100	0
12	6	86	14
20	5	71	29
28	3	43	57
36	0	0	100

Table 4: Toxicity testing at 96 hours

CONC(%WSD)	No. surviving	% alive	% dead
0	7	100	0
4	7	100	0
12	5	71	29
20	4	57	43
28	1	14	86
36	0	0	100

Table 2: Table for 96-hour LC50 determination based on the Arithmetic method of Karber. (adapted by Dede 1992).

conc (% WSD)	conc. difference	No. alive	No. dead	Mean death	Mean death × Does diff
0 (control)	-	7	0	-	0
4	4	7	0	-	0
12	8	5	2	1	8
20	8	4	3	2.5	20
28	8	1	6	5	40
36	8	0	7	6.5	52
					120

96 HOURS LC50 DETERMINATION: Using the Arithmetic method of Karber the LC50 value was determined as follows:

$$LC50 = LC100 - \sum \frac{\text{mean dead} \times \text{conc. diff.}}{\text{No.of organisms per group}}$$

$$= 36 - 120/7$$

$$= 36 - 17.1$$

LC50 = 18.85

Median lethal concentrations of 1, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 99 tests are presented in Table 2. Because mortality (or survival) data are collected for each exposure concentration in a toxicity test at various exposure durations (24, 48, 72, or 96 hours), the data can be plotted in other ways; a straight line of best fit is then drawn through the points. These are time–mortality lines. The LT50 (median lethal survival time) can be estimated for each concentration.

Table 2. Lethal Concentrations ($LC_{1\text{-}99}$) of the water-soluble fraction of diesel oil (WSD)(mean ± Standard Error) depending on time (24-96h) for Beluga.

Point	Concentration (ppt) (95 % of confidence limits)			
	24h	48h	72h	96h
LC_1	3.366 ± 0.82	0.033 ± 0.54	0.001 ± 0.47	0.001 ± 0.44
LC_{10}	11.146 ± 0.82	6.027 ± 0.54	2.765 ± 0.47	1.415 ± 0.44
LC_{20}	14.422 ± 0.82	8.551 ± 0.54	4.719± 0.47	2.927 ± 0.44
LC_{30}	16.784 ± 0.82	10.370 ± 0.54	6.129± 0.47	4.018 ± 0.44

LC_{40}	18.803 ± 0.82	11.925 ± 0.54	7.333 ± 0.47	4.949 ± 0.44
LC_{50}	20.689 ± 0.82	13.379 ± 0.54	8.459 ± 0.47	5.820 ± 0.44
LC_{60}	22.576 ± 0.82	14.832 ± 0.54	9.584 ± 0.47	6.691± 0.44
LC_{70}	24.594 ± 0.82	16.387 ± 0.54	10.789± 0.47	7.623 ± 0.44
LC_{80}	36.957 ± 0.82	18.207 ± 0.54	12.198± 0.47	8.713 ± 0.44
LC_{90}	30.233 ± 0.82	20.730 ± 0.54	14.153± 0.47	10.225 ± 0.44
LC_{99}	38.013 ± 0.82	26.724 ± 0.54	18.795± 0.47	13.816 ± 0.44

Statistical results of 96h exposure for observed mortality, expected response, and prob during probit analysis of results are in Fig. 1.

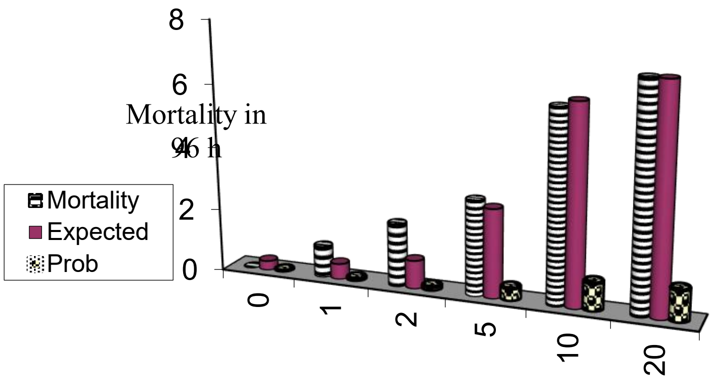

Fig.1. The water-soluble fraction of diesel oil (WSD) response curve of Beluga exposed to 96 h acute toxicity test.

Toxicity Testing Statistical Endpoints are divided into two parts: 1. Hypothesis Testing: Is there a statistically significant difference between the mean response in the treatments and the mean response in the control or reference sample? LOEC.

Lowest Observed Effect Concentration (LOEC); No Observed Effect Concentration (NOEC). 2- Point Estimates: What toxicant concentration will cause a specific effect on the test population? LC50: the median Lethal Concentration. Our results for Toxicity Testing Statistical Endpoints are shown in Fig. 2. The Maximum Acceptable Toxicant Concentration (MATC) was 10 ppt, and the Application Factor (AF) was 0.40 ppt.

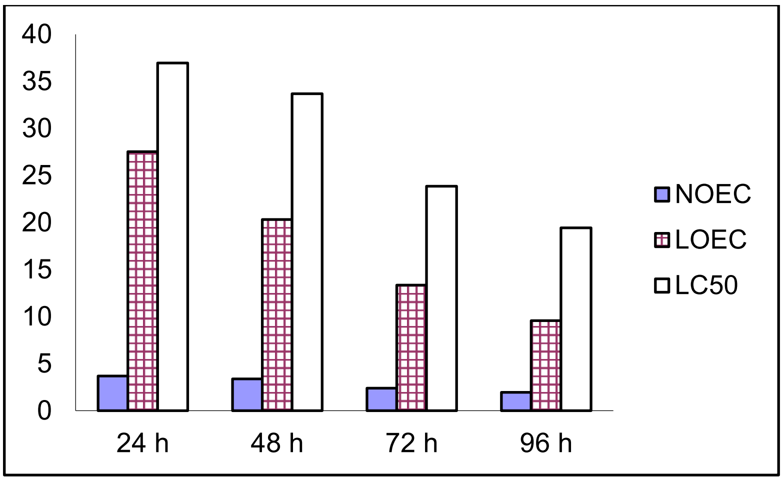

Fig 2. Acute toxicity testing statistical endpoints in Beluga.

Discussion

Diesel oil, like other distilled products of petroleum, shows low solubility in water; therefore, laboratory investigations of its effects involve the preparation of water-soluble fractions. In the present work, fish were exposed to an experimental solution containing 50% of a WSD. The analyses conducted showed that this WSD contained polar compounds and aromatic hydrocarbons from two toxicologically relevant groups: BTX (benzene, toluene, and xylene) and polycyclic aromatic hydrocarbons (PAHs) of low molecular weight, such as naphthalene, fluorene, and phenanthrene. A similar composition of a WSD has been previously reported by others (Pacheco and Santos, 2001).

This study demonstrated that the water-soluble fraction of diesel oil (WSD) can be toxic to Beluga, Huso huso, at concentrations of 5.82 (ppt). This indicates that WSD has moderate to high toxicity against these species. The results of our study exhibited that longer exposure times caused mortality, implying that the sensitivity of non-target organisms to toxicants during prolonged exposure contributes to reduced life expectancy (Yu et al., 1999). The 96h LC50 value of WSD in Beluga was found to be 5.82 mg L-1 in the present work. In aquatic toxicology, if the LC50 (mg L-1) concentration is smaller than 1 mg L-1, the pollution is considered highly toxic, and if it is between 1-10 mg L-1, it is regarded as moderately toxic (Louis et al., 1996). Therefore, we report WSD to be moderately toxic to the fish species used. The LC50 values reported in the present study for WSD were lower than those reported by L. Seuront in 2011 for the same species at 48, 72, and 96h. He reported LC50 values (with 95% confidence limits) of 7.5 and 12.7% WSD, respectively, at 48, 72, and 96h.

References:

1. Boudou, A., Ribeyre, F. 1997. Aquatic ecotoxicology: from the ecosystem to the cellular and molecular levels. Environ. Health Perspect. 105 (Suppl. 1), 21-35.
2. Di Giulio, R.T., Habig, C., Gallagher, E.P., 1993. Effect of black rock harbor sediments on indices of biotransformation, oxidative stress, and DNA integrity in channel catfish. Aquatic Toxicology 26, 1–22.
3. Eisler and Gardener, G. R. 1993. Acute toxicology to an estuarine teleost of mixtures of cadmium, copper, and zinc salts. J. fish. Biol., 5: 131–142.
4. Elia, A.C., Galarini, R., Taticchi, M.I., Dorr, A.J., Mantilacci, L., 2003. Antioxidant responses and bioaccumulation in Ictalurus melas under mercury exposure. Ecotoxicol. Environ. Safety 55, 162–167.
5. Finney, D.J., 1971. Probit Analysis. Univ. Press, Cambridge, p. 333.
6. Gooley, G.J., Gavine, F. M., Dalton, W., De Silva, S. S., Bretherton, M., and Samblebe, M. 2000. Feasibility of aquaculture in dairy manufacturing wastewater to enhance environmental performance and offset costs. Final Report DRDC Project No. MAF001. Marine and Freshwater Resources Institute, Snobs Creek, pp. 84.
7. Gorski. 2007. The effects of trace metals on the Australian abalone, Haliotis rubra Jacquelle. PhD thesis. RMIT University.
8. Gupta, P.K., Khangarot, B.S., Durve, V.S., 1981. The temperature dependence of the acute toxicity of copper to a freshwater pond snail, Viviparus bengalensis L. Hydrobiologia 83, 461–464.
9. Hedayati, A., Safahieh, A., Savar, A., Ghofleh Marammazi, J. 2010a. Detection of mercury chloride acute toxicity in Yellowfin sea bream (Beluga). World Journal of Fish and Marine Science. 2(1): 86-92.
10. Hedayati, A., Safahieh, A., Savar, A., Ghofleh Marammazi, J. 2010b. Assessment of aminotransferase enzymes in Yellowfin sea bream (Beluga) under experimental conditions as biomarkers of mercury pollution. World Journal of Fish and Marine Science. 2(3): 186-192.
11. Hotos, G.N. and N. Vlahos. 1998. Salinity tolerance of Mugil cephalus and Chelon labrosus, Pisces: Mugilidae/fry in experimental conditions. Aquaculture 167-1998.329–338
12. Mohapatra, B.C., Rengarajan, K., 1995. A Manual of Bioassays in the Laboratory and Their Techniques. CMFRI Spec. Pub. 64, CMFRI, Cochin, India, p. 75.
13. Neely, W.B. 1984. An analysis of aquatic toxicity data: water solubility and acute LC50 fish data. Chemosphere 13:813-819.
14. Rathore, R.S., Khangarot, B.S., 2002. Effect of temperature on the sensitivity of sludge worm Tubifex tubifex (Muller) to selected heavy metals. Ecotoxicol. Environ. Saf. 53, 27–36.

15. Tort, L.; Torres, P. 1988. The effects of sub-lethal concentration of cadmium on hematological parameters in the dogfish, Scyliorhinus Caniccula. J. fish. Biol., 32 (2), 277-282.
16. Verep, B., Sibel Besli, E., Altionk, I., and Mutlu, C. 2007. Assessment of Mercuric chloride toxicity on Rainbow trouts and cubs. 2007. Pakistan Journal of Biological Sciences. 10 (7): 1098-1102.
17. Vieira, L.R. Gravato, C. Soares A., Morgado F., Guilhermino. L. 2009. Acute effects of copper and mercury on the estuarine fish Pomatoschistus microps: Linking biomarkers to behavior. Chemosphere 76. 1416–1427.