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# Toxicity Of The Oil And Dimethyl Tin Dichloride, The Ways Of Decrease.

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**ABSTRACT**

We studied the combined effect of oil and dimethyl tin dichloride (DMTC) on the level of lipid peroxidation (LPO) of the liver of the Russian sturgeon (*Acipenser gueldenstaedtii Brandt*) in the conditions of long-running process in vitro and the influence on the fish-breeding, biochemical parameters of the Russian sturgeon juveniles in vivo. In case of combined effect of oil and DMTC pro-oxidant effect is expressed to a greater extent than in case of the separate effect of these toxicants. Increase of the toxic action of oil and DMTC on the young of Russian sturgeon in vivo with the combined influence of xenobiotics was found. Growth kinetic parameters of liver homogenate, erythrocytes membrane LPO, reduction of the rate of hydrogen peroxide decomposition by the fish erythrocytes hemolysate were discovered. The results can be indicative of the general weakening of the system antioxidative protection of young Russian sturgeon under joint influence of toxicants. It was established a decrease in the toxic effect of binary mixture of crude oil and DMTC by adding the (4-hydroxy-3,5-di-*tert*-butylphenyl) methylenediphosphonic acid (DMPA).
**Keywords**: oil, organotin, toxicity, sterically hindered phenol, lipid peroxidation, Russian sturgeon.

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## INTRODUCTION

Currently, the Caspian Sea is one of the most important areas in the world for the development of oil and gas deposits and production of hydrocarbons. The pollution of the sea area with oil and oil products is the major risk factor for the biodiversity of the Caspian Sea and its coastal zone [1].The same significant environmental problem for the Northern Caspian Sea is the pollution of the aquatic environment with heavy metals, among which the most dangerous are such bio-accumulative persistent organic pollutants as organic derivatives of heavy metals, including organotin compounds [2,3].

Now it is established that a diverse toxic effect of heavy metal compounds on living organisms, including hydrobiontes, is based on the development of oxidative stress, which is also one of the key elements of the main component of oil (polycyclic aromatic hydrocarbons, alkylphenols and hydrocarbons) toxicity [4]. Pro-/antioxidant disbalance causes an excessive accumulation of reactive oxygen species (ROS), and an oxidative damage of biomolecules is observed as a result [5,6]. To neutralize ROS produced by the pollutants, to neutralize the oxidative damage caused by the oxidative stress, toxic protectors with antioxidant activity are required. There are numerous data in literature on separate effects of oil [7] and heavy metals [8,9] on vital functions and biochemical parameters of hydrobiontes, including sturgeon fish. While there are few data on the combined effect of oil and other ecotoxicantes, including heavy metals, on fish [10].

The aim of this work was to study the possibility to decrease a toxic effect of binary mixture of crude oil and dimethyl tin dichloride (DMTC) by adding a (4-hydroxy-3,5-di-*tert*-butylphenyl) methylenediphosphonic acid (DMPA) in comparison with the known antioxidant agent 2,6-di-*tert*-butyl-4-methylphenol (BHT).

## MATERIALS AND METHODS

### General

DMPA was synthesized as previously described by the known procedure [11]. BHT (99%) and all other reagents were purchased from Sigma–Aldrich. We studied the effect of oil products from the Tengiz fields, containing about 30% paraffin, 2.5% aromatic hydrocarbons (benzene, toluene) and 0.06% sulfur. Spectrophotometric study was performed by using SF-103 spectrophotometer.

### Experiment in vitro

The level of liver LPO of the Russian sturgeon in vitro in the conditions of long-running LPO has been assessed by the accumulation of carbonyl oxidation by-products, which react with thiobarbituric acid (TBARS), using the traditional method as described previously [12]. Oil and DMTC were dissolved in DMSO and added to the liver homogenate of sturgeons to attain an initial concentration of 0.1 mM in the incubation medium. By special experiment we showed that DMSO did not affect the rate of accumulation of TBARS in the liver homogenate.

### Experiment in vivo

Juvenile Russian sturgeon (75 ± 5 g body wt) were used throughout experiments. The fishery–biological characteristics of were determined as described previously [12]. Russian sturgeon juveniles were fed with an Aller Sturgeon dry granular mixed fodder (grain size 2 mm) without additives and with addition of compounds to a concentration of 150 mg/kg for DMTC and 0.05 mg $L^{-1}$ for oil. Oil and DMTC were dissolved in DMSO, by special experiment we showed that solvent did not affect the fish-breeding and biochemical parameters. The juveniles were grown for 35 days. Russian sturgeon juveniles were placed in 250-L fish-bowls (10–12 individuals per aquarium) equipped with filtration and aeration systems. The amount of feed was calculated using specially developed feed tables on the basis of the body weight and water temperature. The juveniles were fed twice a day manually, and the groats size was consistent with the fish weight. All manipulations were carried out in accordance with Good Laboratory Practice (GLP) standards. The hydrochemical parameters of water remained within the normal ranges during experiments. The results were estimated by the growth rate and survivability of the hydrobionts.

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The level of LPO in the liver homogenate and the erythrocyte membranes of juvenile Russian sturgeon in vivo was evaluated by TBARS according to the standard procedure as described previously (12). The concentrations of TBARS in the enzymatic ($TBARS_e$) and non-enzymatic $Fe^{2+}$-ascorbate induced LPO ($TBARS_n$), as well as the initial concentration of TBARS, were determined as the kinetic parameters of LPO.

**Measurement of the rate of hydrogen peroxide decomposition by the fish erythrocytes hemolysate**

The hemolysate of blood erythrocytes of fish was used for measurement of the rate of $H_2O_2$ decomposition. The fish erythrocytes hemolysate was prepared according to the procedure [13]. The assay for the determination of $H_2O_2$ decomposition rate was determined spectrophotometrically by monitoring disappearance of $H_2O_2$ at 240 nm [14].

**Statistical analysis**

All experiments were repeated three times. The statistical analysis was performed using Statistica for Windows, Version 9.0 (StatSoft, Inc.), the data were presented as mean ± SD. The fish-breeding and biochemical parameters (rate of decomposition of $H_2O_2$ by erythrocytes hemolysate, TBARS concentration) in experiments in vitro, in vivo were analysed using an unpaired Student's t-test. Statistical significance was set up at $p < 0.05$ and $p < 0.0001$.

## RESULTS AND DISCUSSION

**The influence of the tested compounds on the level of oxidative destruction of liver lipids of the Russian sturgeon in vitro**

We studied the combined effect of oil products from the Tengiz fields, DMTC and antioxidants (DMPA, BHT) on the level of oxidative destruction of liver lipids of the Russian sturgeon in vitro under conditions of a long running process (72 hours). As shown in Table 1, it was found that in the control experiment without additives, the TBARS content increased naturally after a while.

**Table 1: Effect of the compounds under study on the level of accumulation of TBARS in liver Russian sturgeon during long-term in vitro oxidation**

| Compounds | Level of accumulation of TBARS in liver Russian sturgeon, nmol $g^{-1}$ | | | |
|---|---|---|---|---|
| | 1 h | 24 h | 48 h | 72 h |
| control | $3.08 \pm 0.11$ | $3.44 \pm 0.17$ | $6.28 \pm 0.23$ | $7.58 \pm 0.01$ |
| oil | $3.57 \pm 0.13$ | $3.92 \pm 0.18$ | $7.06 \pm 0.23$ | $7.93 \pm 0.13$ |
| DMTC | $3.08 \pm 0.12^{*}$ | $4.51 \pm 0.25$ | $9.41 \pm 0.01$ | $9.94 \pm 0.01$ |
| oil+ DMTC | $4.59 \pm 0.14$ | $5.06 \pm 0.23$ | $11.68 \pm 0.70$ | $12.86 \pm 0.06$ |
| BHT | $1.87 \pm 0.06$ | $1.03 \pm 0.12$ | $0.82 \pm 0.06$ | $0.78 \pm 0.01$ |
| oil+ DMTC + BHT | $2.09 \pm 0.01$ | $1.32 \pm 0.04$ | $1.53 \pm 0.22$ | $1.83 \pm 0.01$ |
| DMPA | $0.60 \pm 0.07$ | $0.52 \pm 0.01$ | $0.51 \pm 0.01$ | $0.51 \pm 0.01$ |
| oil+ DMTC+ DMPA | $1.04 \pm 0.03$ | $0.64 \pm 0.03$ | $0.60 \pm 0.04$ | $0.55 \pm 0.01$ |

The average values for a series of experiments are given: differences from the control experimental group ($p < 0.0001$); *differences from the control experimental group ($p > 0.05$). The values are expressed as mean ± SD.

In case of separate effect of toxicants at the initial stage of LPO (1 hour), a greater degree of promotion was observed in presence of oil. At medium and remote stages of the LPO DMTC additive had the greatest promotion effect. In case of combined effect of oil and DMTC at all studied stages of LPO, pro-oxidant effect was expressed to a greater extent than in case of the separate effect of these toxicants.

The presented data on the influence of heavy metals and oil on the accumulation of LPO process products, which is a biomarker of an oxidative stress, indicate that-inhibitors of the oxidative process can be used to reduce the toxicity of these compounds.

As has been shown before DMPA possessed inhibiting activity in reactions of peroxidation of Russian sturgeon liver lipids [15]. It was shown that MDPA possessed the highest efficiency of antioxidating activity, such activity of this compound exceeded the effect of commonly known antioxidants (BHT, trolox). Previously has been also established the ability of DMPA to increase the cryoresistance of Beluga sperm under conditions of the low temperature preservation oxidation of Russian sturgeon liver lipids [16].

The addition of DMPA and BHT in vitro together with oil and DMTC promotes the decrease of TBARS level lower than in the control variant, which confirms the mechanism of toxicity of these compounds connected with the development of the oxidative stress and with the increase of level LPO. The high antioxidant activity of DMPA in comparison with the activity of BHT is shown both in autooxidation and in the promotion of LPO by the combined addition of oil and DMTC. It should be noted that DMPA, unlike BHT, has a prolonged effect – its efficiency does not decrease at the remote stages of the oxidation process.

**The influence of the tested compounds on fish-breeding and biochemical parameters of the Russian sturgeon juveniles in vivo**

The influence of crude oil and DMTC additives on fish-breeding and biochemical parameters of the Russian sturgeon juveniles (the level of LPO of the liver and the erythrocyte membranes of sturgeon fish in an enzymatic and non-enzymatic process, $H_2O_2$ decomposition rate by the fish erythrocytes hemolysate) in vivo is studied.

Artificial feed used in industrial breeding of sturgeon fish may contain organotin compounds, including DMTC. DMTC is used as a stabilizer of polyvinyl chloride and can leach out of plastic pipes, therefore the possibility of entry of this toxicant into pool water during industrial breeding of hydrobiontes should be considered [17].

As shown in Figure 1, it was found that the addition of crude oil and DMTC to fish feed both separately and combined led to inhibition of the growth of juvenile fish, moreover when the addition of toxicants was combined, a synergic effect was observed, which led to the decrease in the survival rate of juveniles up to 80%.

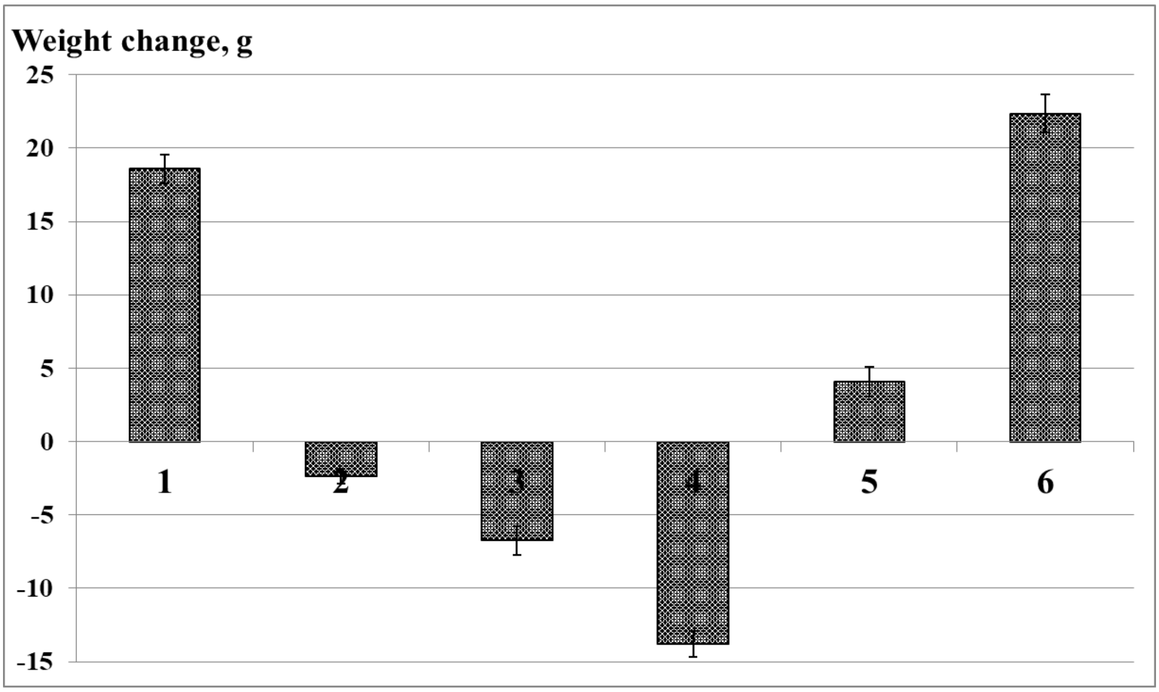


**Fig 1: Change in the body weight of young sturgeon (g) upon the addition of toxicants in the feed (the growing period is 35 days): 1 – control; 2 – oil; 3 – DMTC; 4 – oil + DMTC; 5 – oil + DMTC + DMPA; 6 – DMPA. The average values for a series of experiments are given: differences from the control experimental group ($p < 0.05$). The values are expressed as mean ± SD.**

The addition of DMPA in concentration of 100 mg $kg^{-1}$ to fish feed increases the growth of the juvenile of hydrobiontes. It was detected that the combined addition of toxicants and DMPA decreased the toxic effect of xenobiotics, since there was an increase in the growth of juveniles, unlike the group of fish receiving feed with additives of toxicants.

The level of LPO of the liver of sturgeon fish in an enzymatic and non-enzymatic process, was determined. As shown in Figure 2, an increase in the level of enzymatic ($TBARS_e$) and non-enzymatic LPO ($TBARS_n$) and in the initial level of TBARS in fish liver homogenates which consumed fish feed with additives of crude oil and DMTC was found.

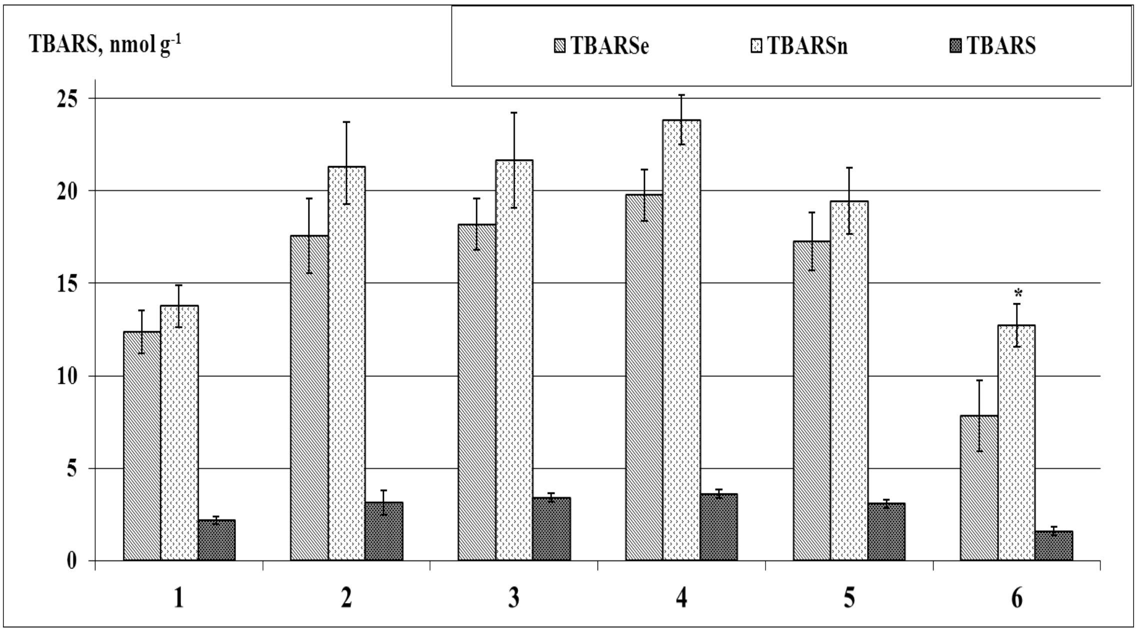


**Fig 2: Effect of the compounds under study on the level of accumulation of TBARS in liver Russian sturgeon in vivo: 1 – control; 2 – oil; 3 – DMTC; 4 – oil + DMTC; 5 –oil + DMTC + DMPA; 6 – DMPA. The average values for a series of experiments are given: differences from the control experimental group ($p < 0.005$); *differences from the control experimental group ($p > 0.05$). The values are expressed as mean ± SD.**

A separate addition of toxicants into fish feed leads to an increase in the level of TBARS in liver homogenate approximately by 45-55%, combined addition – by 60-75% in comparison with the control variant. Adding DMPA to the fish feed leads to a decrease in the level of enzymatic, non-enzymatic LPO and the initial level of TBARS by 37%, 8% and 27% respectively. Combined addition of DMPA and toxicants results in a decrease in the promotion activity of the latter. The results obtained in the work correspond the literature data on the ability of antioxidants to reduce the toxicity of heavy metals by inhibiting the oxidative process [18].

According to the literature data-fish blood is sensitive to pollution-induced stress; and changes to the haematological parameters can be used to monitor stress caused by pollutants including oil and heavy metals in fishes erythrocytes [19]. Tin compounds are able to accumulate in erythrocytes and cause their hemolysis, induce morphological changes in erythrocytes [20].

It is known that the lipid membranes of fish erythrocytes are characterized by a high degree of unsaturated fatty acids, which are highly exposed to peroxide oxidation. Considering this fact, it is rational to clarify the molecular mechanisms of erythrocyte protection from the oxidative stress signs. As shown in Table 2, it was found that upon separate addition of crude oil and DMTC into fish feed of sturgeon juveniles, non-enzymatic LPO of blood erythrocyte membranes increased by 27 and 54% respectively, the combined addition led to the promotion of LPO by 84%.

**Table 2: The dependence of the rate of $H_2O_2$ utilization by erythrocytes hemolysate of the Russian sturgeon and on the level of accumulation of TBARS of erythrocyte membranes on the additives of the test compounds in vivo**

| Compounds | Level of TBARS accumulation of erythrocyte membranes, nmol $g^{-1}$ | | Rate of $H_2O_2$ utilization by erythrocytes hemolysate, mmol $min^{-1}$ |
|---|---|---|---|
| | $TBARS_e$ | $TBARS_n$ | |
| control | 19.35 ± 0.56 | 13.78 ± 1.04 | 0.025 ± 0.002 |
| oil | 21.69 ± 1.90* | 21.33 ± 0.97 | 0.016 ± 0.001 |
| DMTC | 26.77 ± 3.50 | 21.65 ± 0.95 | 0.014 ± 0.001 |
| oil + DMTC | 31.61 ± 3.61 | 23.85 ± 1.75 | 0.012 ± 0.001 |
| oil + DMTC + DMPA | 27.46 ± 2.06 | 19.46 ± 0.97 | 0.013 ± 0.001 |
| DMPA | 18.46 ± 1.41** | 12.71 ± 1.50** | 0.018 ± 0.001 |

The average values for a series of experiments are given: differences from the control experimental group ($p < 0.005$); *differences from the control experimental group ($p < 0.05$); **differences from the control experimental group ($p > 0.05$). The values are expressed as mean ± SD.

Thus, in the experiments in vivo, as well as in the experiments in vitro, the synergistic effect was observed under the combined effect of toxicants. Most likely, this effect is possible in the case of mixtures containing reactive toxins with different mechanisms of toxicity [21].

$TBARS_e$ level increases upon separate addition of crude oil by 12%, DMTC – 38% and upon combined addition of toxicants – 63%. The feed additive DMPA decreases the level of $TBARS_e$ by 5% and $TBARS_n$ by 16%. Fish feeding with DMPA additives and toxicants resulted in a slight decrease in the level of LPO in comparison with the combined addition of toxicants, but in comparison with the control variant there is an increase in parameters.

It is known that $H_2O_2$ is the most stable form of ROS, which is constantly formed in various cells, including erythrocytes [22]. It has several useful functions for a cell, but at high concentrations has a toxic effect. In our experiment, the rate of $H_2O_2$ decomposition by hemolysate of sturgeon juvenile erythrocytes showed 1.7 times decrease when crude oil and DMTC were added separately, and 2 times decrease in the case of combined additives, as shown in Table 2. Reduction of the rate of $H_2O_2$ decomposition by hemolysate of erythrocytes in the presence of ecotoxicantes may be associated with a decrease in the activity of antioxidant enzymes under the influence of toxicants, with the interaction DMTC with hemoglobin.

The biochemical parameters studied in the work are often used as biomarkers of water pollution with various substances [23]. It is known that under the effect of various pollutants the activity of antioxidant enzymes can be increased and decreased and LPO products in fish tissues can be increase [24].

DMPA additive also contributed to the decrease in the rate of $H_2O_2$ utilization by the hemolysate of fish blood erythrocytes, so at combined addition with DMPA toxicants, de-toxic effect was not recorded. The decrease in the rate of $H_2O_2$ decomposition of fish blood erythrocytes hemolysate of may indicate a general weakening of the antioxidant protection system of the Russian sturgeon juveniles, exposed to the toxic effect of lipophilic chemical pollutants, which can easily penetrate through the biological membranes into the cells of living organisms.

## CONCLUSIONS

Thus, the increased toxic effect with the combined addition of crude oil and dimethyl tin chloride on the growth, development and biochemical parameters of the juveniles of the Russian sturgeon in vivo was determined in the work. The increase in the LPO level and the decrease in the ability of hemolysate of fish blood erythrocytes to utilize $H_2O_2$ with the combined addition of the given toxicants indicate damage to the antioxidant system of the organism of fish, which adversely affects the juveniles of sturgeon fish.

The conducted experiments show that sturgeon fish possessing relatively high protective mechanisms of detoxification cannot always resist the increased anthropogenic load, even alongside with the strengthening of antioxidant systems. For the purpose to increase the antioxidant defense system of hydrobiontes efficient anti-oxidants should be used as additives to fish feed in artificial fish breeding. Increasing the resistance of sturgeon fish to stressful environmental situations will allow preserving valuable species of fish.

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