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Mortality of embryos, developmental disorders and changes in biochemical parameters in marsh frog *(Rana ridibunda)* tadpoles exposed to the water-soluble fraction of Kazakhstan crude oil and O-Xylene

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ABSTRACT

The effects of different concentrations of water-soluble fraction of crude oil (WSFO) from the Zhanazhol oil field (Aktobe region, Kazakhstan) and compared to o-xylene, prevalent in this oil, on growth and development of marsh frog *(Rana ridibunda)* were assessed. In subchronic experiments (7 d), a dose-related increase in mortality and incidence of deformities in embryos were observed. In chronic experiments (60 d; starting from the Gosner stage 26), a dose-dependent decrease in body weight, size and developmental delay by 3–4 stages were also detected. In addition, the content of lipid hyperoxide (LHO) and malondialdehyde (MDA), as well as activities of superoxide dismutase (SOD) and catalase (CAT) enzymes in liver of the tadpoles were determined at the end of chronic experiment. Exposure to 0.5 mg/L or 1.5 mg/L WSFO elevated the content of LHO by 76% and 86%, and MDA by 47% and 58% but decreased SOD activity by 26% and 49%, and CAT by 35% and 46%, respectively. A less pronounced adverse effect was found after chronic exposure to the same concentrations of o-xylene. In tadpole liver exposed to o-xylene levels of LHO was increased by 40% and 51%, MDA by 11% and 29%, while the activity of SOD was lowered by 18% and 41%, and CAT – by 13% and 37% in the 0.5 mg/L and 1.5 mg/L treatment groups, respectively. Data demonstrated the embryotoxic and teratogenic effects attributed to WSFO and o-xylene exposure which may involve oxidative stress mechanisms.

KEYWORDS

Rana ridibunda; water-soluble fraction of oil; o-xylene; development; oxidative stress

Introduction

The increasing global demand and growth of oil production and processing lead to increased environmental pollution by associated waste (Wu et al. 2014). Kazakhstan holds approximately 2% of the world's oil reserves, and oil production in recent years was approximately 18% of the GDP (Akhmadi Invest 2016). Thus, vast territories of the country are under development for oil and gas production. A consequence of this process is deterioration of the ecosystems in oil-producing regions (Askarova and Mussagaliyeva 2014). Intensive pollution of the environment by oil and petroleum products leads to the reduction of natural animal populations and the decrease in biodiversity (Kolesnikov et al. 2011; Neuparth et al. 2014). According to national ecological reports (Unified Environmental Internet Resource 2018), the content of petroleum products in waters of the country may be up to 36-fold higher than the maximum permissible concentrations in water (MPCW) (Ministry of Fisheries of the USSR 1990). Thus, assessment of toxicity of oil is highly relevant.

There are a number of toxicological studies of oil and petroleum products in Kazakhstan (Mahmoud, Shalakhmetova, and Umbayev 2012; Shalakhmetova, Mahmoud, and Umbayev 2012; Shalakhmetova et al. 2015; Shametov et al. 2015; Suvorova et al. 2015), but most of these investigations were conducted with either lab rodents (rats, mice) or natural rodent populations inhabiting the oil-producing regions, such as great gerbil (*Rhombomys opimus*) or yellow ground squirrel (*Spermophilus fulvus*). Many oil-producing regions in Kazakhstan contain numerous rivers and small lakes, hence these waterways may be contaminated by oil hydrocarbons; however little is apparently known regarding potential impacts on aquatic ecosystems. Due to their biological characteristics, such as

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semi-permeable skin and life cycle involving water and terrestrial stages, amphibians are a convenient model system for assessing the state of both terrestrial and aquatic ecosystems under conditions of anthropogenic transformation and pollution of the environment (ENSR International 2004; Gardner et al. 2016; Gutleb et al. 1999). The Eurasian marsh frog (*Rana ridibunda*) is a reliable target species due to its prevalence in most areas of Kazakhstan, including oil-producing regions (Duysebaeva et al. 2005). *R. ridibunda* is an integral link in food webs, serving as both an important predator and prey species; therefore, reducing the number of individuals could disrupt the balance within the ecosystem (Johari et al. 2015).

Exposure to oil and petroleum products results in relatively high mortality, reduced growth, and incidences of various malformations in amphibians (Sparling 2010). The effects of petroleum on early life stages were examined previously in many anuran amphibians species, such as African clawed frog (*Xenopus laevis*), wood frog (*Lithobates sylvaticus*), leopard frog (*Lithobates pipiens*), American green tree frog (*Hyla cinerea*) and others. Naphthenic acids, present in Oil Sands Process Affected Water (OSPW) exhibit acute toxic effects as evidenced by high mortality rates and significant delay in growth and development of *L. sylvaticus* (Melvin and Trudeau 2012a). Polycyclic aromatic hydrocarbon (PAH) fluoranthene induced severe malformations in *X. laevis* and *L. pipiens* in addition to increased mortality frequency (Hatch and Burton, Jr. 1998). Previous studies using *R. ridibunda* have investigated the effects of monochromatic light (Ruchin and Lobachev 2005) and heavy metals (Loumbourdis, Kyriakopoulou-Sklavounou, and Zachariadis 1999; Shiyan 2011). However, to our knowledge, no studies have examined the impacts of oil or petroleum products on this species. Further, no other apparent research has been conducted to determine the effects of oil from Kazakhstan.

Amphibian larvae possess a low level of tolerance and are susceptible to low concentrations of chemical compounds (Gardner et al. 2018). The effect of these substances is expressed not only in the appearance of developmental defects but also in the modification of a number of cytological, morphological, and biochemical parameters (Falfushynska et al. 2015; Salin et al. 2012; Salvaterra et al. 2013). Melvin et al. (2013) demonstrated that sub-lethal concentrations of a commercial naphthenic acids preparation disrupted normal metabolic function (glycogen and triglycerides storage in liver) in *L. pipiens*. It was also shown that presence of petroleum products leads to morphological deformities in tadpoles, accompanied by changes in the functioning of the antioxidant system (Amaeze, Onadeko, and Nwosu 2014; Wu et al. 2017). Although alterations in enzymes involved in the antioxidant system are not a specific response to the action of specific toxicants, the use of these biomarkers helps to confirm the effects of anthropogenic action at the biochemical level (Falfushinska et al. 2008; Venturino and Pechen de D'Angelo 2005).

The aim of this study was to examine the growth and development of *R. ridibunda* in conditions of water polluted with oil and oil products. Subchronic and chronic exposure to various concentrations of a water-soluble fraction of crude oil were compared to the effect of o-xylene.

Materials and methods

Preparation of water-soluble fraction of oil

Preparation of water-soluble fraction of oil (WSFO) was carried out according to Anderson et al. (1974), taking into account the recommendations of Singer et al. (2000). Crude oil from the Zhanazhol oil field (Aktobe region of the western part of the Republic of Kazakhstan) was mixed with water in a 1:9 ratio (100 ml oil per 900 ml distilled water). The resulting mixture was placed in a 1 L flask with a tightly closed stopper and stirred on a magnetic stirrer in the dark for 18 h avoiding deterioration of the oil film integrity and emulsification (Singer et al. 2000). After stirring, the resulting mixture was allowed to stand at room temperature for 6 h. Further, the water-soluble fraction was extracted using a separatory funnel and stored at 4°C. Before use in exposure experiments, the water-soluble fraction was acclimated to room temperature (21–23°C).

The prepared WSFO was analyzed by gas chromatography-mass spectrometry (GC/MS) method for hydrocarbon content (according to Holowenko, MacKinnon, and Fedorak 2002) (Figure 1). Preparation of WSFO samples for analysis was carried out as follows: 500 ml of a sample were extracted with 50 ml of hexane using liquid-liquid extraction for 30

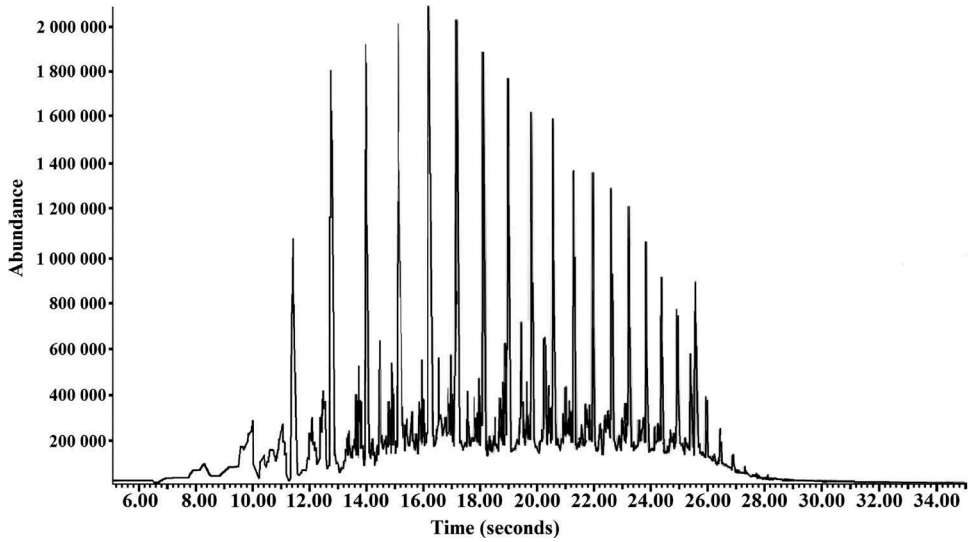

Figure 1. Chromatogram of the sample of water-soluble fraction of oil from the Zhanazhol oil field.

min. After extraction, the organic phase was separated using a separatory funnel, the total volume of the extract was measured with a measuring cylinder. All the obtained extracts of 1.5 ml were transferred to 2 ml glass vials with plastic screw caps and ultra-clean Teflon/silicone gaskets. Next, the samples were analyzed by GC/MS. Using a microsyringe for an autosampler Combi-PAL (CTC Analytics, Switzerland), 1 µl sample was injected into a gas chromatograph coupled to the 6890N/5973N mass spectrometry detector (Agilent, USA) in Splitless mode. Chromatography was performed using a DB-5MS capillary column with a length of 30 m, an inner diameter of 0.25 mm and a film thickness of 0.25 µm. The Agilent MSD ChemStation software (version 1701EA) was used to control the entire gas chromatographic system and to record and process the chromatographic data. Data processing included the determination of retention times, heights, and peak areas, as well as the processing of spectral information obtained using a mass spectrometric detector. The Wiley 7th edition and NIST'02 libraries were used to decipher the mass spectra obtained (the total number of spectra in the libraries is more than 550 thousand).

The WSFO contained a total of 1.72 mg/L petroleum hydrocarbons. The major component was o-xylene (Table 1). Therefore, additional experiments were conducted to determine the effects of o-xylene alone on *R. ridibunda* embryos and tadpoles. The o-xylene was purchased from Sigma–Aldrich (Cat No. 294780).

Table 1. Proportions of the oil hydrocarbons detected in the sample of WSFO from the Zhanazhol oil field.

Compounds	Content	Compounds	Content
Nonane	3.0%	3,5-dimethyl-octane	2.4%
Decane	4.2%	4-methyl-hexadecane	1.6%
Dodecane	4.7%	2-ethyl-1,4-dimethyl-benzene	3.9%
Tridecane	1.8%	1,2,4,5-tetramethyl-benzene	2.8%
Pentadecane	2.5%	n-nonylcyclohexane	5.1%
Hexadecane	1.1%	Pentyl-cyclohexane	2.5%
Heptadecane	0.8%	Decyl-cyclohexane	1.3%
Octadecane	0.7%	1,4-dimethyl-, cis-cyclohexane	3.4%
Nonadecane	1.2%	1,2-dimethyl-, trans-cyclohexane	1.4%
Eicosane	0.4%	4-ethyl- cyclohexanone	3.3%
Heneicosane	1.6%	2-propenyl-cyclohexane	1.9%
Tetracosane	0.9%	Benz(o)pyrene	9.2%
Pentacosane	1.1%	o-Xylene	26.3%
Octacosane	0.8%	p-Xylene	8.1%
Pentatriacontane	0.3%	Mesitylene	1.0%
2,6,10,14-tetramethyl-pentadecane	0.7%		

Obtaining *R. ridibunda* eggs

Fifteen mature specimens of *R. ridibunda* (nine males and six females) were caught in April 2017 from the river Emba (Aktobe region, the Republic of Kazakhstan) and brought to the Ecotoxicology Laboratory of the Faculty of Biology and Biotechnology of the Al-Farabi Kazakh National University. Adult males and females were placed separately in 100 L aquaria with algae and branches to create conditions similar to natural

spawning substrate. The water temperature was maintained at 20 ± 2°C. Frogs were fed crickets and kept for two weeks to acclimatize before induction of spawning.

For the production of eggs, both males and females were injected (5 µl/g body weight) with a mixture of a gonadotropin-releasing hormone agonist (0.4 µg/g) and metoclopramide (10 µg/g) according to the AMPHIPLEX method described by Trudeau et al. (2010) except drugs were dissolved in saline. Adult frogs were divided into groups of five individuals (three males and two females) into separate aquaria (three aquaria in total). After 2–3 d, three clutches of fertilized eggs were obtained. For experiments on embryotoxicity, eggs were taken randomly from each of the clutches and placed in Petri dishes after they reached the Gosner stage (Gs) 8–11 (Gosner 1960). The residual eggs were transferred into 100 L aquaria with clean dechlorinated aerated water at 23 ± 2°C for further development. Once tadpoles reached Gs 26, when they all began to feed and swim independently, they were randomly caught and allocated to 18 L aquaria (15 per tank) filled with 15 L of dechlorinated water for conducting a chronic experiment.

Effect of the WSFO and O-Xylene on embryonic development

Early development of *R. ridibunda* was investigated under the conditions of exposure of eggs to three concentrations of WSFO or o-xylene (0.05, 0.5 or 1.5 mg/L) in separate experiments. The concentrations for WSFO treatments were selected according to maximum permissible concentrations of oil hydrocarbons in water (MPCW) accepted in the Republic of Kazakhstan (Ministry of Fisheries of the USSR 1990). In our study, concentrations employed were equal to the MPCW (0.05 mg/L), and were exceeded by 10 fold (0.5 mg/L) and 30 fold (1.5 mg/L). The highest concentration was selected as 30 fold MPCW as it was not possible to obtain 100 fold MPCW from our WSFO samples which contained 1.72 mg/L oil hydrocarbons. *R. ridibunda* embryos were divided into four groups: control (aerated dechlorinated water), 0.05, 0.5, and 1.5 mg/L WSFO. Each group consisted of 15 eggs at the Gs 8–11 stage in an 80 mm Petri dish containing 15 ml of corresponding medium: dechlorinated water (control), ethanol (solvent control), WSFO or o-xylene. Four replicates were set for each group (60 embryos in total for each group of the experiment). Embryos in each group were incubated at 22 ± 2°C for 7 d. The dishes were examined every 24 hr to identify and remove dead embryos and larvae, and replace the water and add the appropriate concentrations of WSFO or o-xylene. On day 7, the surviving larvae were euthanized in a buffered solution of the MS-222 anesthetic (Tricaine Methane Sulfonate; Sigma–Aldrich) and fixed in formalin, and subsequently examined for developmental disorders using a stereoscopic microscope (Motic DM 143, China).

The o-xylene only exposures were conducted the same way as the WSFO treatments except the solvent control (ethanol, final concentration 0.005%) was used instead of water control.

Chronic exposure experiments

For the experiment, Gs 26 stage tadpoles of a similar size capable of independent swimming and feeding were selected which corresponds to the Gs 26 stage. Tadpoles were exposed to control (dechlorinated water), 0.05, 0.5, and 1.5 mg/L WSFO for 60 d. There were three replicates for each treatment group, each containing 15 tadpoles. The tadpoles were placed into 18 L aquaria filled with 15 L of aerated dechlorinated water (t = 23 ± 2°C), and the appropriate concentrations of ethanol, WSFO or o-xylene added. The tadpoles were fed boiled lettuce and seaweed each day, *ad libitum*, and feces and food waste was removed daily. Eighty % of the water was replaced every two days, followed by the addition of appropriate concentrations of WSFO and o-xylene. At the end of the experiment (60 d), tadpoles were euthanized in a buffered solution of the MS-222 anesthetic (Tricaine Methane Sulfonate; Sigma–Aldrich), wet weight was measured, photographs were made using a stereoscopic microscope (Motic DM 143, China) to measure the morphometric parameters (snout-vent length (SVL) and total body length) and liver samples were flash frozen and stored at −80°C for further biochemical analysis. In addition, the presence of morphological abnormalities was also noted.

Chronic effects of o-xylene on *R. ridibunda* tadpoles were studied the same way as the WSFO treatments except the solvent control (ethanol, final concentration 0.005%) was used instead of water control.

Assessment of biochemical indicators

For all assays, data on the liver were expressed per mg protein. The content of lipid peroxidation products (lipid hydroperoxides (LHO) and MDA) was determined using the Lipid Hydroperoxide (LPO) Assay Kit (Cat No. 437639, Merck) and Lipid Peroxidation (MDA) Assay Kit (Cat No. MAK085, Sigma-Aldrich). The LPO assay is based on detection of ferric ions produced as a result of the reaction of LHO with ferrous ions. In this method, thiocyanate ion is used as chromogen. The LHO were extracted from homogenized liver samples with deoxygenated chloroform. For LHO assay 500 µL chloroform extract, 450 µL chloroform:methanol mixture and 50 µL chromogen were mixed in test tubes and incubated at room temperature for 5 min. The absorbance of each tube was measured at 500 nm using quartz cuvettes against chloroform:methanol mixture as a blank. MDA assay is based on its reaction with thiobarbituric acid (TBA). Tissue samples were homogenized in 300 µl of the MDA Lysis Buffer containing 3 µl of butylated hydroxytoluene, followed by centrifugation at 13,000 g for 10 min. TBA was added to homogenate followed by incubation at 95°C for 60 min, then cooling to room temperature on an ice bath. The absorbance was measured colorimetrically at 532 nm.

The activity of antioxidant enzymes was determined using superoxide dismutase (SOD) Assay Kit (Cat No. 19160, Sigma-Aldrich) and Catalase (CAT) Assay Kit (Cat No. CAT100, Sigma-Aldrich) . The SOD assay is based on the quantitative measurement of red formazan. It is formed as a result of the reduction of Dojindo's highly water-soluble tetrazolium salt (WST), which are generated by xanthine oxidase. Fifty percent inhibition of this system corresponds to one conventional unit of activity of SOD. Homogenated liver samples were centrifuged at 12000 g for 10 min. The SOD activity was determined by adding 20 µL supernatant and 20 µL enzyme working solution to 200 µL WST solution followed by 20 min incubation at 37°C. The absorbance was read at 450 nm using a microplate reader. CAT assay method is based on the measurement of the hydrogen peroxide substrate remaining after the action of catalase. The reaction was initiated by adding 25 µL 3% H_2O_2 to 20 µL liver lysate diluted in 55 µL 500 mM potassium phosphate buffer (pH 7.0) and stopped in 5 min by adding 900 µL 15 mM sodium azide. Then, 10 µL of the obtained aliquot was mixed with 1 mL color reagent (150 mM potassium phosphate buffer, pH 7.0, containing 0.25 mM 4-aminoantipyrine and 2 mM 3,5-dichloro-2-hydroxybenzenesulfonic acid). The absorbance was measured in 15 min at 520 nm.

Statistical analyses

Data were analyzed for statistical significance with Fisher's exact test and one-way ANOVA using SPSS version 23 (IBM Inc., Chicago, USA), with α set as 0.05. To compare the mortality levels in control and experimental groups the Fisher's exact test was used. The morphometric measurements (SVL, total body length), developmental stage (Gs), and biochemical analyses results (LHO and MDA content, and SOD and CAT activity) at the time of sampling (day 60) Levene's tests indicated homogeneity of variance so the data were analyzed using one-way ANOVA followed by Tukey's *post-hoc* test. In all tests, WSFO treatments were compared to water control, whereas the o-xylene treatments were compared to the solvent control. Data on SVL, total body length, developmental stage, LHO, and MDA content, and SOD and CAT activity are presented as mean ± SEM.

Results

Subchronic exposure to WSFO or O-Xylene

Exposure to the increasing levels of WSFO resulted in significantly higher mortality rate (Table 2), as well as developmental delay (Figure 2) of larvae compared to control and solvent control. Mortality of embryos at the end of the experiment (168 hr) (Table 2) in control group was 8%, whereas in groups incubated in water containing 0.05, 0.5, and 1.5 mg/L WSFO it was 15%, 28%, and 37%, respectively. Almost the same increase in embryo mortality

Table 2. Ratio of dead and surviving *R. ridibunda* embryos exposed to 0 (control), 0.05 mg/L, 0.5 mg/L, and 1.5 mg/L of WSFO for 168-h post-fertilization.

	Duration of exposure (h) Mortality (dead/alive ratio)							
Concentration (mg/L)	0	24	48	72	96	120	144	168
0	0/60	0/60	2/52	3/57	4/56	4/56	5/55	5/55
0.05	0/60	0/60	2/58	3/57	5/55	6/54	9/51	9/51
0.5	0/60	2/58	5/55	8/52	11/49	16/44	17/43	17/43**
1.5	0/60	3/57	6/54	12/48	17/43	22/38	22/38	22/38**

Note: significance was analyzed by Fisher's exact test.
**$p < 0.01$.

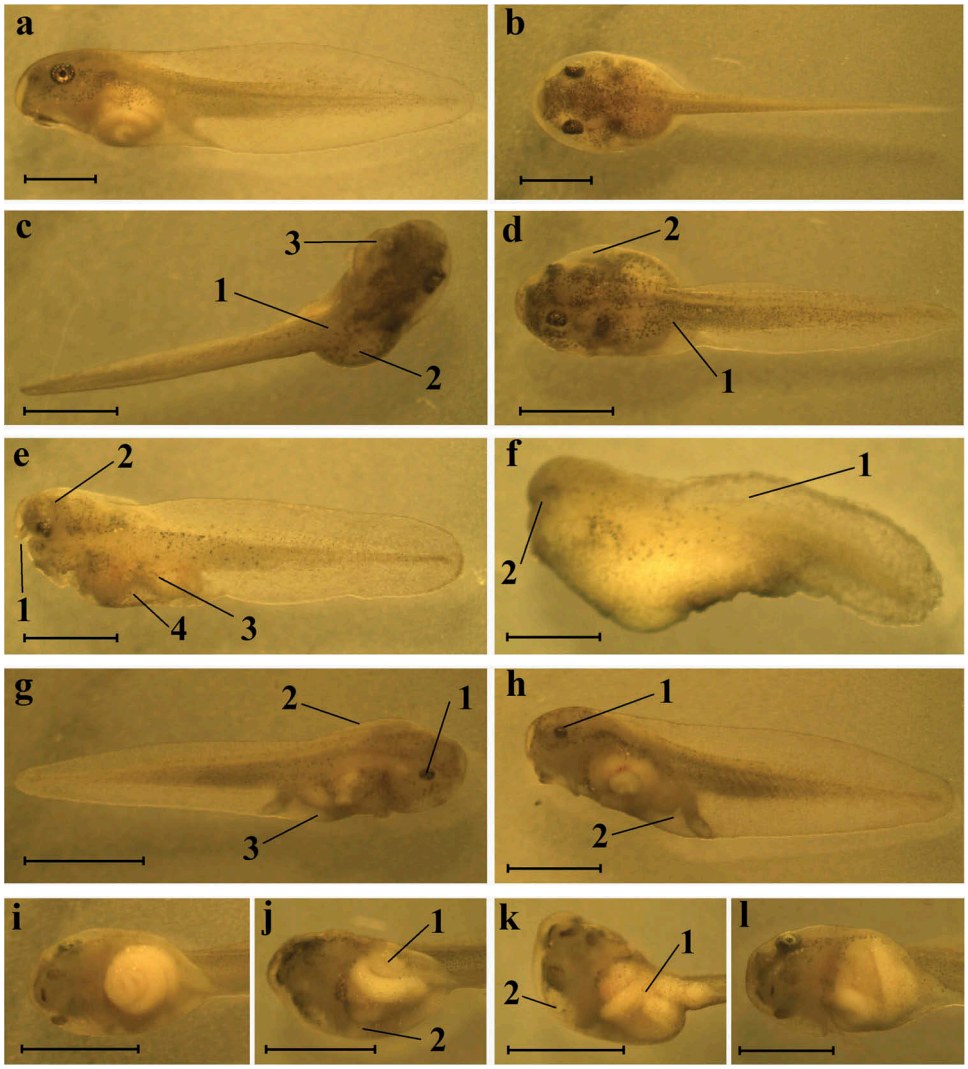

Figure 2. Larvae of *R. ridibunda* after 7 d of exposure to WSFO or o-xylene. **a** and **b –** lateral and dorsal view of a tadpole from the control group; **c** – axial curvature (1), edema (2) and eye malformation (3) in the tadpole from the group 0.05 mg/L of the WSFO, **d** – axial curvature (1) and edema (2) in the tadpole from the 0.05 mg/L o-xylene group; **e** – eye malformation (1), mouth malformation (2), and gut malformation (3), and visceral edema (4) in tadpole from the 0.5 mg/L WSFO group, **f –** axial curvature (1), head malformation (2) in tadpoles from the 0.5 mg/L o-xylene group; **g** – microphthalmia (1), edema (2), gut malformation (3) in tadpole from the 1.5 mg/L WSFO group, **h** – microphthalmia (1), gut malformation (2) in tadpoles from 1.5 mg/L o-xylene group; **i –** a normally twisted gut in a tadpole from a control group; **j** – diverted gut (1), edema (2) in the tadpole from the group 0.5 mg/L of WSFO group; **k** – gut uncoiling (1), edema (2) in the tadpole from the 1.5 mg/L of WSFO group; **l** – gut uncoiling in a tadpole from the group 1.5 mg/L of o-xylene group. The scales indicate 1 mm.

was observed in o-xylene-treated groups: mortality rates were 5% in solvent control, 12% at 0.05 mg/L, 22% at 0.5 mg/L, and 30% at 1.5 mg/L (Table 3).

The effect of WSFO or o-xylene exerted a marked teratogenic action on *R. ridibunda* larvae. Photos of the larvae after 7 d of exposure to WSFO or o-xylene are illustrated in Figure 2. Table 4 presents the number of various abnormalities observed in tadpoles from WSFO and o-xylene treatments. By day 7 larvae in 0.05 mg/L WSFO or 0.05 mg/L o-xylene groups reached the stage Gs 23–25 and, on average, did not differ markedly from control or solvent control. The larvae exhibited only a few cases of axial curvatures and small visceral edema, which is comparable to the incidence of the same abnormalities in control or solvent control (Figure 2c–d). With an increase in concentration of toxicants (0.5 mg/L WSFO and 1.5 mg/L o-xylene), various developmental anomalies and morphological disturbances such as developmental delay, abnormal gut coiling, axial curvature, microphthalmia, edema, hemorrhages were noted (Figure 2e–h, j–l).

In all experimental groups, an increase in morphological deformities was observed relative to control (Table 4). In tadpoles exposed to 0.5 mg/L WSFO, abnormalities such as axial curvature, diverted guts, and gut uncoiling accompanied by visceral edema, occurred most frequently (Table 4). In addition, there were various disorders in the development of the head including microcephaly and eye and mouth malformations. In the 1.5 mg/L group, 47% of surviving tadpoles experienced delayed development, reaching only Gs 17–19 by day 7(Figure 4c). In this as well as the 0.5 mg/L group, common disorders included axial curvature, gut malformations, and edema, but the incidence of these alterations was higher (74%, 54%, and 52%, respectively) (Table 4). When exposed to o-xylene developmental delay and axial curvatures were also often detected in tadpoles. In groups exposed to o-xylene concentrations of 0.5 mg/L and 1.5 mg/L, the incidence of gut uncoiling and edema was the highest (39% and 42%, respectively) (Table 4).

Chronic effects of WSFO or O-Xylene

In contrast to the subchronic experiment, there was no significant difference in survival rate between control and treated in chronic experiment (60 d). The rate of mortality did not exceed 5% in control and solvent control, 8% in WSFO and 7%

Table 3. Ratio of dead and surviving *R. ridibunda* embryos exposed to 0 (control), 0.05 mg/L, 0.5 mg/L, and 1.5 mg/L of o-xylene for 168-h post-fertilization.

	Duration of exposure (h) Mortality (dead/alive ratio)							
Concentration (mg/L)	0	24	48	72	96	120	144	168
0 (control, EtOH 0.005%)	0/60	0/60	0/60	1/59	1/59	3/57	3/57	3/57
0.05	0/60	0/60	0/60	2/58	3/57	5/55	6/54	7/53*
0.5	0/60	4/56	7/53	7/53	9/51	10/50	12/48	13/47*
1.5	0/60	5/55	8/52	11/49	13/47	15/45	18/42	18/42**

Note: significance was analyzed by Fisher's exact test.
*$p < 0.05$.
**$p < 0.01$.

Table 4. Total number and percentage of incidence of malformations observed in surviving tadpoles at the end (day 7) of the subchronic experiment.

	Treatment							
	WSFO				O-xylene			
Type of malformation	Control	0.05 mg/L	0.5 mg/L	1.5 mg/L	Solvent control	0.05 mg/L	0.5 mg/L	1.5 mg/L
Stuntedness	-	1 (2%)	8 (18%)	18 (47%)	-	-	7 (14%)	15 (35%)
Axial malformations	2 (3%)	1(2%)	10 (24%)	28 (74%)	1 (2%)	1 (2%)	8 (17%)	13 (32%)
Microcephaly	-	-	5 (11%)	8 (21%)	-	-	3 (6%)	5 (12%)
Eye malformation	1 (2%)	-	6 (13%)	13 (33%)	-	1 (2%)	3 (6%)	5 (12%)
Gut malformation	1 (2%)	1 (2%)	7 (17%)	21 (54%)	2 (4%)	1 (2%)	8 (17%)	16 (39%)
Edema	2 (3%)	1 (2%)	7(17%)	20 (52%)	2 (4%)	2 (4%)	10 (21%)	18 (42%)
Total number of surviving tadpoles	55	51	43	38	57	53	47	42

in o-xylene groups. However, other measured parameters such as weight, SVL, total body length, developmental stage differed between experimental groups and control, especially when exposed to higher concentrations of WSFO or o-xylene.

Exposure to the 0.05 mg/L of WSFO or o-xylene exerted no significant effect on *R. ridibunda* tadpoles wet weight (Figure 4). Tadpoles chronically exposed to 0.5 or 1.5 mg/L of WSFO displayed a significantly (28% and 43%) lower weight compared to control groups (Figure 3a). Similarly, the body weight of the tadpoles exposed to 0.5 or 1.5 mg/L o-xylene was found to be significantly (28% and 48%) lower (Figure 3b).

Measurements of SVL (Figure 4a) and total body length (Figure 4b) showed a marked decrease in the WSFO compared to with control. Treatment with 0.05, 0.5 or 1.5 mg/L resulted in 5%, 21%, and 42% shorter SVL compared to control, respectively. The total body length was decreased by 2%, 23%, and 37% in 0.05, 0.5 or 1.5 mg/L WSFO groups compared to control, respectively. At the time of measurement, most animals in the control group reached Gs 36–37, which are characterized by separation of toes in the hindlimbs (Figure 4c). There were no significant delays in the development of tadpoles in the 0.05 mg/L group but the development of tadpoles in 0.5 and 1.5 mg/L animals was delayed on average by approximately 3 and 4 stages compared to control, respectively (Figure 4c). For example, many animals in 1.5 mg/ . were at Gs 31–32, when the hindlimbs are at the foot paddle stage of development (Gosner 1960).

Similar results were obtained for SVL (Figure 5a) and total body length (Figure 5b) in tadpoles exposed to o-xylene. The SVL and total body length of solvent control and 0.05 mg/L o-xylene group did not differ markedly. In treatment groups 0.5 and 1.5 mg/L o-xylene, these parameters were decreased, respectively, by 17% and 33% compared to the solvent control. At the time of measurement, most animals in the control group reached Gs 37, which is characterized by complete separation of toes in the hindlimbs. There was also a significant developmental delay on average by approximately four stages in the 1.5 mg/L o-xylene group (Figure 5c) and many tadpoles were at Gs 33, which is typified by indentations of the developing foot paddle becoming evident (Gosner 1960).

Hepatic antioxidant system after chronic exposure to WSFO or O-Xylene

The hepatic concentrations of primary and secondary products of LPO (LHO and MDA) and activity of antioxidant defense enzymes (SOD and CAT) exposed to WSFO are shown in Figure 6. Differences in the levels of LHO and MDA between tadpoles exposed to 0.05 mg/L WSFO and those of control were not s significant (Figure 6a–b). The levels of primary product of LPO – LHO in tadpoles treated with WSFO at a concentration of 0.5 or 1.5 mg/L WSFO was significantly increased by 76% and 86%, respectively, in comparison with control (Figure 6a). In the liver of tadpoles of the same experimental groups, a statistically significant rise in concentrations of secondary LPO product: MDA was found to be 47%

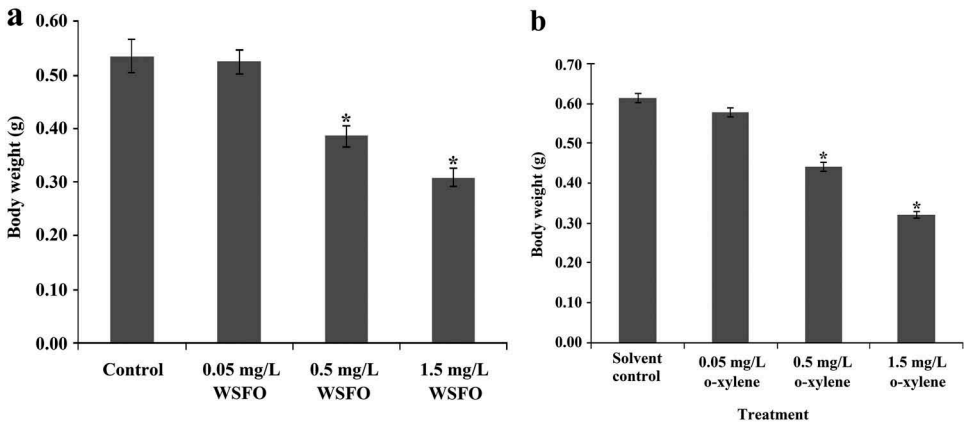

Figure 3. Body weight of *R. ridibunda* tadpoles after 60 d of exposure to WSFO (a) and o-xylene (b). Bars represent mean ± SEM (n = 3). Asterisk indicates data statistically different from control ($p < 0.05$).

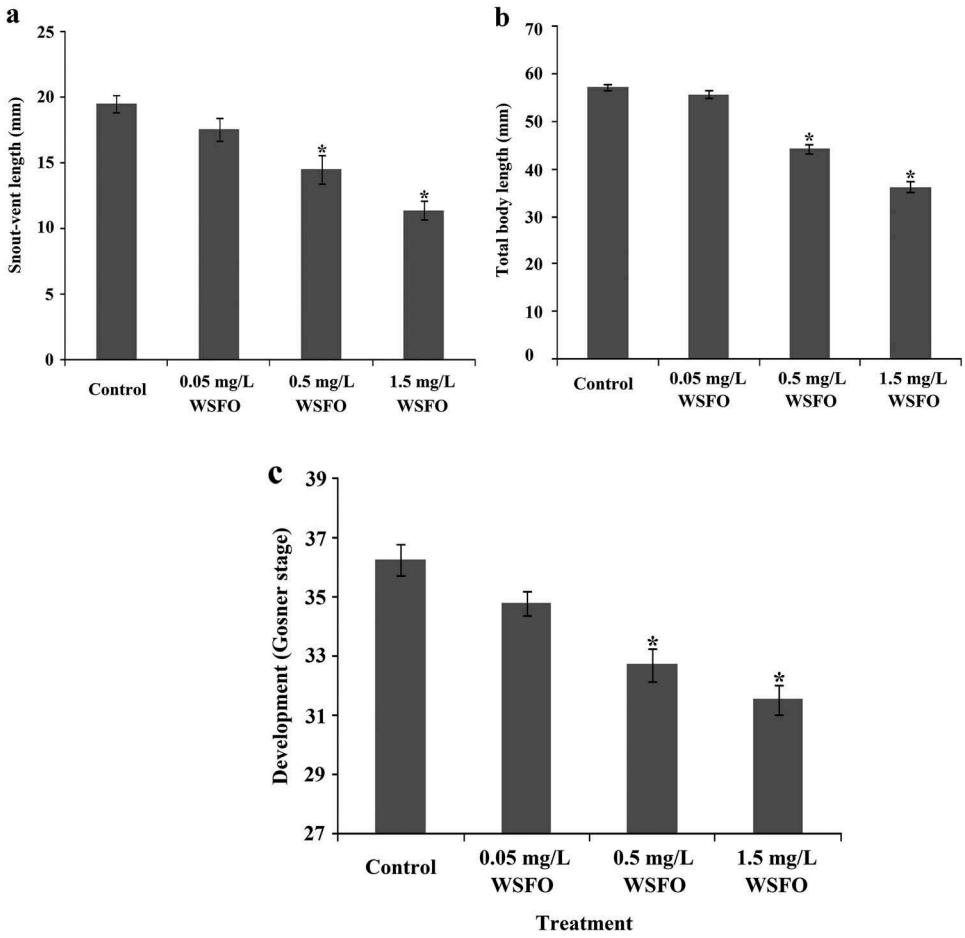

Figure 4. SVL (a), total body length (b), and development (Gosner stage) (c) of *R. ridibunda* tadpoles after 60 d of exposure to WSFO. Bars represent mean ± SEM (n = 3). Asterisk indicates data statistically different from control ($p < 0.05$).

and 58% higher than control, respectively (Figure 6b). The activity of hepatic antioxidant enzymes SOD and CAT exposed to 0.05 mg/L WSFO did not differ markedly from control (Figure 6c–d). A similar trend was observed in CAT activity with decreased by 35% in tadpoles exposed to 0.5 mg/L of WSFO, and by 46% in tadpoles exposed to 1.5 mg/L compared to the control (Figure 6d).

Data on the content of LPO products and activity of antioxidant defense enzymes in liver of tadpoles exposed to o-xylene are presented in Figure 7. The levels of LHO and MDA after chronic exposure to 0.05 mg/L o-xylene did not differ markedly from solvent control. The effect of 0.5 or 1.5 mg/L of o-xylene produced a significant elevation in levels of LHO by 40% and 51%, respectively (Figure 7a). Similarly, exposure to 0.5 or 1.5 mg/L o-xylene resulted in significantly increased MDA levels by 11% and 29% compared to solvent control, respectively (Figure 7b). Hepatic SOD activity of tadpoles exposed to 0.5 or 1.5 mg/L o-xylene was markedly reduced by 18% and 40%, respectively (Figure 7c). CAT activity in liver of tadpoles exposed to 0.5 or 1.5 mg/L o-xylene was also significantly lower compared to solvent control by 13% and 37%, respectively (Figure 7d).

Discussion

Data demonstrated that environmentally relevant concentrations of WSFO or o-xylene caused a dose-dependent increase in mortality rate in *R.ridibunda* embryos. The increased mortality is a general response of exposure of embryos and larvae to toxicants, which indicates an adverse effect attributed to contaminants (Peltzer et al. 2013). An increased mortality rate was observed in *L. sylvaticus* tadpoles exposed to low concentrations of commercial naphthenic acids (Melvin and Trudeau 2012b), and in *X. laevis, L. pipiens* and *A. maculatum* tadpoles exposed to fluoranthene

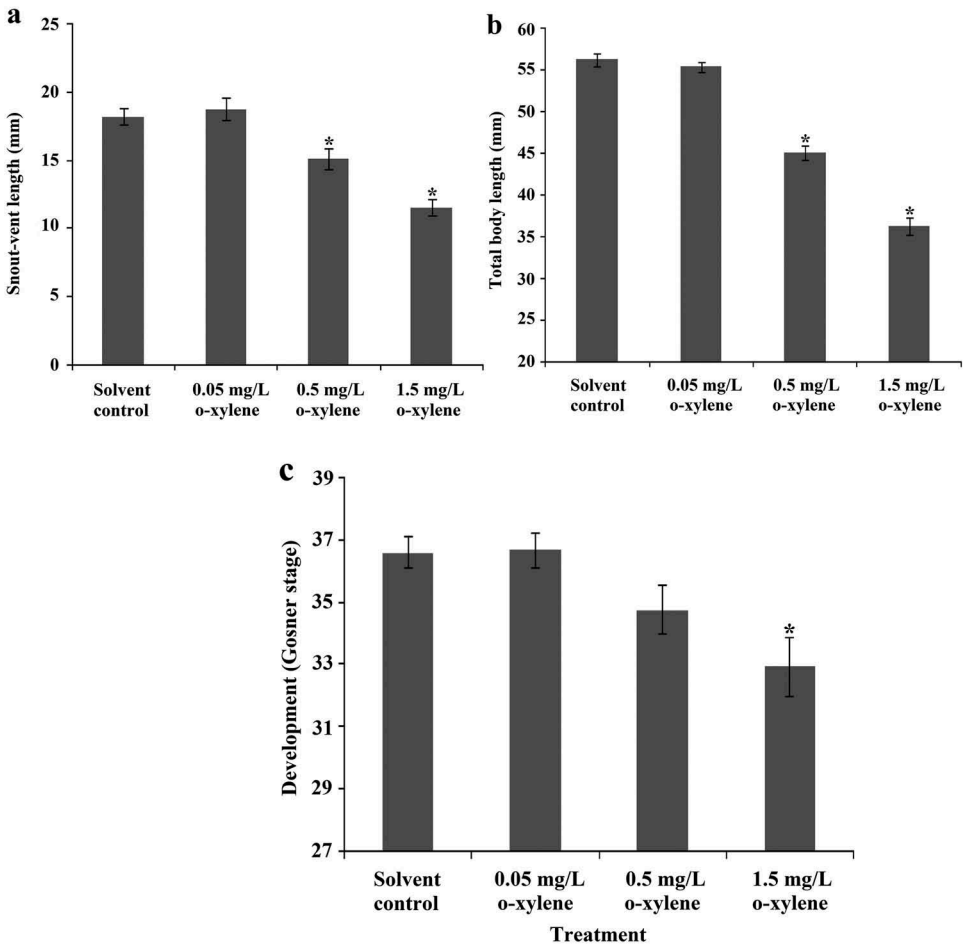

Figure 5. SVL (a), total body length (b), and development (Gosner stage) (c) of *R. ridibunda* tadpoles after 60 d of exposure to o-xylene. Bars represent mean ± SEM (n = 3). Asterisk indicates data statistically different from control ($p < 0.05$).

(Hatch and Burton 1998). The findings of these and our researches demonstrate that the presence of petroleum-related products in water may be a significant risk factor for developing amphibians. Thus, petroleum contamination may contribute to amphibian populations decline in areas with active oil production. The toxicity of various pollutants, including oil and petroleum products, has also been observed for other aquatic organisms, for example, fish (Marentette et al. 2015; Pampanin et al. 2016), amphipods (Neuparth et al. 2014); mollusks (Wessel et al. 2007), crustaceans (Hansen et al. 2016, 2015). The risk of exposure of embryos to contaminants from oil spill film is higher for those species of amphibians and fish that lay eggs close to the water surface in the immediate vicinity of the oil film (Mahaney 1994), where the concentrations of water-soluble hydrocarbons are the highest. The types of the morphological deformities of *R. ridibunda* larvae that were observed in the present study are similar to the developmental anomalies that arise in other species of amphibians, exposed to various chemicals, such as benzo[a]pyrene (Saka 2004), endosulfan (Brunelli et al. 2009), cetylpyridinium chloride (Park et al. 2016), or in amphibians from polluted natural sites (Peltzer et al. 2013). It should be noted that the incidence of abnormalities, as well as mortality, rose with increasing concentrations of oil and o-xylene. Probably, the presence of 1.5 mg/L WSFO or 1.5 mg/L o-xylene caused appearance of anomalies in *R. ridibunda* embryos incompatible with further development. There are data according to which the sensitivity of fish to the action of toxicants is the highest in the early stages of development (embryos and larvae) and decreases in juvenile and adult individuals (McIntosh et al. 2010). Similar results were noted in amphibians (Gulteb A. et al. 1999; Marquis et al. 2006b; Salin et al. 2012). In our study a relatively high rate of mortality was found when *R. ridibunda* embryos were exposed to medium (0.5 mg/L) and high (1.5 mg/L) concentrations of

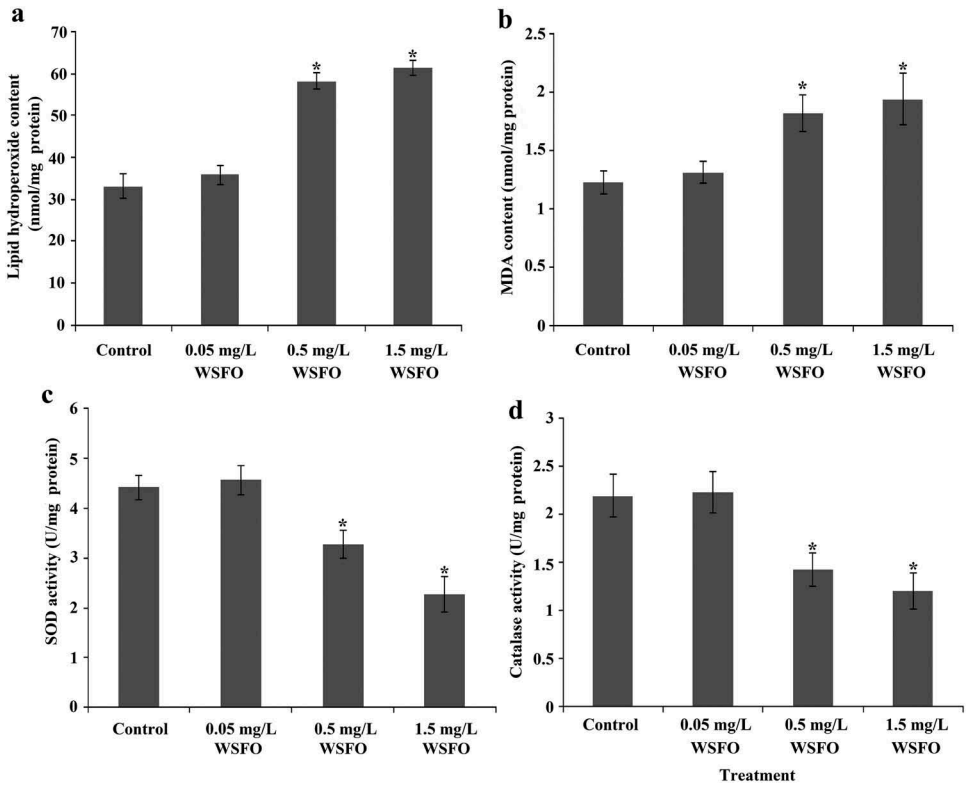

Figure 6. LHO (a) and MDA (b) content, SOD (c) and CAT (d) activity in liver of tadpoles after 60 d of exposure to WSFO. Bars represent mean ± SEM (n = 3). Asterisk indicates data statistically different from control ($p < 0.05$).

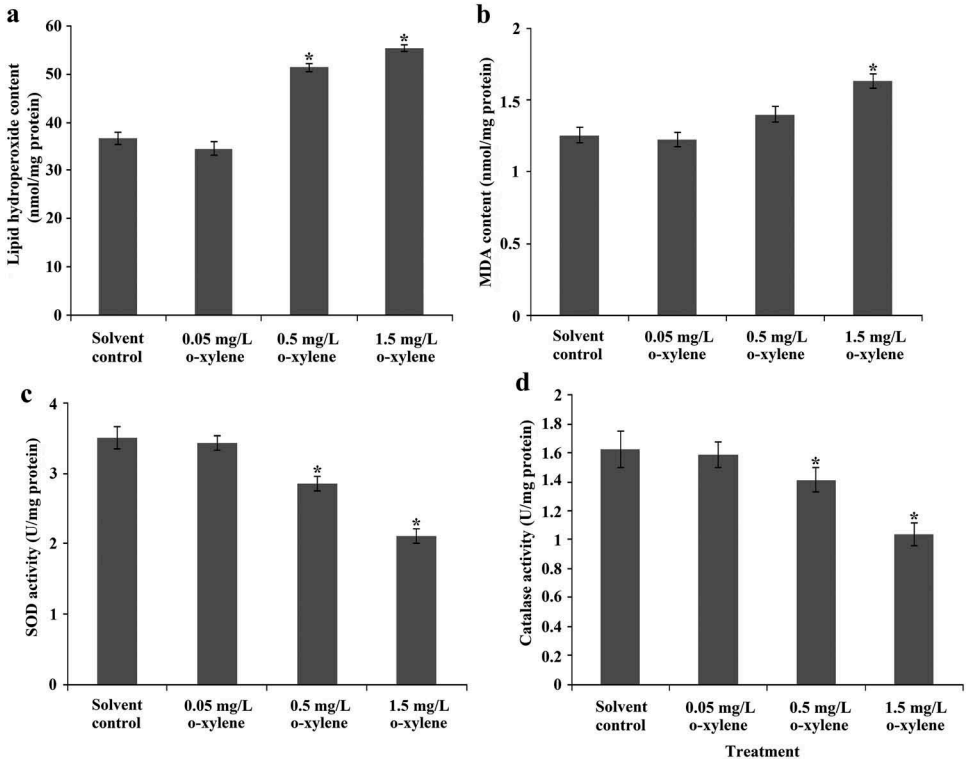

Figure 7. LHO (a) and MDA (b) content, SOD (c) and CAT (d) activity in liver of tadpoles after 60 d of exposure to o-xylene. Bars represent ± SEM ($n = 3$). Asterisk indicates data statistically different from control ($p < 0.05$).

WSFO or o-xylene, while these toxicants did not markedly affect the survival when treated as tadpoles. Similarly, a higher mortality rate of embryos in comparison to larvae was reported by Marquis et al. (2006b) who examined the toxicity of various solvents to early life stages of *Rana temporaria*. The authors suggested a hypothesis that some components of jelly coat might react with solvents becoming toxic to the embryos. However, the same group of researchers reported a protective role of the jelly against three PAHs (naphthalene, phenanthrene, and pyrene) dissolved in water (Marquis et al. 2006a). Taking into account that composition and thickness of the jelly coat differ among amphibian species (Duellman and Trueb 1994), embryonic sensitivity to pollutants may vary among species. Another important factor is the content of dissolved oxygen (DO) which is essential for embryos and larvae development (Seymour et al. 2000). Mills and Barnhart (1999) reported no lethal effect of hypoxia in two *Ambystoma* (*A. maculatum, A. annulatum*) and two *Rana* (*R. sphenocephala, R. palustris*) species, however, in *Ambystoma* slowed development and delayed hatching were observed, whereas *Rana* hatched sooner. In other study (Seymour et al. 2000) eggs of Australian frog, *Crinia georgiana* were incubated at a range of oxygen partial pressure (Po_2) between 2 and 25 kPa. Development rate to stage 26 between 5 and 2 kPa was not affected, but hatching occurred earlier at lower Po_2. Also at 2 kPa, there was a delay in growth and development of embryos, and morphological anomalies appeared. In our study, the difference between chronic and acute experiments was that aquaria with tadpoles were aerated and Petri dishes with embryos were not. This difference may account for the higher mortality rate in acute compared to chronic experiment. The incidences of malformations also may indicate low DO level. However, it should be noted that the dishes were filled with aerated water, which was refreshed daily, and hence embryos presumably did not suffer acute hypoxia. Moreover, there are many studies showing various tolerance to hypoxia among amphibian species, such as study of Seymour (1995) reporting no obvious developmental retardation in *R. sylvatica* below 5 kPa, and study of Bradford and Seymour (1988) showing developmental retardation in *P. bibronii* at 12.2 kPa, and death at 6.9 kPa and below. Thus, it would be incorrect to state unequivocally that the changes we observed were due solely to the action of petroleum products or hypoxia. This question requires further study.

The results of the chronic experiments demonstrated a dose-dependent delay in growth and development of *R. ridibunda* as evidenced by reduced weight, decreased SVL and total body length with increasing concentration of WSFO and o-xylene from 0.05 to 1.5 mg/L. In addition, the tadpoles in groups 0.5 and 1.5 mg/L exhibited developmental delay by 3–4 stages. Suppression of growth is not a specific indicator for the impact of oil and oil products, but several researchers suggested, that this is one of the most sensitive markers of toxic effects (Brunelli et al. 2009; Eriyamremu et al. 2008; Richards and Kendall 2003). Our data are consistent with the results of researches on the effects of naphthenic acids on anuran amphibians tadpoles (Hersikorn, Ciborowski, and Smits 2010; Melvin et al. 2013). It should be noted that the sensitivity of different types of amphibians to oil may not be equal, and may also depend upon the composition of the particular oil under study due to variations in content of hydrocarbons in different types of oil and so further work is needed to investigate the effects of different types of oil on amphibians development not only in laboratory experiments but in petroleum-contaminated natural sites.

In nature in addition to a high death rate of embryos due to intoxication with petroleum products, tadpoles able to successfully undergo metamorphosis may be affected due to their small size, where large tadpoles find it easier to compete for food and avoid predator attacks (Bacon et al. 2006). In addition, if the size of the frogs after metamorphosis is less than the natural rate, it becomes more difficult for them to find and catch food of suitable size, which may lead to death from hunger. A similar negative effect may also be manifested in other species of amphibians, contributing to the reduction of their populations.

The main structural components of cell membranes are lipids, which are easily oxidized by reactive oxygen species (ROS). One of the main indicators of the status of cell membranes is the level of lipid peroxidation (LPO). This is a physiological process, and an imbalance in radicals and ROS generation lead to a disruption in the integrity of cell membranes (Adeyemi 2014;

Georgieva 2005; Oruç and Usta 2007). The degree of LPO is determined from the levels of primary and secondary lipid peroxidation products in the tissues, lipid hydroperoxides (LHO) and malondialdehyde (MDA). Our observations noted that WSFO or o-xylene elevated hepatic LPO products indicative of an enhanced formation of ROS in the long-term presence of petroleum hydrocarbons in water. Our data are consistent with those of Eriyamremu et al. (2008), who investigated the influence of Bonny light crude oil on *X. laevis* tadpoles and found that prolonged exposure to crude oil resulted in increased accumulation of MDA and inhibition of SOD and glutathione reductase activity.

In our studies, the chronic effect of WSFO on *R. ridibunda* tadpoles produced a dose-dependent suppression of SOD and CAT activity. In the case of o-xylene, similar changes were observed. The decrease in SOD and CAT activity during chronic exposure to high concentrations of WSFO or o-xylene indicates the damage and death of liver cells of treated tadpoles. This assumption was put forward by many researchers in the studies of oxidative stress induced by xenobiotics (Burraco and Gomez-Mestre 2016; Ferrari et al. 2011; Kalra, Mantha, and Prasad 1994; Neuparth et al. 2014; Stefani Margarido et al. 2013).

The effect of o-xylene on growth and development of *R. ridibunda* was similar to that of WSFO. Since the main composition of the fraction is aromatic hydrocarbons, including the prevailing o-xylene, our results indicate that aromatic hydrocarbons exposure produced a disturbance in development. It should be noted that the medium concentration of o-xylene used in the study (0.5 mg/L) corresponds to the drinking water limit concentration according to WHO (Gorchev and Ozolins 2011). Both 0.5 and 1.5 mg/L o-xylene concentrations exerted a negative effect which may be due to an enhanced sensitivity of this species *R. ridibunda*. At the same time, although o-xylene was the most abundant component in the samples of WSFO from Zhanazhol oil field, this does not indicate its higher toxicity compared to other components. Presumably, its prevalence may account for similar trends in the observed effects in WSFO and o-xylene experiments. Analysis of literature revealed a paucity of information regarding the effects of xylenes on any species of amphibians which makes it difficult to draw any conclusion at this point.

Conclusions

Data showed that both the WSFO from the Zhanazhol oil field (Aktobe region, the Republic of Kazakhstan) and aromatic compound o-xylene exert embryotoxic and teratogenic effects on *R. ridibunda* in subchronic (7 d) experiments. Chronic exposure to WSFO and o-xylene (60 d) induced developmental delay and oxidative stress in liver of tadpoles as evidenced by increased lipid peroxidation and inhibition of SOD and CAT activities, the antioxidant defense mechanism.

Conflict of interest

The authors declare no conflict of interest.

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References

Adeyemi, J. A. 2014. Oxidative stress and antioxidant enzymes activities in the African catfish, *Clarias Gariepinus*, experimentally challenged with *Escherichia coli* and *Vibrio fischeri*. *Fish. Physiol. Biochem.* 40:347–54. doi:10.1007/s10695-013-9847-x.

Akhmadi Invest. 2016. The share of the oil and gas industry in Kazakhstan's GDP decreased from 25% to 17%. Accessed May 23, 2018. http://www.akhmadi-invest.com/2016/06/15/dolya-neftegazovoj-otrasli-v-vvp-kazaxstana-snizilas-s-25-do-17/.

Amaeze, N. H., A. Onadeko, and C. C. Nwosu. 2014. Comparative acute toxicity and oxidative stress responses in tadpoles of *Amietophrynus regularis* exposed to refined petroleum products, unused and spent engine oils. *Afr. J. Biotechnol.* 13:4251–58. doi:10.5897/AJB2014.14093.

Anderson, J. W., J. M. Neff, B. A. Cox, H. E. Tatem, and G. M. Hightower. 1974. Characteristics of dispersions and water-soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. *Mar. Biol.* 27:75–88. doi:10.1007/BF00394763.

Askarova, M. A., and A. N. Mussagaliyeva. 2014. The ecological situation in contaminated areas of oil and gas

exploration in Atyrau region. *Procedia. Social. Behav. Sci.* 120:455–59. doi:10.1016/j.sbspro.2014.02.124.

Bacon, J. P., D. W. Linzey, R. L. Rogers, and D. J. Fort. 2006. Deformities in cane toad (*Bufo marinus*) populations in Bermuda: Part I. Frequencies and distribution of abnormalities. *Appl. Herpetol.* 3 (1):39–65. doi:10.1163/157075406775247049.

Bradford, D. F., and R. S. Seymour. 1988. Influence of environmental Po2 on embryonic oxygen consumption, rate of development, and hatching in the frog Pseudophryne bibroni. *Physiol. Zool.* 61:475–82. doi:10.1086/physzool.61.6.30156155.

Brunelli, E., I. Bernabò, C. Berg, K. Lundstedt-Enkel, A. Bonacci, and S. Tripepi. 2009. Environmentally relevant concentrations of endosulfan impair development, metamorphosis and behaviour in *Bufo bufo* tadpoles. *Aquat. Toxicol.* 91:135–42. doi:10.1016/j.aquatox.2008.09.006.

Burraco, P., and I. Gomez-Mestre. 2016. Physiological stress responses in amphibian larvae to multiple stressors reveal marked anthropogenic effects even below lethal levels. *Physiol. Biochem. Zool.* 89:462–72. doi:10.1086/688737.

Duellman, W. E., and L. Trueb. 1994. *Biology of amphibians*. London: John Hopkins University Press.

Duysebaeva, T. N., N. N. Berezovikov, Z. K. Brushko, R. A. Kubykin, and V. A. Khromov. 2005. Marsh frog (*Rana Ridibunda Pallas*, 1771) in Kazakhstan: Range changing and recent distribution. *Contemp. Herpetol.* 3:29–59. (Translated from Russian).

Eriyamremu, G. E., V. E. Osagie, S. E. Omoregie, and C. O. Omofoma. 2008. Alterations in glutathione reductase, superoxide dismutase, and lipid peroxidation of tadpoles (*Xenopus laevis*) exposed to Bonny light crude oil and its fractions. *Ecotoxicol. Environ. Saf.* 71:284–90. doi:10.1016/j.ecoenv.2007.08.009.

Falfushinska, H., N. Loumbourdis, L. Romanchuk, and O. Stolyar. 2008. Validation of oxidative stress responses in two populations of frogs from western Ukraine. *Chemosphere* 73:1096–101. doi:10.1016/j.chemosphere.2008.07.060.

Falfushynska, H., L. Gnatyshyna, O. Fedoruk, N. Mitina, A. Zaichenko, O. Stoliar, and R. Stoika. 2015. Hepatic metallothioneins in molecular responses to cobalt, zinc, and their nanoscale polymeric composites in frog *Rana ridibunda*. *Comp. Biochem. Physiol C: Toxicol. Pharmacol.* 172–173:45–56. doi:10.1016/j.cbpc.2015.04.006.

Ferrari, A., C. Lascano, A. M. P. De D'Angelo, A. Venturino, A. Hamaguchi, M. I. Homsi-Brandeburgo, M. V. Sousa, E. N. Aquino, M. S. Castro, F. de Oliveira, et al. 2011. Effects of azinphos methyl and carbaryl on *Rhinella arenarum* Larvae Esterases and Antioxidant Enzymes. *Comp. Biochem. Physiol C. Toxicol. Pharmacol.* 153:34–39. doi:10.1016/j.cbpc.2010.08.003.

Gardner, S., G. Cline, N. Mwebi, and J. Rayburn. 2018. Natural tissue concentrations in adult Ambystoma maculatum and larval DNA damage from exposure to arsenic and chromium. *J. Toxicol. Environ. Health A* 81:512–24. doi:10.1080/15287394.2018.1455611.

Gardner, S. T., A. T. Wood, R. Lester, P. E. Onkst, N. Burnham, D. H. Perygin, and J. Rayburn. 2016. Assessing differences in toxicity and teratogenicity of three phthalates, Diethyl phthalate, Di-n-propyl phthalate, and Di-n-butyl phthalate, using Xenopus laevis embryos. *J. Toxicol. Environ. Health A* 79:71–82. doi:10.1080/15287394.2015.1106994.

Georgieva, N. V. 2005. Oxidative stress as a factor of disrupted ecological oxidative balance in biological systems – A review. *Bulg. J. Vet. Med.* 8:1–11.

Gorchev, H. G., and G. Ozolins. 2011. WHO Guidelines for drinking-water quality. *WHO Chron.* 38:104–08. doi:10.1016/S1462-0758(00)00006-6.

Gosner, K. L. 1960. A Simplified Table for Staging Anuran Embryos and Larvae with Notes on Identification. *Herpetologica* 16:183–90.

Gutleb, A. C., J. Appelman, M. C. Bronkhorst, J. H. J. van Den Berg, A. Spenkelink, A. Brouwer, and A. J. Murk. 1999. Delayed effects of pre- and early-life time exposure to polychlorinated biphenyls on tadpoles of two amphibian species (*Xenopus laevis* and *Rana temporaria*). *Environ. Toxicol. Pharmacol.* 8:1–14. doi:10.1016/S1382-6689(99)00023-X.

Hansen, B. H., I. Salaberria, A. J. Olsen, K. E. Read, I. B. Overjordet, K. M. Hammer, D. Altin, and T. Nordtug. 2015. Reproduction dynamics in Copepods following exposure to chemically and mechanically dispersed crude oil. *Environ. Sci.Technol.* 49:3822–29. doi:10.1021/es504903k.

Hansen, B. H., T. Jager, D. Altin, I. B. Øverjordet, A. J. Olsen, I. Salaberria, and T. Nordtug. 2016. Acute toxicity of dispersed crude oil on the cold-water copepod Calanus finmarchicus: Elusive implications of lipid content. *J. Toxicol. Environ. Health A* 79:549–57. doi:10.1080/15287394.2016.1171981.

Hatch, A. C., and G. A. Burton Jr. 1998. Effects of photoinduced toxicity of fluoranthene on amphibian embryos and larvae. *Environ. Toxicol. Chem.* 9:1777–85. doi:10.1002/etc.5620170918.

Hersikorn, B. D., J. J. C. Ciborowski, and J. E. G. Smits. 2010. The effects of oil sands wetlands on wood frogs (*Rana sylvatica*). *Toxicol. Environ. Chem.* 92:1513–27. doi:10.1080/02772240903471245.

Holowenko, F. M., M. D. MacKinnon, and P. M. Fedorak. 2002. Characterization of naphthenic acids in oil sands wastewaters by gas chromatography-mass spectrometry. *Water Res.* 36:2843–55. doi:10.1016/S0043-1354(01)00492-4.

Johari, S. A., I. Sourinejad, S. Asghari, and N. Bärsch. 2015. Toxicity comparison of silver nanoparticles synthesized by physical and chemical methods to tadpole (*Rana ridibunda*). *Caspian. J. Environ. Sci.* 13:383–90.

Kalra, J., S. V. Mantha, and K. Prasad. 1994. Oxygen free radicals: Key factors in clinical diseases. *Lab. Med. Int.* 11:9–13.

Kolesnikov, S. I., N. A. Spivakova, L. S. Vezdeneeva, Y. S. Kuznetsova, and K. S. Kazeev. 2011. Modeling the Effect of chemical pollution on biological properties of hydromorphic solonchaks in the dry steppe zone of

Southern Russia. *Arid. Ecosys.* 1:83–86. doi:10.1134/S207909611102003X.

Loumbourdis, N. S., P. Kyriakopoulou-Sklavounou, and G. Zachariadis. 1999. Effects of cadmium exposure on bioaccumulation and larval growth in the frog *Rana ridibunda*. *Environ. Pollut* 104:429–33. doi:10.1016/S0269-7491(98)00172-9.

Mahaney, P. A. 1994. Effects of freshwater petroleum contamination on amphibian hatching and metamorphosis. *Environ. Toxicol. Chem.* 13:259–65. doi:10.1002/etc.5620130210.

Mahmoud, K. E., T. M. Shalakhmetova, and B. A. Umbayev. 2012. Short term effects of crude oil, vanadium and nickel intoxication on rats liver antioxidant defence system. KazNU Bulletin. *Biol. Ser.* 2:32–37.

Marentette, J. R., R. A. Frank, A. J. Bartlett, P. L. Gillis, L. M. Hewitt, K. M. Peru, J. V. Headley, P. Brunswick, D. Shang, and J. L. Parrott. 2015. Toxicity of naphthenic acid fraction components extracted from fresh and aged oil sands process-affected waters, and commercial naphthenic acid mixtures, to fathead minnow (*Pimephales promelas*) embryos. *Aquat. Toxicol.* 164:108–17. doi:10.1016/j.aquatox.2015.04.024.

Marquis, O., A. Millery, S. Guittonneau, and C. Miaud. 2006a. Toxicity of PAHs and jelly protection of eggs in the common frog. *Rana temporaria*. *Amphibia-Reptilia* 27:472–75. doi:10.1163/156853806778189945.

Marquis, O., A. Millery, S. Guittonneau, and C. Miaud. 2006b. Solvent toxicity to amphibian embryos and larvae. *Chemosphere* 63:889–92. doi:10.1016/j.chemosphere.2005.07.063.

McIntosh, S., T. King, D. Wu, and P. V. Hodson. 2010. Toxicity of dispersed weathered crude oil to early life stages of Atlantic herring (*Clupea harengus*). *Environ. Toxicol. Chem.* 29:1160–67. doi:10.1002/etc.134.

Melvin, S. D., C. M. Lanctôt, P. M. Craig, T. W. Moon, K. M. Peru, J. V. Headley, and V. L. Trudeau. 2013. Effects of naphthenic acid exposure on development and liver metabolic processes in anuran tadpoles. *Environ. Pollut.* 177:22–27. doi:10.1016/j.envpol.2013.02.003.

Melvin, S. D., and V. L. Trudeau. 2012a. Toxicity of naphthenic acids to wood frog tadpoles (*Lithobates sylvaticus*). *J. Toxicol. Environ. Health A* 75:170–73. doi:10.1080/15287394.2012.640092.

Melvin, S. D., and V. L. Trudeau. 2012b. Growth, development and incidence of deformities in amphibian larvae exposed as embryos to naphthenic acid concentrations detected in the canadian oil sands region. *Environ. Pollut.* 167:178–83. doi:10.1016/j.envpol.2012.04.002.

Mills, N. E., and M. C. Barnhart. 1999. Effects of Hypoxia on Embryonic Development in Two Ambystoma and Two Rana Species. *Physiol. Biochem. Zool: Ecol. Evol. Approaches* 72:179–88. doi:10.1086/316657.

Ministry of Fisheries of the USSR. 1990. *Generalized list of maximum permissible concentrations (MPCs) and approximately safe levels of exposure (ASLE) of hazardous substances for water in fishery reservoirs*, 49. Moscow: USSR.

Neuparth, T., R. Capela, S. P. P. Pereira, S. M. Moreira, M. M. Santos, and M. A. Reis-Henriques. 2014. Toxicity Effects of Hazardous and Noxious Substances (HNS) to Marine Organisms: Acute and Chronic Toxicity of p-Xylene to the Amphipod Gammarus locusta. *J. Toxicol. Environ. Health A* 77:1210–21. doi:10.1080/15287394.2014.921867.

Oruç, E. Ö., and D. Usta. 2007. Evaluation of oxidative stress responses and neurotoxicity potential of diazinon in different tissues of *Cyprinus carpio*. *Environ. Toxicol. Pharmacol.* 23:48–55. doi:10.1016/j.etap.2006.06.005.

Pampanin, D. M., J. Le Goff, K. Skogland, C. K. Marcucci, K. B. Øysæd, M. Lorentzen, K. B. Jørgensen, and M. O. Sydnes. 2016. Biological effects of PAHs and their first metabolic products in *in vivo* exposed Atlantic cod (*Gadus morhua*). *J. Toxicol. Environ. Health A* 79:633–46. doi:10.1080/15287394.2016.1171993.

Park, C. J., S. H. Song, D. H. Kim, and M. C. Gye. 2016. Developmental and acute toxicity of cetylpyridinium chloride in *Bombina orientalis* (Amphibia: Anura). *Aquat. Toxicol.* 177:446–53. doi:10.1016/j.aquatox.2016.06.022.

Peltzer, P. M., R. C. Lajmanovich, A. M. Attademo, C. M. Junges, M. C. Cabagna-Zenklusen, M. R. Repetti, M. E. Sigrist, and H. Beldoménico. 2013. Effect of exposure to contaminated pond sediments on survival, development, and enzyme and blood biomarkers in veined treefrog *(Trachycephalus typhonius)* tadpoles. *Ecotoxicol. Environ. Saf.* 98:142–51. doi:10.1016/j.ecoenv.2013.09.010.

Richards, S. M., and R. J. Kendall. 2003. Physical effects of chlorpyrifos on two stages of *Xenopus laevis*. *J. Toxicol. Environ. Health A* 66:75–91. doi:10.1080/15287390390155769.

Ruchin, A. B., and E. A. Lobachev. 2005. Influence of monochromatic light on growth and development of the lake frog, *Rana ridibunda*. *Contemp. Science-Intensive. Technol.* 8:13–16. (Translated from Russian).

Saka, M. 2004. Developmental toxicity of p,p′-dichlorodiphenyltrichloroethane, 2,4,6-trinitrotoluene, their metabolites, and benzo[a]pyrene in xenopus laevis embryos. *Environmental Toxicology and Chemistry* 23:1065–1073. doi: 10.1897/03-272.

Salin, K., E. Luquet, B. Rey, D. Roussel, and Y. Voituron. 2012. Alteration of mitochondrial efficiency affects oxidative balance, development and growth in frog (*Rana temporaria*) tadpoles. *J. Exp. Biol.* 215:863–69. doi:10.1242/jeb.062745.

Salvaterra, T., M. G. Alves, I. Domingues, R. Pereira, M. G. Rasteiro, R. A. Carvalho, A. M. V. M. Soares, and I. Lopes. 2013. Biochemical and metabolic effects of a short-term exposure to nanoparticles of titanium silicate in tadpoles of *Pelophylax perezi* (Seoane). *Aquat. Toxicol.* 128–129:190–92. doi:10.1016/j.aquatox.2012.12.014.

Seymour, R. S. 1995. Oxygen uptake by embryos in gelatinous egg masses of *Rana sylvatica*: The roles of diffusion and convection. *Copeia* 1995:626–35. doi:10.2307/1446759.

Seymour, R. S., J. D. Roberts, N. J. Mitchell, and A. J. Blaylock. 2000. Influence of Environmental Oxygen on Development and Hatching of Aquatic Eggs of the

Australian Frog, *Crinia georgiana*. *Physiol. Biochem. Zool: Ecol. Evol. Approaches* 73:501–07. doi:10.1086/317739.

Shalakhmetova, T. M., K. E. Mahmoud, and B. A. Umbayev. 2012. Biochemical changes in male albino rats following single exposure to crude oil and ciprofloxacin. *KazNU Bulletin. Ecol. Ser.* 34:87–93.

Shalakhmetova, T. M., M. A. Suvorova, L. R. Sutuyeva, A. S. Ondassynova, M. S. Zhankulova, and K. A. Mukhatayeva. 2015. The inhalation effects of vapors of crude oil from the Kumkol fields in rats. *KazNU Bulletin. Ecol. Ser.* 45:283–91. (Translated from Russian).

Shametov, A. K., A. N. Kozhakhmetova, A. B. Bigaliev, R. K. Bigalieva, and B. O. Bekmanov. 2015. Molecular genetic studies of rodents from the area of the tailings Koshkar-Ata. *Contemp. Prob. Sci. Educ.* 2:559–66. (Translated from Russian).

Shiyan, A. A. 2011. Influence of metal oxide in nanoform on success of larval stages of development of lake frog (*Rana ridibunda Pall.*). *Sci. J. KubSAU*. 66:289–299. (Translated from Russian).

Singer, M. M., D. Aurand, G. E. Bragin, J. R. Clark, G. M. Coelho, M. L. Sowby, and R. S. Tjeerdema. 2000. Standardization of the preparation and quantitation of water-accommodated fractions of petroleum for toxicity testing. *Mar. Pollut. Bull.* 40:1007–16. doi:10.1016/S0025-326X(00)00045-X.

Sparling, D. W. 2010. Ecotoxicology of organic contaminants to amphibians. In *Ecotoxicology of amphibians and reptiles*, ed. D. W. Sparling, G. Linder, C. A. Bishop, and S. K. Krest, 261–88. 2nd ed. Pensacola, FL: SETAC Press.

Stefani Margarido, T. C., A. A. Felício, D. de Cerqueira Rossa-Feres, and E. A. de Almeida. 2013. Biochemical biomarkers in scinax fuscovarius tadpoles exposed to a commercial formulation of the pesticide fipronil. *Mar. Environ. Res.* 91:61–67. doi:10.1016/j.marenvres.2013.02.001.

Suvorova, M. A., T. M. Shalakhmetova, L. R. Sutuyeva, A. S. Ondassynova, M. S. Zhankulova, and R. Imankulova. 2015. The inhalation effects of vapors of crude oil from the Kumkol fields in rats. *KazNU Bulletin. Ecol. Ser.* 45:276–82. (Translated from Russian).

Trudeau, V. L., G. M. Somoza, G. S. Natale, B. Pauli, J. Wignall, P. Jackman, K. Doe, and F. W. Schueler. 2010. Hormonal induction of spawning in 4 species of frogs by coinjection with a gonadotropin-releasing hormone agonist and a dopamine antagonist. *Reprod. Biol. Endocrinol.* 8:2–10. doi:10.1186/1477-7827-8-36.

Unified Environmental Internet Resource. 2018. Information bulletins on the state of the environment of The Republic of Kazakhstan. Accessed May 30, 2018. http://ecogosfond.kz/orhusskaja-konvencija/dostup-k-jekologicheskoj-informacii/jekologijaly-zha-daj/orsha-an-otrany-zhaj-k-jituraly-a-paratty-bjulletender

Venturino, A., and A. M. Pechen de D'Angelo. 2005. Biochemical targets of xenobiotics: Biomarkers in amphibian ecotoxicology. *Appl. Herpetol.* 2:335–53. doi:10.1163/1570754054507433.

Wessel, N., S. Rousseau, X. Caisey, F. Quiniou, and F. Akcha. 2007. Investigating the relationship between embryotoxic and genotoxic effects of benzo[a]pyrene, 17α-ethinylestradiol and endosulfan on *Crassostrea gigas* embryos. *Aquat. Toxicol.* 85:133–42. doi:10.1016/j.aquatox.2007.08.007.

Wu, B., T. Lan, D. Lu, and Z. Liu. 2014. Ecological and enzymatic responses to petroleum contamination. *Environ. Sci: Processes. Impacts* 16:1501–09. doi:10.1039/c3em00731f.

Wu, C., Y. Zhang, L. Chai, and H. Wang. 2017. Oxidative stress, endocrine disruption, and malformation of *Bufo gargarizans* embryo exposed to sub-lethal cadmium concentrations. *Environ. Toxicol. Pharmacol.* 49:97–104. doi:10.1016/j.etap.2016.12.005.