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Short Communication

Effects of Crude Oil and Oil Cleaner Mixture on Rainbow Trout in Early Ontogenesis

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Abstract

The aim of our study was to investigate acute and chronic effects of the oil cleaner Simple Green (SG), crude oil (oil), crude oil and SG mixture (oil-SG) on rainbow trout (*Oncorhynchus mykiss*) in early development stages (embryos, larvae). The acute effects of SG, oil, and oil-SG mixture on rainbow trout embryos and larvae were investigated. The 96-hour LC_{50} values for embryos/larvae were: SG – 1,270/1,040; oil – 39,280/21,610; and oil-SG – 24,430/14,040 mg/l. Larvae were more sensitive for these toxicants than embryos. The chronic effect of oil-SG mixture on rainbow trout in early stages of development showed that the mixture slightly increased the mortality of embryos, and influenced more the mortality of larvae. The mortality of larvae depends on the duration of exposure and oil concentration in the mixture. Comparative studies on the acute and chronic effects of oil and the oil-SG mixture on rainbow trout in early stages of development showed that the mixture has a more toxic effect on embryos and larvae than oil alone.

Keywords: crude oil, Simple Green, mixture, acute and chronic toxicity, embryos, and larvae

Introduction

Negative consequences of oil spills for aquatic flora and fauna, and the use of chemicals (dispersants or surfactants) for mitigating the overall adverse environmental impact, have been widely investigated. During the last years, dispersants have been applied in about half of the cases of major oil spills [1, 2]. The toxicity of oil dispersants to living organisms reported in the literature is highly variable [1, 2]. The cleaner CRYSTAL Simple Green (SG) is an oil dispersant designed for cleaning the ground, various surfaces polluted with oil products, and oil spills in open waters [3]. The first opinions about the use of the chemical substance SG in Lithuania were expressed after oil spills in the Baltic Sea in March and April 2001. However, few studies on the biological effects of this chemical have been performed. The toxic effects of oil and the oil-SG mixture on rainbow trout at different stages of development were determined and compared under laboratory conditions. Studies revealed that oil-SG mixture could induce significant deleterious changes in the most sensitive systems of the fish [4].

The aim of our study was to investigate acute and chronic effects of Simple Green (SG), crude oil (oil), and the crude oil and SG (oil-SG) mixture on rainbow trout in early developmental stages (embryos, larvae).

Experimental Procedures

The toxicity tests were carried out under semi-static conditions. Deep well water of high quality was used for storing control embryos and larvae. Average hardness of water was approximately 250 mg/l as $CaCO_3$, dissolved oxygen concentration, and pH were not less than 7 mg/l and 7.9-8.1, respectively. The temperature of water in the aquaria was optimal for rainbow trout and maintained at

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10±0.5ºC. Control water and solutions in aquaria were renewed on alternate days.

CRYSTAL SG is an aqueous-based organic cleaner with a pH of 9.3. It consists of a number of aliphatic hydrocarbons <10.0% and non-ionic surfactants <5.0% [3]. The solution of SG (concentration 0.5 mg/l) was prepared in distilled water and was directly inserted into the aquarium water with oil.

The total concentrations of oil (Lithuanian oil) introduced into the aquarium water were 110-110,880 mg/l. The total concentrations of SG introduced into the aquarium water were 220-3,500 mg/l. In order to determine the effect of SG on the concentrations of water-soluble and thin-dispersed fractions of petroleum hydrocarbons, a series of studies was performed [4].

Acute (96-hour) toxicity tests of SG, oil, and oil-SG mixture were conducted using eyed-egg stage embryos (10-day test prior to hatching), and larvae of rainbow trout. Chronic (52-58-day) toxicity tests were conducted with embryos and larvae (including hatching period). One hundred embryos were exposed to each concentration of SG, oil, and oil-SG mixture. Studies with embryos and larvae were performed in four replications.

The median acutely lethal concentration (LC_{50}) values and their 95% confidence intervals (CI) were estimated by the trimmed Spearman-Karber method [5]. The significance of all the data obtained was determined by use of Student's t-test with $P \leq 0.05$.

Results and Discussion

Acute Toxicity

The calculated 96-hour LC_{50} values of SG, oil, and oil-SG mixture for rainbow trout embryos/larvae were: SG – 1,270 (95% CI 1,130-1,430)/1,040 (940-1,140) mg/l; oil – 39,280 (33,720-45,750)/21,610 (19,020-24,560) mg/l; and oil-SG – 24,430 (21,930-27,220)/14,040 (12,820-15,380) mg/l. The calculated 96-hour LC_{50} of SG only for marine fish are: for mud minnow (*Fundulus heteroclitus*) – 1,574 mg/l, and whitebait (*Galaxias maculates*) – 210 mg/l. The calculated 96-hour LC_{50} of oil for marine fish mud minnow (*Fundulus heteroclitus*) was 45,300 mg/l [3].

Fish at early stages of development are more sensitive to toxicants [8]. A higher sensitivity of larvae than that of embryos to the impact of oil was confirmed by alterations in physiological parameters [6]. These results are consistent with the data on the sensitivity of early life stages of pink salmon, Coho salmon (*Oncorhynchus kisutch*) [7], and rainbow trout, which demonstrated that larvae of rainbow trout were more sensitive to oil exposure, with acute toxicity occurring at concentrations causing sublethal damage in embryos. The difference in sensitivities is most likely due to chorion protection of embryos [6]. Waterborne petroleum hydrocarbons crossed the chorion of embryonic rainbow fish, reducing survival and hatchability [8].

Chronic Toxicity

The solution of SG (0.5 mg/l) did not affect the mortality of embryos and larvae of rainbow trout during the exposure period (Table 1). A slight increase in the mortality of embryos was induced by oil-SG mixture at the concentrations 1,730-6,930-0.5 mg/l, and in the mortality of larvae at concentrations of 220-6,930-0.5 mg/l after a 10-day exposure was recorded. It was observed that the duration of exposure of the mixture influenced more the mortality of larvae: at the 870-0.5 mg/l mixture concentration after 10-day exposure mortality became 24.9%, and after 35-day – 78.5%. Equally, we might say that the mortality of larvae depends on the concentration of oil in the mixture (Table 1). With an increase in oil concentrations in the mixture of up to 1,730 mg/l, the mortality of larvae increased and reached a peak (74.7-76.5%) there. However, as oil concentrations

Table 1. Chronic toxicity of SG, oil, and oil-SG mixture on the mortality (%) of rainbow trout embryos and larvae depending on the concentration and the duration of exposure (M±SD, N=200).

Concentration, mg/l	Mortality, %		
	Embryos	Larvae	
	Exposure		
	10-day	10-day	35-day
SG			
0.5	5.9±0.4	12.6±1.0	16.4±0.4
Oil [6]			
6,930	13.0±0.3	26.4±1.4*	81.0±1.2*
3,460	10.4±0.5	22.8±1.6*	57.9±1.8*
1,730	11.0±1.5	31.3±1.5*	50.3±1.6*
870	5.0±0.3	27.4±0.8*	51.2±0.8*
430	2.1±0.3	12.6±0.4	22.3±1.5*
220	2.5±0.3	12.5±0.6	14.7±1.2
Oil-SG mixture			
6,930+0.5	16.4±0.4	38.6±1.7*	52.5 ±0.8*#
3,460+0.5	15.4±0.5	29.4±0.7*	53.7±0.9*#
1,730+0.5	13.0 ±0.2	30.4±0.8*	74.8±1.6*#
870+0.5	8.5±0.2	24.6±1.0*	76.7±0.7*#
430+0.5	5.0±0.8	10.6±0.8	39.1±1.0*#
220+0.5	3.5±0.2	12.2±0.6	26.6±1.9*#
110+0.5	2.5±0.7	12.1±0.4	14.6±0.2
Control			
	2.0±0.4	10.3±0.3	14.3±0.3

*significant differences from control ($P \leq 0.05$),
#significant differences from test data (($P \leq 0.05$).

in the mixture were increased from 3,460 to 6,930 mg/l the mortality of larvae decreased to 52.8-53.8% (Table 1).

Oil toxicity studies showed the opposite result. A slight increase in the mortality of embryos was induced only by 6,930 mg/l concentration of oil. An increasing mortality of larvae was directly related to an increase in oil concentration (Table 1) [6]. Such differences in the toxicity of oil and the oil-SG mixture can be associated with many factors, including composition and amount of dispersant, physical and chemical oil properties, amount of oil, dispersant-to-oil ratio, action mechanism of dispersant, degradation level, and various environmental indices such as temperature, salinity, etc. [9].

Consequently, our comparative studies on the acute and chronic effects of oil and the oil-SG mixture on rainbow trout in early stages of development showed that the mixture has a more toxic effect on embryos and larvae than oil alone. The results observed in this study are consistent with results from other published reports [4, 10].

The chemical agents emulsifying oil increase aqueous concentrations of petroleum hydrocarbons, raise their water solubility, enhance bioavailability and, consequently, increase their negative effects on aquatic life [11]. Hence, we predict that in nature reduced survival, disturbed development, and hatchability of fish in early life stages can cause negative consequences for the quantity and quality of the fish in the second generation and actually for the well-being of the population.

Conclusions

1. The acute effect of toxicants on rainbow trout in early stages of development showed that 96-hour LC_{50} values of SG, oil, and oil-SG mixture for embryos/larvae were: SG – 1,270/1,040; oil – 39,280/21,610; and oil-SG – 24,430/14,040 mg/l. Larvae were more sensitive to these toxicants than embryos.
2. The chronic effect of the solution of SG (0.5 mg/l) on rainbow trout in early stages of development showed that this solution did not affect the mortality of embryos and larvae during the exposure period.
3. The chronic effect of the oil-SG mixture on rainbow trout in early stages of development showed that the mixture slightly increased mortality of embryos, and increased the mortality of larvae more. The mortality of larvae depends on the duration of exposure and oil concentration in the mixture.
4. Comparative studies on the acute and chronic effects of oil and the oil-SG mixture on rainbow trout in early stages of development showed that the mixture has a more toxic effect on embryos and larvae than oil alone.

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