EKOLOGIJA. 2006. Nr. 2. P. 15–20

Toxicity of road maintenance salts to rainbow trout *Oncorhynchus mykiss*

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Studies of the acute and long-term toxicity of salt (RMS) used for road maintenance in Lithuania to rainbow trout were performed by using a set of biological parameters. The calculated 96-hour LC50 value for rainbow trout juveniles was 18.25 g/l. No significant changes were found in the weight of RMS-exposed fish, while the liver weight and hepato-somatic index of fish exposed to 4.56 g/l and 1.14 g/l of RMS significantly differed from controls. Blood parameters (erythrocyte count, haemoglobin concentration, haematocrit level, leukocyte count) differed by their sentitivity to RMS effect. Erythrocyte count decreased in the blood of fish exposed to all concentrations of RMS studied.

Key words: road maintenance salt, rainbow trout, toxicity

INTRODUCTION

Road maintenance salts (RMS), widely used in Lithuania in cold seasons can negatively affect roadside environment and its components. The impact of chlorides and other traffic pollutants falling into snow was comprehensively studied in countries using salts for road maintenance. Salt concentrations were evaluated in snow cover (Yakhnin et al., 1997), soil (Norrstrom, Jacks, 1998; Backstrom et al., 2004), underground waters (Williams, 1999; Thunqvist, 2004), plants and trees (Kayama et al., 2003; Viskari, Karenlampi, 2000).

Snow cover pollution by salts and traffic pollutants in Lithuania was first evaluated in 1995–1996. The measured concentrations of chloride in snow cover near Vilnius city streets ranged from 1911 to 2571 mg/l and in rain sewage during snow melting from 438 to 1223 mg/l, respectively (Laurinavičius, Čygas, 1996). The Institute of Geology and the Geology Survey of Lithuania in 1995–1996 periodically determined the pollution of snow cover, and among other elements the levels of RMS was been determined also. According to the data of these authors, the geochemical situation in Lithuania was found to be an intermediate between the "clean" Scandinavian and "polluted" Median European countries (Kadūnas et al., 1999). The latest studies performed at Vilnius Gediminas Technical University Department of Environmental Protection (2003–2004) demonstrated that salts were mostly concentrated near the edge of the roads and up to 1 m away from it; the highest salt concentrations in snow melt water were found near the Vilnius–Kaunas–Klaipėda highway, where they rang from 1933 to 3200 mg/l, while chloride concentrations in snow melt water near Vilnius were lower, reaching a level of about 17 mg/l. The highest chloride concentrations were found in snow at a distance of up to 20 m from the border of the road (Storpirštytė et al., 2004; Балтренас, Казлаускене, 2005). These data suggest the possibility of large amounts of RMS to enter open water bodies with melting snow.

An evaluation of the negative impact of RMS on aquatic ecosystems according to diatom flora biodiversity was performed at Vilnius Gediminas Technical University in 2004. The data obtained suggested that these salts could influence the spreading of diatom flora which is specific of saline waters (Oškinis, Kasperovičius, 2005).

In order to show the impact of road maintenance salts (RMS) on fish, different studies were performed under laboratory conditions. Short-term and long-term toxicity tests were conducted on rainbow trout *Oncorhynchus mykiss,* using a set of biological parameters.

MATERIALS AND METHODS

Toxicity tests were conducted at the Laboratory of Ecology and Physiology of Hydrobionts (Institute of Ecology of Vilnius University). Rainbow trout (*Oncorhynchus mykiss*) juveniles were obtained from the Žeimena hatchery and kept in holding tanks of about 3000 l capacity, supplied with flow-through artesian aerated water. The weight of fish under study ranged from 12.9 to 14.4 g. During long-term studies fish were fed until satiety with commercial DANA FEED fish food.

Artesian water of high quality was used for dilution. The average hardness of dilution water was approximately 284 mg/l as $CaCO_3$, alkalinity was 244 mg/l as HCO_3^-, the mean pH was 8.0, temperature ranged from 12° C to 13.5° C, and the oxygen concentration ranged from 8 to 10 mg/l.

The effect of the RMS that are in use in Vilnius during autumn and winter seasons was investigated. A chemical analysis of salt solution was performed by ICP–MS (inductively coupled plasma mass spectrometry), and analytical data revealed the metal ion composition of the salt under study (Table 1).

Table 1. **Concentrations of chemical constituents in RMS solution (g/l)**

Elements	g/l
Cl	0.6632
Na	0.5603
Ca	0.0017
Mg	0.00015
K	0.0002
Elements	**mg/l**
V	0.0019
Sr	0.0176
Br	0.0632
As	0.0019
P	0.0439
Si	0.0307
Mn	0.09
Zn	0.002

Acute (96 hours) toxicity studies were performed under semi–static conditions according to the LST ISO 7346–2:1999. The system included six 40-l aquaria. For the experiments, seven fish were transferred from holding tanks to each aquarium and kept in the new medium until acclimation, i.e. till they started to swim freely and feed well. Different amounts of RMS stock solution were added to five aquaria and nothing was added to the last one (control). Each test was replicated twice. Every day the water was changed, and the number of dead fish was recorded. Dead fish were weighed individually (after being lightly blotted dry). No mortality was observed among control fish. At the same time acute toxicity tests were conducted using the common salt (CS) (Sodium Chloride – NaCl) and the same stock solution concentrations.

Long-term (14 days) toxicity tests were also performed under semi–static conditions. The fish were transferred from holding tanks into six 100-l aquaria. The concentrations of salt in long-term studies were diluted four times and constituted 4.56; 2.28; 1.14; 0.1825 g/l, respectively. For a comparison of the toxicity of additional components in the RMS, to the sixth aquarium CS was added (4.56 g/l). Control water and salt solutions were renewed daily. During the tests fish were fed every day.

The body weights were measured at the start and at the end of exposure. After 14 days of exposure fish were caught gently in a small net, avoiding stress as much as possible, and their biological parameters were measured. The following morphological parameters were evaluated: total weight and liver weight. The hepatosomatic index was calculated according to Vosylienė, Svecevičius, (1997). Blood samples were collected by punction of caudal blood vessels. Heparin sodium salt was used for stabilization of the fish blood. Blood was sampled from 10 fish of each exposure group. Erythrocytes (RBC, $10^6 \times mm^{-3}$), haemoglobin concentration (Hb, g/l), the haematocrit level (Hct, l/l) and leukocyte counts (WBC, $10^3 \times mm^{-3}$) were determined using routine methods (Diagnostics, Prevention, and Therapy of Fishes Diseases and Intoxications, 1991).

The Median acutely lethal concentration (LC_{50}) values and their 95% confidence intervals were estimated by the trimmed Spearman–Karber method (Hamilton et al., 1977).

A statistical analysis of the data was performed using SPSS 10.0 software and significance of the data was determined by the one-way ANOVA test.

RESULTS

Acute toxicity studies demonstrated differences in the sensitivity of rainbow trout juveniles to RMS and CS. After introducing different amounts of stock RMS solution into the aquaria, fish began to die within the first 24 hours of the exposure, and their mortality depended on the salt concentration present. The same amount of fish (57.12%) died at 20 and 22 g/l concentrations of RMS, respectively. Total mortality (100%) was registered at 22 g/l of RMS at the end of exposure (96 hours). No mortality was registered at the lowest RMS concentration studied (14 g/l) during the whole period of exposure. The calculated 96-hour LC50 value for RMS was reached at 18.25 g/l (Table 2). The mortality of fish induced by CS during the first 24 hours was registered only at the highest concentration studied (22 g/l). No mortality was registered at lower CS concentrations (14, 16, or 18 g/l) during the total duration of exposure. The method applied did not permit to calculate the 24-hour and 48-hour LC_{50} values of both salts studied.

Table 2. **The calculated median lethal 96–hour LC_{50} of RMS and CS for rainbow trout in acute toxicity tests**

Chemicals	LC50 (g/l)	95% confidence intervals (g/l)
RMS	18.25	17.40 ÷ 19.13
CS	20.38	19.30 ÷ 20.88

No sharp boundary between the lethal and sublethal effects of RMS was registered. Fish (14.28%) began dying at lower concentrations (16 g/l) already during the first 24 hours and more fish died until the end of exposure.

Based on the data obtained from the first experiments, sublethal effects of RMS on rainbow trout juveniles were further studied using concentrations starting from 0.25 96-hour LC_{50} i.e. 4.56 g/l and correspondingly diluted concentrations.

No significant changes in the length and weight of rainbow trout juveniles were found at the end of the tests. The weight of fish at the initiation of exposure ranged from 12.9 ± 0.5 to 14.4 ± 0.4 g, and at the end of the experiment no significant differences were determined in the weight of the controls and fish exposed to RMS or CS. The mean weight of control fish was 22.3 ± 0.7 g, while to weight of exposed fish ranged from 21.0 ± 0.7 to 22.9 ± 1.0 g. A significant difference was found in the liver weight of fish exposed to 4.56 g/l and 1.14 g/l concentrations of RMS and in the hepato-somatic index of fish exposed to the same concentrations of RMS solution (Table 3).

Changes in blood parameters (erythrocyte count, haemoglobin concentration, haematocrit level, leukocyte count) revealed sublethal toxic effects of RMS on the fish. Erythrocyte count decreased significantly ($p < 0.01$) in the blood of fish exposed to any RMS concentration studied. A significantly reduced haemoglobin concentration was found only in the blood of fish exposed to 4.56 g/l RMS. Haematocrit level changes were similar to alterations in RBC count. White blood cell count decreased significantly in the fish exposed to 4.56 or 2.28 g/l of RMS compared to the control (Table 4). The highest concentration of CS induced a significant drop of RBC count compared to the control; however, no significant changes in the other blood parameters of exposed fish were found.

DISCUSSION

At present, no data are available in the literature on the effects of RMS on the physiological state of fish. Meanwhile, the chemical analysis of the RMS in use in the Vilnius city indicated that road maintenance salt contains various elements (vanadium, strontium, arsenic, zinc, manganese etc.) which could be toxic to fish. Furthermore, the specification of the RMS revealed the presence of small amounts of potassium ferricyanide added to this salt. Cyanide is one of the chemicals most toxic to fish. Fish are approximately one thousand times more sensitive to cyanide than are humans. Dose levels as low as 0.03 mg/l HCN can be fatal to sensitive species, while 0.2 mg/l is lethal to most species. In any case, levels less than lethal do provoke physiological and pathological responses that reduce swimming ability, interfere with reproductive capacity and can lead to seriously deformed offspring, and also make fish more vulnerable to predators (Ingles, 1982). In addition to the total cyanide level in the water, a number of other factors associated with water chemistry exert a modifying effect on acute toxicity, among them is water salinity and other dissolved constituents (e.g., zinc, ammonia). Seventeen parts per thousand chloride ion (17‰) decreased the survival time in acute toxicity tests (Hynes et al., 1998).

However, comparative studies of the lethal toxicity of RMS and CS did not reveal clear significant

Table 3. **Morphological parameters of rainbow trout exposed to sublethal concentrations of RMS and CS (N = 10)**

Concentration, g/l	Length, cm	Weight, g	Liver weight, g	HSI
4.56	11.9 ± 0.18	22.5 ± 0.7	0.33 ± 0.01*	1.53 ± 0.11*
2.28	11.6 ± 0.19	22.5 ± 0.9	0.35 ± 0.01	1.78 ± 0.04
1.14	11.7 ± 0.19	22.9 ± 1.0	0.33 ± 0.01*	1.69 ± 0.06*
0.18	11.5 ± 0.12	21.0 ± 0.7	0.34 ± 0.03	1.79 ± 0.12
Control	11.8 ± 0.19	22.3 ± 0.7	0.38 ± 0.02	1.92 ± 0.08
4.56 (CS)	12.0 ± 0.14	22.9 ± 0.5	0.38 ± 0.03	1.84 ± 0.13

* Values significantly different from controls ($P < 0.01$).

Table 4. **Haematological parameters of rainbow trout exposed to sublethal concentrations of RMS and TS (N = 10)**

Concentration, g/l	RBC count, $10^6 \times mm^{-3}$	Haemoglobin, g/l	Haematocrit, l/l	WBC count, $10^3 \times mm^{-3}$
4.56	0.60 ± 0.04*	72.8 ± 6.9*	0.48 ± 0.01*	15.9 ± 2.7*
2.28	0.67 ± 0.05*	84.4 ± 2.8	0.49 ± 0.02*	16.9 ± 1.9*
1.14	0.64 ± 0.04*	92.4 ± 2.1	0.45 ± 0.04	18.9 ± 1.6
0.18	0.68 ± 0.04*	–	0.46 ± 0.01*	19.7 ± 1.4
Control	0.84 ± 0.04	81.6 ± 3.7	0.42 ± 0.01	22.1 ± 1.8
4.56 (TS)	0.68 ± 0.04*	84.0 ± 3.9	0.42 ± 0.01	24.7 ± 2,2

* Values significantly different from controls ($P < 0.01$).

Table 5. **Concentrations of constituents in RMS solutions tested and their MPC accepted for Lithuanian inland waters (Anonymous, 2000)**

Elements	Concentrations		MPC, mg/l
	4.56 g/l	0.18 g (180 mg/l)	
Cl^-	3.02	0.119 (119)	300
Na	2.56	0.10 (100)	120
Ca	0.0079	0.0003 (0.3)	180
Mg	0.0007	0.00003 (0.03)	40
K	0.0002	0.000008 (0.004)	50
	mg/l	mg/l	mg/l
V	0.0085	0.0003	–
Sr	0.08	0.0032	–
Br	0.288	0.0114	–
As	0.0086	0.0003	–
P	0.2	0.008	0.2
Si	0.14	0.006	–
Mn	0.04	0.007	0.01
Zn	0.009	0.0004	0.1

differences in the 96-hour LC_{50} of these chemicals. The 96-hour LC_{50} of the RMS was 18.25 g/l, and CS – 20.38 g/l. Probably, these differences in LC_{50} were caused by all elements present in RMS. Furthermore, some specific differences in the toxic effects of these salts were observed within the exposure period. Mortality during the first 24 h of exposure was only registered for fish exposed to the highest CS concentrations – 20.0 g/l and 22.0 g/l. Lethal effects due to RMS during 24 h occurred already at lower concentrations (16 g/l) and at all subsequent higher concentrations of this salt.

Acute toxicity tests were performed in order to determine the basic toxic characteristics of the salts studied, and long-term studies were started with a RMS concentration of 4.56 g/l. This and the subsequent RMS concentrations were closer to the ones found in studies performed by the VGTU Department of Environmental Protection (Балтренас, Казлаускене, 2005). According to the data obtained in this study, chloride concentrations in snow melt water at the roadside of highways in the western part of Lithuania ranged from 9400 to 13600 mg/l at the border of the road and within 1840–9136 mg/l at a distance of 1–2 m from the road. Concentrations of sodium ranged from 1030 to 1983 mg/l, and at a distance of 1–2 m from the road 543–1378 mg/l (Lithuanian state roads, 2004). The authors explained these high salt concentrations in snow cover by unfavorable weather conditions in that year and enormous amounts of salt used for maintenance of the roads. Mixed with snow melt water, the salt penetrates into the soil and could enter water bodies, rivers and lakes. However, salt water is soon diluted with water flow, and its toxic effects on aquatic organisms are questionable, while salt that enters ponds and lakes might induce a negative impact on aquatic biota. According to US EPA, organisms in a water body are not typically experiencing a constant, steady exposure but rather fluctuating exposures, including periods of high concentrations, which may have adverse effects (US EPA 1999). Common freshwater fish (roach, perch, and bream) are very sensitive to changes of water salinity, and many studies reported an influence of water salinity on fish development and growth (Evans, 1997).

The sensitivity of fish to changes in water salinity was confirmed in the present study also, as higher concentrations of RMS and 4.56 g/l of CS induced significant changes in the morphological and haematological parameters studied. It is in accordance with generally recognised data that sodium, chloride, potassium and calcium are primarily responsible for osmoregulation in fishes, either by exerting osmotic effects (sodium and chloride) or by affecting uptake and excretion (calcium and potassium) (Evans, 1997). Rainbow trout (*Oncorhynchus mykiss*) was used in these studies as one of the fish species most sensitive to the impacts of xenobiotics; besides, this fish is a representative of *Salmonidae* species which may withstand some fluctuations of water salinity. However, even rather low concentration of RMS (0.18 g/l) reduced erythrocyte count and resulted in an increased haematocrit level in the exposed fish. We suggest two possible explanations for the changes in the parameters studied: first, though the concentrations of chemical constituents of RMS were lower than their Maximum Permissible Concentrations (MPC) accepted for Lithuanian inland waters (Anonymous 2000) or their limiting values permitted in other countries (e.g., for vanadium, strontium) (Irwin, 1997a, b; Gravenmier et al., 2005), salinity could enhance their toxic effects. This is consistent with data showing that the alterations in the bioavailability and toxicity of chemicals to aquatic organisms depend on water salinity (Van Leeuwen, Hermens, 1995; Četkauskaitė, 1999; Jezierska, Witeska, 2001). The second possible explanation could be the synergetic effects of pollutant mixtures. According to Van Leeuwen, Hermens (1995), some chemicals may interact resulting in a higher or lower toxicity of one or more of the chemicals involved (Montvydienė, Marčiulionienė, 2003); our previous studies also demonstrated that heavy metals in mixtures at low concentrations were more toxic than single ones (Vosylienė et al., 2003).

Our data confirm that even low levels of salts widely used for road maintenance can induce adverse effects in fish. Toxic effects caused by this salt depend on its concentrations and specific composition. The negative impact of this salt in natural waters will depend firstly on their concentrations in water, water characteristics and living aquatic animals.

ACKNOWLEDGEMENT

Authors thank Dr. Kęstutis Jokšas from the Institute of Geology and Geography for performing measurements of chemical constituents in road maintenance salt solutions.

Received 19 January 2006
Accepted 15 March 2006

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KELIŲ PRIEŽIŪRAI NAUDOJAMOS DRUSKOS POVEIKIS VAIVORYKŠTINIAM UPĖTAKIUI *ONCORHYNCHUS MYKISS*

S a n t r a u k a

Druskos, naudojamos kelių priežiūrai (KPD) šaltuoju metų laiku, ūminio ir ilgalaikio toksiškumo vaivorykštiniam upėtakiui tyrimai buvo atlikti, naudojant kompleksą biologinių parametrų. Apskaičiuota 96 val. LC50 KPD vertė vaivorykštinio upėtakio jaunikliams sudarė 18,25 g/l. Ilgalaikiuose tyrimuose nebuvo nustatyta patikimų KPD paveiktų žuvų masės skirtumų, lyginant su kontrole. Tuo tarpu 4,56 g/l ir 1,14 g/l koncentracijų KPD paveiktų žuvų kepenų masė ir kepenų somatinis indeksas patikimai skyrėsi nuo kontrolės. Kraujo rodiklių (eritrocitų skaičius, hemoglobino koncentracija, hematokrito lygis ir leukocitų skaičius) skyrėsi savo jautrumu KPD poveikiui. Žuvų, paveiktų visų tirtų koncentracijų KPD, kraujyje eritrocitų skaičius sumažėjo.

Raktažodžiai: druska kelių priežiūrai, upėtakis, toksiškumas