EEET ECOLOGICAL ENGINEERING & ENVIRONMENTAL TECHNOLOGY

Ecological Engineering & Environmental Technology 2022, 23(6), 111–117
https://doi.org/10.12912/27197050/152920
ISSN 2719-7050, License CC-BY 4.0

Received: 2022.08.09
Accepted: 2022.08.23
Published: 2022.09.07

Ecotoxicological Assessment of Waste from Oil Production

Pavlo Pysarenko[1], Maryna Samojlik[1], Anna Taranenko[1*], Inna Lavrinenko[1], Ivan Mostoviak[2]

[1] Poltava State Agrarian University, 1/3 Skovorody St., Poltava, 36003, Ukraine
[2] Uman National University of Horticulture, 1 Instytutska St., Uman, 20305, Ukraine
* Corresponding author's e-mail: anna.taranenko@pdaa.edu.ua

ABSTRACT

Utilization of mineralized fossil water (MFW), as a by-product of oil production is a special environmental problem in oil and gas areas. In Ukraine the annual production of mineralized fossil water together with oil is estimated at about 20 million tons. Taking into account that MFW is an aby-product of oil production, they are usually placed in waste disposal sites and can contaminate environment. It was determined that toxicological properties of mineralized fossil water have not been studied so far. There is the need for study of toxicological features of the MFW which will allow to determine its hazard class and to develop some measures for safe usage of this drug for making the agriculture more ecological. Thus, in the work we have made the assessment of ecotoxicological properties of MFW as an environmentally safe substitute for agrochemicals. It was established that MFW is referred to low-toxic compounds. LD_{50} for oral introduction of the compound in the bodies of white female rats is more than 21,000 mg/kg, female mice – 31,000 mg/kg, male mice – more than 20,000 mg/kg; when applied to the skin of rats – more than 8000 mg/kg. The value of LD_{50} when applied to the inhalation routes of entry for female rats is more than 5000 mg/kg. It was justified that by oral route of entry and skin penetration the MFWs belong to the IV class of danger, and by inhalation route of entry – to III class. It was also determined that MFW does not have an irritating effect on the skin and mucous membranes of the eyes. There is also no sensitizing effect on the body of guinea pigs. Studies have shown that the cumulative properties of MFW are weakly represented. The accumulation coefficient is greater than 5. At a dose of 4260 mg/kg MFW has cardiotoxic effect, it affects the process of erythropoiesis and increases filtration and excretory function of the kidneys. Ineffective dose for rats in a subchronic experiment is 2130 mg/kg. These results of ecotoxicological study of MFW make it possible to confirm that its usage is safe in the agricultural system, especially as environmentally friendly organo-mineral fertilizer, herbicide, and fungicide.

Keywords: waste from oil production, ecotoxicological assessment, mineralized fossil water, cumulative properties, toxicity, sensitizing effect.

INTRODUCTION

During the extraction of oil and gas the large quantities of mineralized fossil water (MFW), which is a by-product, come to the surface. The problem of utilization of large amounts of these waters is very considerable, taking into account the fact that getting a lot of fossil water on the ground, leads to significant salinization of the soil, deterioration of its structure and destruction of the vegetation.

Study [Rudko and Skatinskiy, 2002] have shown very high mineralization of fossil waters (up to 200 g/L), which causes oil pollution, intensive salinization (from 0.08 to 0.63% of total mineralization), increasing sodium content (from 2.3 to 52.4% from the amount of exchange bases in the soil layer 0–20 cm), a decrease of phosphorus mobility by 1.7–2.8 times.

Thus, a special environmental problem of oil and gas area is the utilization of mineralized fossil water, as a by-product of oil production, as well as restoring the fertility of soil in the areas that have undergone uncontrolled release of MFW.

As studies [Pisarenko et. al. 2018; Pisarenko et. al. 2020] have shown, the mineralized fossil waters, extracted in Poltava region are recommended to be used for greening the agriculture, especially for the replacement of recommended agrochemicals with ecologically safe natural compounds which would reduce the pesticide impact on the soil, to control more effectively the phytosanitary condition of crops and to provide environmentally friendly products. The expediency of development of the MFW usage in agricultural production is determined not only by obvious economic efficiency, but also by the environmental necessity [Yaron 1980; Abd-Elmabod et. al. 2017]. It is important to note that the studied problem is relevant for both oil and gas industry and for agriculture.

At the same time, till today the toxicological properties of mineralized fossil water have not been studied. There is a need to conduct the study of toxicological properties of MFW. Thus, the purpose of the article was to make an assessment of ecotoxicological properties of MFW as an environmentally friendly substitute to agrochemicals.

MATERIALS AND METHODS

The object of study is mineralized fossil water, a by-product of oil production. MFW was characterized by: pH 8.7–8.9; Na+K 45.8–50.2 $g \cdot dm^{-3}$; Ca^{2+} 10.9–11.1 $g \cdot dm^{-3}$; Mg^{2+} 0.9–1.0 $g \cdot dm^{-3}$; Cl^- 95.6–105.2 $g \cdot dm^{-3}$; SO_4^{2-} 6.8–7.0 $g \cdot dm^{-3}$; HCO_3^- 0.82–1.15 $g \cdot dm^{-3}$; the oil-hydrocarbon content – 3–5%.

According to the methodical recommendations for the assessment of new pesticides [Methodical instructions for the hygienic assessment of new pesticides,1998] before the start of state testing of new chemical plant protection products an initial ecotoxicological assessment is necessary, which will determine the hazard class and will develop some measures for the safe usage of this drug.

In toxicological studies we have used white rats, white mice, guinea pigs, rabbits that have undergone a 14-day quarantine in the vivarium. The acute toxicity of MFW was studied in white Wistar rats and nonlinear white mice. To the experimental animals we have applied MFW using a metal probe. For 14 days a clinical examination was performed, and the dynamics of animal body weight were determined. The main criterion of toxicity was the dose that caused the death of 50% animals (LD_{50}). The average lethal dose of MFW was determined by probit analysis of mortality curves [Prozorovskiy 1961]. In the case of impossibility to calculate LD_{50} we have established the amount of the drug that did not cause the death of animals.

Acute inhalation toxicity research was performed in a chamber for dynamic seed. The needed concentration was achieved by dripping the drug through the nozzle and simultaneous air supply at a pressure of 2 atm. This provided a creation of a finely dispersed aerosol of MFW in a chamber. The selection of samples was made through a special cartridge with an air flow rate of 10 liters per minute for 10 minutes. The concentration of MFW in the chamber was determined by weight method.

The resorptive-toxic effect of MFW was studied in white Wistar rats [Kundiev 1964]. Skin irritation was studied on white rats and rabbits by drip application of the drug to the skin of animals. Study of irritant action on the mucous membranes of the eyes of rabbits were performed by dripping the MFW in native condition into a conjunctival sac.

The study of sensitizing effects MFW were held on Guinea white pigs weighing 350–380 g when applying 0.2 mL of the substance in native condition on the cut areas of the skin for 20 days. The testing of animals was performed on the 21st day in an experimental way by intradermal introduction of 0.02 mL of the studied substance in native form in the ear of the experimental and control animals. Observations of animals were performed in 1, 3, 6 and 24 hours. The skin reaction was visually evaluated using a 5-point unified scale [Dueva 1989]. 24 hours after the introduction of the studied substance, the study of immunological indicators - RSLL (leukocyte lysis sensitization reaction) and RDTC (mast cell destruction reaction) [Methods of laboratory specific diagnostics of occupational diseases of chemical etiology 1980] was performed.

To clarify the cumulative effect of MFW, the experiment was performed on white Wistar rats. The doses corresponding to 1/5 and 1/10 of LD_{50} were injected in the animals every day for 2 months. The degree of accumulation was evaluated by cumulation coefficient calculated by the method of Y.S. Kagan [Kagan 1976].

The functional state of the liver was evaluated by the total amount of protein in serum, determined by the biuret method [Menshikova 1987], the urea content, the activity of alkaline phosphatase [Bodansky 1953] and enzymes of

reamination of ALT (alanine aminotransferase) and AST (aspartate aminotransferase) in serum [Reitman 1957], the intensity of the processes of demethylation of amidopyrine in liver [Nash 1953]. The state of the processes of lipid peroxidation was evaluated by the accumulation of malondialdehyde in liver hormones [Stalnaya 1977]. The excretory and filtration functions of the kidneys were assessed by daily diuresis, the amount of protein, urea, and chloride in the urine.

To determine the nature of the MFW effect on the blood system, a study of several indicators of the morphological composition of peripheral blood was conducted by the usage of unified clinical and laboratory methods. Morphological composition of peripheral blood was assessed in the same animals 2 months after the experiment.

RESULTS AND DISCUSSION

The study of acute oral toxicity of mineralized fossil water for laboratory animals

In adult female rats weighing 225–240 g we have injected the MFW at the rate of 21.300 mg/kg body weight. No animal deaths under the experiment were observed. In the first 2–3 hours after application of the MFW we have observed the clinical symptoms of intoxication in two animals in the form of depression, immobility, and accelerated breath. The dynamics of body weight of experimental animals did not differ from control ones (Table 1). During the autopsy of animals on the 14th day of the experiment there were no visible changes in internal organs. Thus, LD_{50} of MFW for rats by oral application is more than 21.300 mg/kg.

In the adult white mice females weighing 20–30 g we have injected the MFW at the rate of 24.000 mg/kg, 34.000 and 36.000 mg/kg. After the application of MFW to mice animal mortality and clinical symptoms of intoxication were observed at all doses. In a group of animals, after introduction of MFW at the rate of 24000 mg/kg the drug killed 3 animals, 34000 mg/kg – 5 animals were killed and 36000 mg/kg - 6 animals killed out of 10. General condition of the surviving mice was tolerable. On the 7th day after drug application there was a decrease in weight gain of animals by 10% (Table 1). When dissecting mice 14 days after the experiment no visible changes in the internal organs were observed.

There were no deaths at oral action of MFW on an organism of mice males at a dose of 20.000 mg/kg. Clinical signs of intoxication were observed within 2–3 hours after drug injection. During the dissection of bodies on 14th day no macroscopic changes of internal organs were detected. LD_{50} for male mice is more than 20.000 mg/kg of body weight. Thus, according to the parameters of acute oral toxicity for female rats and mice males and females MFW belongs to low-toxic substances which is defined by GOST 12.1.007 - 76. The variability in species and sexual sensitivity to the drug is not expressed.

Study of acute inhalation toxicity of mineralized fossil water

Acute inhalation toxicity of MFW was studied in a single 4-hour effect on the body of white female rats weighing 190.0–225.0 g (average

Table 1. The results of the study of acute oral and inhalation toxicity of MFW

The group of animals	Research period, days		
	0	7	14
The average weight of rats with acute oral exposure to MFW			
Control	229.2 ± 6.2	238.3 ± 6.2	246.7 ± 5.3
Experimental	235.0 ± 2.6	240.8 ± 2.6	250.0 ± 4.4
Dynamics of body weight of female mice with acute oral action of MFW			
Control	22.6 ± 0.31	26.9 ± 0.92	24.8 ± 0.22
24000 mg/kg	21.7 ± 0.25	26.0 ± 0.56	23.7 ± 0.57
34000 mg/kg	22.1 ± 1.30	26.4 ± 0.22	24.1 ± 0.63
36000 mg/kg	21.9 ±0.54	26.0 ± 0.32	23.9 ±1.02
Dynamics of body weight of female rats during acute inhalation of MFW			
Control	153.3 ± 1.8	159.2 ± 2.7	165.0 ± 1.8
Experimental	153.3 ± 1.8	159.6 ± 3.6	166.7 ± 2.7

Table 2. Dynamics of body weight of rats with subchronic action of MFW (2 months)

Group of animals	Period of research, days				
	0	10	20	30	40
Control	105.8 ± 4.4	136.7 ± 3.5	146.7 ± 3.5	151.7 ± 3.5	159.2 ± 1.8
0.02 LD_{50}	102.1 ± 2.3	135.8 ± 2.3	146.7 ± 0.9	154.2 ± 1.9	161.3 ± 1.9
0.1 LD_{50}	104.6 ± 2.8	131.7 ± 2.8	142.5 ± 2.8	151.3 ± 1.9	157.9 ± 1.9

concentration was 5000±84 mg/kg). After the action of MFW the appearance and the behavior of experimental animals did not differ from the control ones. Mass dynamics of animal bodies are submitted in Table 1. At the end of the two-week period macroscopic changes in internal organs were not detected.

Thus, MFW did not have a toxic effect on the animals in the highest achievable concentration of 5000 mg/kg. According to GOST 12.1.007-76 by inhalation route of entry into the organism MFW belongs to the III class of danger – moderately dangerous compounds.

Investigation of resorptive - toxic action of MFW

Resorptive-toxic effect of MFW was studied using 6 female Wistar rats weighing 250 g. The criterion of our study was the presence or absence of deaths, the time and degree of severity of intoxication. The drug in its native form was precisely applied to the cut areas of the skin of rats at a rate of 8000 mg/kg. The general condition of animals, their behavior did not differ from those measured among the control animals. There was no irritant action on the place of application of MFW.

Thus, MFW did not have resorptive toxic effect on animals when applied to the skin. LD_{50} of MFW for rats by skin applications is more than 8000 mg/kg of body weight. According to GOST 12.1.007-76 MFW belongs to the IV class of danger – low-hazardous compounds.

The study of the irritating effect of MFW on the skin

MFW in its native form was carefully applied to the cut areas of rabbit skin in a volume of 0.5 ml. After application of the drug on the skin on the day of the experiment and after 7 days the redness of the skin, fever, increase of skin fold thickness and other visible changes were not observed. Cut areas of skin were evenly filled up with hair. In another experiment on a group of rats weighting 180–200 g MFW in native state was applied for 2 weeks in a volume of 0.2 ml. Throughout the experiment, the irritating effect of MFW on the skin of rats was also not observed.

Thus, because of the experiments it was found out that MFW does not have an irritating effect on the skin of rabbits and female rats.

The study of the irritating effect of MFW on the mucous membranes of the eyes

MFW in its native form in the quantity of 2 drops (0.1 ml) was added to the conjunctival sac of an eye of three rabbits of the breed chinchilla. Monitoring the condition of mucous membranes of the eyes was performed after 15 minutes, 1, 24, 48 and 72 hours. It was defined that MFW does not have an irritating effect on the mucous membranes of the eyes.

Study of the cumulative properties of MFW at doses of 4260 and 2130 mg/kg of body weight was performed on white female Wistar rats with an initial weight of 100.0–120.0 g. The drug was introduced orally in the native form every day for two months using a probe. The effect was evaluated by the number of deaths.

During the experiment, the general condition of the animals was satisfactory. There were no external symptoms of intoxication and death among the experimental animals. The dynamics of body weight did not differ from the dynamics among the animals of control group (Table 2). At the end of the experiment, the relative mass of animals' internal organs was determined.

According to the data of Table 3, a statistically significant increase in the relative heart weight was observed in animals exposed to MFW at a dose of 4260 mg/kg. There were no other changes of the relative mass of other organs.

As can be seen from the Table 4, protein-synthesizing, detoxifying, and ferent-forming liver function were not disturbed. It was statistically defined that there may be an increase in daily diuresis (by 81%) and chloride content in urine in

Table 3. The relative mass of animal organs in the subchronic action of MFW (2 months)

Organ	Control	0.02 LD_{50}	0.1 LD_{50}
Liver	39.6 ± 1.5	39.8 ± 1.8	39.7 ± 1.1
Lungs	10.7 ± 0.5	9.0 ± 1.0	12.0 ± 1.1
Heart	3.6 ± 0.3	4.3 ± 0.2	4.1 ± 0.2
Kidneys	7.0 ± 0.2	7.2 ± 0.1	7.1 ± 0.4
Brain	10.9 ± 0.2	10.2 ± 0.4	10.2 ± 0.4
Adrenal glands	0.29 ± 0.01	0.26 ± 0.02	0.31 ± 0.01
Spleen	4.9 ± 0.3	4.9 ± 0.2	4.4 ± 0.2

Note: Changes are statistically significant at $p< 0.05$.

Table 4. The results of the study of the cumulative properties of MFW

Indicators	Control	4260 mg/kg	2130 mg/kg
Some biochemical parameters of blood serum under a subchronic action (2 months)			
Alkaline phosphatase, mmol/(L.h)	1.92 ± 0.10	1.96 ± 0.14	2.19 ± 0.09
Alanine aminotransferase, mmol/(L.h	0.64 ± 0.05	0.59 ± 0.07	0.70 ± 0.07
Aspartate aminotransferase, mmol/(L.h)	0.84 ± 0.09	0.87 ± 0.04	0.87 ± 0.03
Influence of mineralized formation water on protein metabolism under a subchronic action (2 months)			
The content of total protein, mg/L	79.3 ± 1.6	76.5 ± 3.5	77.7 ± 0.7
Urea content, mmol/L	5.3 ± 6.0	6.0 ± 0.3	5.8 ± 0.7
Influence of mineralized formation water on the state of processes of lipid peroxidation and MOGS under a subchronic action (2 months)			
Malondialdehyde, mmol/(g.h)	36.7±3.8	45.5 ± 6.0	36.4 ± 5,3
Dialdehyde, mmol/(g.h)	0.94 ± 0.10	0.98 ± 0,20	0.93 ± 0.05
Some indicators of the functional state of the kidneys of rats under a subchronic action of MFW (2 months)			
Daily diuresis, ml	2.38 ± 0.21	4.30 ± 0.5	2.76 ± 0.45
Ph	6.64 + 0.09	6.58 + 0.09	6.62 + 0.09
Chlorides, mmol/diuresis	31.6 ± 2.3	51.6 ± 4.6	34.3 ± 3.9
Urea, mmol/l	441.2 ± 35.7	491.2 ± 44.7	449.6 ± 35.7
Protein, mg/g	123.0 ± 4.3	104.0 ± 13.9	120.0 ± 7.5
Morphological composition of peripheral blood of white rats under a subchronic action of MFW (2 months)			
Erythrocytes, terra/L	8.54 ± 0.08	8.32 ± 0.23	8.14 ± 0.22
Hemoglobin, mmol / l	9.69 ± 0.25	9.58 ± 0.49	9.98 ± 0.19
Reticulocytes, % 0	32.60 ± 3.65	32.09 ± 6.22	29.60 ± 4.08
Leukocytes, g/L	22.94 ± 4.36	19.90 ± 3.80	23.80 ± 3.78
Platelets, g/L	917.64 ± 88.53	1038.15 ± 54.82	1000.25 ± 80.40

Table 5. The state of immunological parameters in guinea pigs with sensitization of MFW

No.	Skin reaction to the antigen's action, points		RSLL, %		RDTK, %	
	Control	Experiment	Control	Experiment	Control	Experiment
1	0	0	7.0	7.0	4.0	0
2	0	0	8.0	0	0	0
3	0	0	3.0	9.0	4.	4.0
4	0	0	7.0	9.0	0	8.0
5	0	0	7.0	9.0	8.0	4.0
6	0	0	7.0	9.0	4.0	8.0
7	-	0	-	9.0	-	8.0
8	-	0	-	2.0	-	4.0
9	-	0	-	3.0	-	8.0
10	-	0	-	2.0	-	4.0
$M_{\pm m}$	0	0	5.7 ± 1.0	5.2 ± 1.2	3.3 ± 1.2	4.8 ± 1.0

the group of animals exposed to MFW at a dose of 4260 mg/kg body weight. Probable changes in the quantitative indicators of blood after the action of MFW were not revealed.

Thus, MFW at the dose of 4260 and 2130 mg/kg of body weight did not cause any deaths of animals. Throughout the experiment there were no clinical signs of intoxication. The accumulation coefficient is greater than 5. Under the action of MFW on the body of rats at a dose of 4260 mg/kg the drug has a toxic effect on the heart and irritation effect on erythropoiesis, increasing the filtration and excretory function of the kidneys. Ineffective dose for rats in a subchronic experiment is 2130 mg/kg of body weight.

When conducting the research on the sensitizing properties of MFW by 20x skin applications it was found out that throughout the experiment the skin had remained clean. The skin reaction to the drug was 0 points in all experimental animals. The results of RSLL (leukocyte lysis sensitization reaction) and RDTC (mast cell destruction reaction) are shown in Table 5. The obtained results indicate that in the conditions of this experiment MFW does not have a sensitizing effect on guinea pig body.

CONCLUSIONS

Thus, mineralized fossil water (MFW) appertains to low-toxic compounds. LD_{50} is more than 21.000 mg/kg when introduced orally into white female rats, into female mice – 31.000 mg/kg, into mice males – more than 20.000 mg/kg, when applied to the skin of rats more than 8000 mg/kg. The value of LD_{50} for female rats is more than 5000 mg/kg. According to the system of standards GOST 12.1.007-76 for oral introduction and when penetrating through the skin MFW belongs to class IV of danger, and when introduced through the inhalation – to class III.

It was determined that MFW does not have an irritating effect on the skin and mucous membranes of the eyes. Sensitizing effect on the body of guinea pigs was not detected. The cumulative properties of MFW are weakly expressed. The cumulation coefficient is higher than 5. At a dose of 4260 mg/kg MFW has a cardiotoxic effect, irritates the process of erythropoiesis and increases the filtration and excretory function of the kidneys. Ineffective dose for rats in the subchronic experiment is at 2130 mg/kg. These results of ecotoxicological study of MFW make it possible to confirm that its usage is safe in the agricultural system, as environmentally friendly organo-mineral fertilizer, herbicide, and fungicide.

REFERENCES

1. Rudko G.I., Skatinskiy Y.P. 2002..Environmental impact assessment of oil and gas facilities in difficult environmental conditions (on the example of Bitkovsky and Pasichnyansky fields). Visnyk ekonomichnoi nauky Ukrainy, 7, 54–56. (in Ukrainian)
2. Pisarenko P.V., Samoylik M.S., Kolesnikova L.A., Plaksienko I..L. 2018. Dynamics and diagnosis of toxic effects of oil-contaminated soil on seedling stability. Agrology, 1(3), 240–246. (in Ukrainian). DOI: 10.32819/2617-6106.2018.13002
3. Pisarenko P.V. 2020. Scientific approaches to the greening the technology of growing spring barley in conditions of the left – bank forest-steppe. Visnyk PDAA, 4, 142–149. (in Ukrainian) DOI: 10.31210/visnyk2020.04.17
4. Yaron D. 1980. A model for optimal scheduling with saline water. Water Resources Research, 2, 112–117.
5. Abd-Elmabod S.K., Jordan A., Fleskens L.A., Phillips J.D., Muñoz-Rojas M., Van der Ploeg M., Anaya-Romero M., De la Rosa D. 2017. Modelling agricultural suitability along soil transects under current conditions and improved scenario of soil factors. Soil Mapping and Process Modeling for Sustainable Land Use Management. Elsevier: Amsterdam, The Netherland, 193–219. DOI: 10.1016/B978-0-12-805200-6.00007-4
6. Methodical instructions for the hygienic assessment of new pesticides. 1998. Kiev: Minzdrav SSSR, 209. (in Russian)
7. Prozorovskiy V.B. 1961. Using the least squares method for probit analysis of lethality curves. Farmakologiyaitoksikilogiya, 1, 115–120. (in Russian)
8. Kundiev Y.I. 1964. Methods for studying the penetration of chemicals through intact skin. Gigienaisanitariya, 10, 71–78. (in Russian)
9. Dueva L.A., Kogan V.Yu., Suvorov S.V., Shterengarts R.Y. 1989. Industrial allergens. Moscov, 78. (in Russian)
10. Methods of laboratory specific diagnostics of occupational diseases of chemical etiology. 1980. Moskva: AMN SSSR, 214. (in Russian)
11. Kagan Y.S. 1976. Cumulation, criteria and methods for its assessment, predicting chronic concentrations. Moskva, 85. (in Russian)
12. Menshikova V.V. 1987. Laboratory research methods in the clinic. Spravochnik. Moskva:

Meditsina, 315. (in Russian)

13. Bodansky V. 1953. Serum phosphogenase isomerase. J. Biol. Chem, 202, 829–830.
14. Reitman S., Frankel S. 1957. A colorimetric method for determination of serum glutamicoxalacetic and glutamicopyruvic transaminases. Amer. J. Clin. Phath, 28, 56–60.
15. Nash T. 1953. The colorimetric estimation of formaldegide by means of the Hautzsch reaction. Bioch. J., 55, 416–421.
16. Stalnaya I.D., Garishvili T.G. 1977. Method for the determination of malonic dialdehyde using thiobarbituric acid. Sovremennye metody v biokhimii, 66–68. (in Russian)