International Journal of Veterinary Science and Agriculture Research
Volume 1 Issue 1, May 2019
Available at www.ijvsar.com

Acute Toxic Effects of the Antifreeze (Ethylene Glycol) On Rainbow Trout (*Oncorhynchus mykiss*)

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Keywords: *Antifreeze, acute toxicity, rainbow trout, LC50.*

Abstract: Antifreeze (Ethylene glycol: EG), a chemical substance derivatized from alcohol and contaminating aquatic ecosystems as a potential toxic pollutant, was investigated in the present study for acute toxicity. Ethylene glycol is used to prevent freezing in the motor vehicle engine cooling systems and hot water production installations sunny, some hot water heating installations in the world. So there is a risk of contamination of the aquatic environment with the environment in which EG is used. Therefore, the toxic effects of this compound on aquatic insights must be examined.

Rainbow trout (Oncorhynchus mykiss) were selected for the bioassay experiments. The 96-h LC50 was determined for the Rainbow trout. The static acute toxicity tests were replicated as three times and a total of 300 Rainbow trout were used in bioassay tests. In addition, behavioral changes at each ethylene glycol concentration was observed for the individuals. Data obtained from the Antifreeze acute toxicity test results were evaluated using the Probit Analysis Statistical Method based on Finney's Probit Analysis using a computer program. The 96-h LC50 value for the rainbow trout was estimated as 14.21 g/L.

I. INTRODUCTION

Fish and wildlife populations have been dramatically affected by environmental pollutants. One of the best-known examples is the response of wildlife populations to the use of pollutants such as heavy metals, pesticides and the other chemical substances [1]. There are a large number of pollutants currently in use, with a wide range of physicochemical properties and belonging to a wide variety of chemical classes [2].

Antifreeze (ethylene glycol) is a chemical substance derivatized from alcohol and contaminating aquatic ecosystems as a potential toxic pollutant. Environmental pollution in water affects fish and other aquatic animals [3].

Ethylene glycol is metabolized by alcohol dehydrogenase to glycoaldehyde, which is then metabolized to glycolic, glyoxylic, and oxalic acids. These acids, along with excess lactic acid, are responsible for the anion gap metabolic acidosis. Oxalate readily precipitates with calcium to form insoluble calcium oxalate crystals. Tissue injury is caused by widespread deposition of oxalate crystals and the toxic effects of glycolic and glyoxylic acids.

Ethylene glycol is the most widely used glycol in the world and its high production is mainly used in the production of antifreeze and antifreeze mixtures with primary use and polyester in a lower proportion. While the use of propylene glycol from other glycols is concentrated in the production of resin and polyester, diethylene glycol is mainly used as a dehydrator in the natural gas industry [4].

Automobiles with radiator cooling system use ethylene glycol and propylene glycol antifreeze to reduce the freezing point of water. Propylene glycol is preferred in building heating systems although ethylene glycol is used in the automotive sector. Both types of antifreeze contain anti-corrosion agents to prevent metal corrosion. Most anti-corrosion and antifoam inhibitors are toxic. In addition, the cooling engine and pipes (lead, phosphorus and cadmium as toxic) may take the wear of metals [5].

Ethylene glycol is a low toxic compounds to aquatic organisms. Acute toxicity tests with aquatic invertebrates where a value could be determined show LC50s above 20000 mg/L, and those with fish show LC50s above 17800 mg/L. An amphibian test showed an LC50 for tadpoles at 17 000 mg/L. A no observed-effect concentration (NOEC) for chronic tests on daphnids of 8590 mg/L (for reproductive endpoints) has been reported. A NOEC following short-term exposure of fish has been reported at 15 380 mg/L for growth [6].

In terms of glycoles, algae in aquatic environment are more sensitive than vertebrates or invertebrates and have relatively low toxicity. The most sensitive response to Ethylene glycol exposure was 96-h LOEC 1923.4 mg/L for the *Selenastrum capricornutum* from green algae. Anti-icing fluids used in aircraft are more toxic than pure glycols. When the most sensitive response was exposed to a propylene glycol-based anti-icing liquid, the 96-h LC_{50} value for *Pimephales promelas* was 18 mg/L [4].

The present study was conducted to determine the acute toxicity of ethylene glycol on the rainbow trout in a static aquaria system. This species was selected for bioassays because it can easily adapt to the laboratory conditions and it is available throughout the year.

II. Material and Method

Experimental fish and design

Juvenile rainbow trout Oncorhynchus mykiss [3.2±0.6 g body weight (BW) and 7.0±0.6 cm total length (TL)] was obtained from a local trout breeding farm and then transferred to the Aquatic Toxicology Laboratory in the Faculty of Fisheries and Aquatic Science, Recep Tayyip Erdoğan University (Fig. 1). Fish were allowed to acclimate to laboratory conditions in two glass aquaria (600 L) containing total of 500 fish for 2 weeks prior to experiments [7,8]. During acclimation, aquaria water had following characteristics: dissolved oxygen, 8.40±0.30 mgL^{-1}; temperature, 12.0±0.3 ◦C; pH 7.40±0.23 (Table 1).

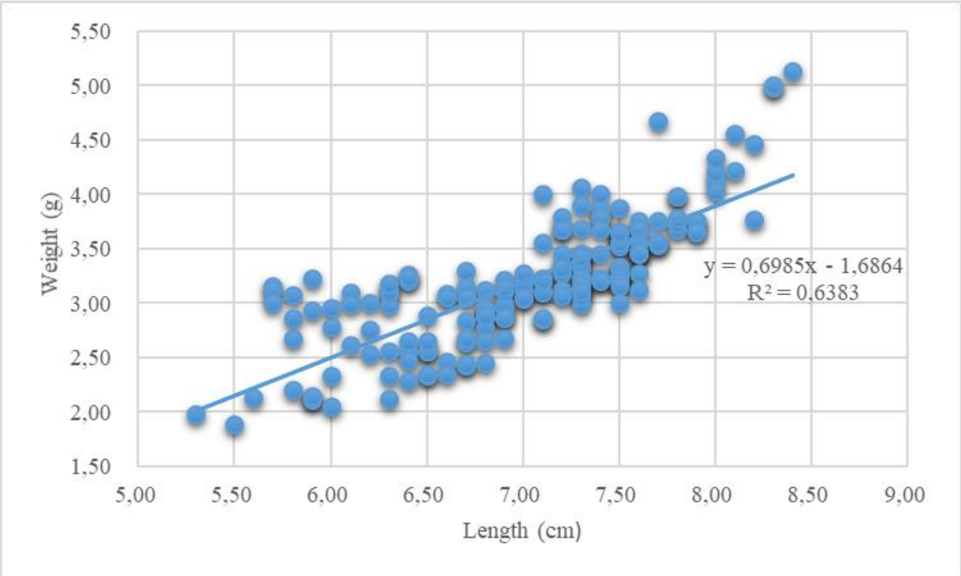

Figure 1. Length and weight relation of experimental fish

Table 1. Physicochemical properties of the test water and test chemical used.

Water Quality Parameters	Bioassay experiments medium
Temperature (°C)	12.0±0.3°C
pH	7.40±0.23
Dissolved oxygen (mg L-1)	8.40±0.30
Conductivity (µS cm-1)	60.1-65.9
Total hardness (as mg CaCO3L-1)	64-65
TDS (mg L-1)	29-32
Parameters of E.glycol as a test chemical	Property
Empirical formula	C2H6O2
Molecular weight	62.07
CAS registry number	107-21-1
Physical state (25 °C)	Colourless liquid
Melting point (°C)	13
Boiling point (°C)	197.6
Density (gr/mL)	1.1135 (20 °C)
Vapor pressure (mmHg)	0.05 (20 °C)
Log Koc	2.14
Log Kow	1.93
Solubility in water	Miscible

Source: [4,9]

Determination of LC_{50}, behavioral changes and statistical analyzes

Before toxicological tests, the fish were determined to be free of bacteria and external parasites [10]. Triplicate random groups of 10 fish each were exposed for 96 h in static fresh water containing 2.5, 5.0, 10.0, 15.0, and 25.0 g/L Ethylene Glycol (Table 2). Test solutions of Ethylene Glycol were prepared from commercial formulations (OMV Petrol Office, Turkey). During the toxicity experiment, water in each aquarium was aerated and measured the characteristics. At the end of the 96-h of acute toxicity tests, died fish were discarded and the nominal concentration of ethylene glycol estimated to kill 50% of rainbow trout within 96-h (96-h LC50) was calculated by probity analysis according to the maximum-likelihood procedure [11,12].

Table 2. The concentrations of ethylene glycol used for bioassay experiments

Antifreeze (Ethylene glycol; EG)	Concentrations (g L-1)					
	0	2.5	5.0	10.0	15.0	25.0

III. Results

Evaluations of lethal concentrations and acute toxicological parameters

No fish died during the acclimation period before ethylene glycol exposure, and no control fish died during toxicity tests. When fish were exposed to 5 g/L ethylene glycol or higher concentrations, fish exhibited signs of restlessness, erratic swimming, dark pigmentation, flashing, convulsions, increasing respiratory rates, and then lose of balance. Fish also had excessive mucus. At the time of death, mouth and opercula were open. The concentrations of ethylene glycol that killed 50% of the rainbow trout (3.2±0.6 g) within 96 h (96 h; LC50) were 14.2± 1.5 g/L (95% confidence limits) (Fig. 2, 3) (Table 3). The highest concentration at which no fish die was less than 10 g/L (Fig. 3). On the other hand, the lowest concentration of all fish died above 23 g/L (Fig. 3). None of the unexposed control fish died.

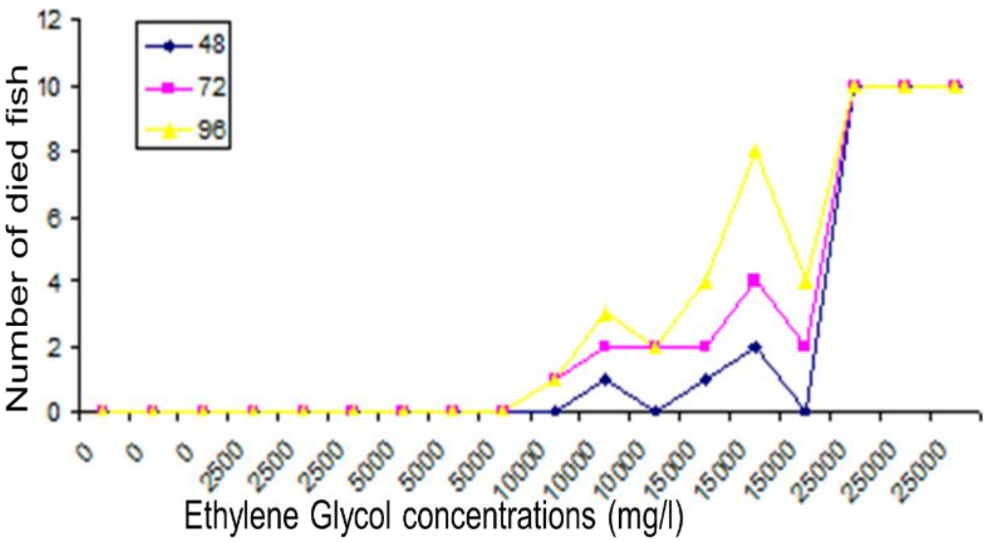

Figure 2. Number of died fish in the bioassay results

Table 3. Calculated lethal concentrations and 95% confidence limits

LC Level	Concentration (mg/L)	%95 Confidence Limits	
		Lower	Upper
LC/EC_{20}	10828	9019	12152
LC/EC_{30}	12102	10618	13450
LC/EC_{40}	13191	11850	14693
LC/EC_{50}	14208	12889	15967
LC/EC_{85}	18372	16488	21831
LC/EC_{90}	19356	17270	23288
LC/EC_{95}	20815	18410	25465
LC/EC_{99}	23553	20511	29587

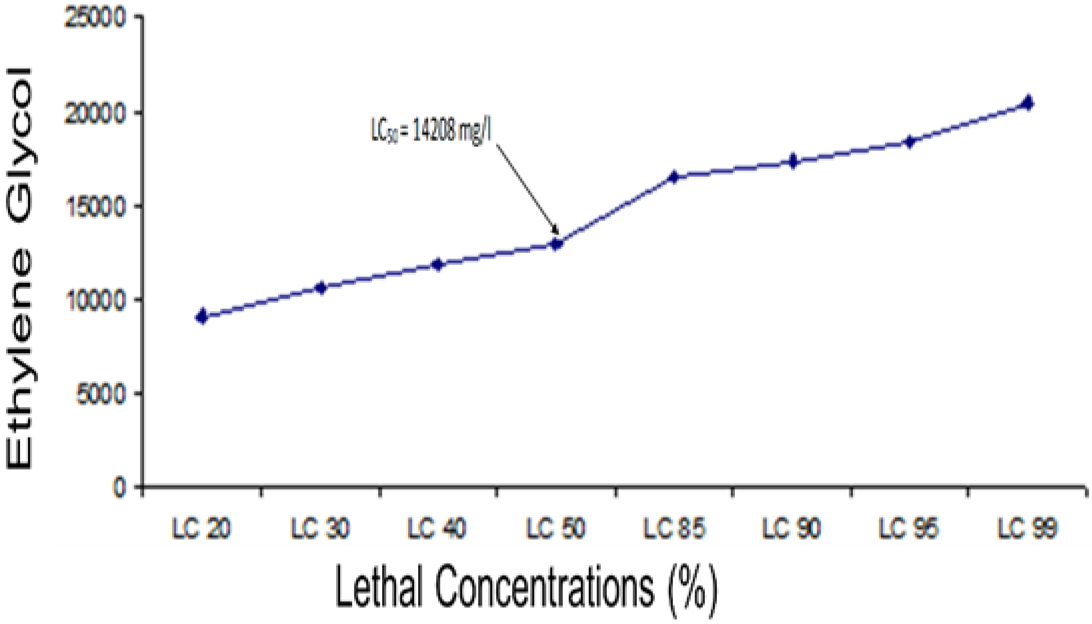

Figure 3. Lethal concentrations in the bioassay results

IV. Discussion

Water can be contaminated by the inappropriate storage, use or disposal of ethylene glycol which is also low toxic to fish. The concentrations at which a compound is lethal can depend upon many contributing factors including species and water quality. Ethylene glycol has generally low toxicity to aquatic organisms. Toxic thresholds for microorganisms are above 1000 mg/L. EC50s for growth in microalgae are 6500 mg/L or higher. Acute toxicity tests with aquatic invertebrates where a value could be determined show LC50s above 20,000 mg/L, and those with fish show LC50s above 17,800 mg/L. An amphibian test showed an LC50 for tadpoles at 17,000 mg/L. A no-observed-effect concentration (NOEC) for chronic tests on daphnids of 8,590 mg/L (for reproductive end-points) has been reported. A NOEC following short-term exposure of fish has been reported at 15,380 mg/L for growth [6].

It has been reported that even rainwater contaminated by glicol derivatives used to prevent icing on Canadian airports and airplanes has a lethal effect of 40% in Oncorhynchus mykiss and 30% in Daphia magna [13]. These kinds of chemicals are related to behavioral changes and to the drop in swimming performance in larvae and juveniles of several species of fish exposed to acute intoxications. It can be used as an additive in petroleum production, although it has been reported that it affects on some fish swimming performances despite the low level of toxicity on fish [14].

Ethylene glycol LC_{50} values have been reported for goldfish (*Carassius auratus*) 24-h LC_{50} >5,000 mg/L [15], for rainbow trout (*Onchorhynchus mykiss*) 96-h 41,000 mg/L; (monomethyl ether) 96-h 16,000 mg/L (14,000-18,000) [16]; 96-h > 18,500 mg/L [17]; 17800-45600 mg/L [18], for guppy (*Poecilia reticulate*) 168-h 49,300 mg/L [19], fathead minnow (*Pimephales promelas*) 96-h fry 53,000 mg/L, juvenile 49,000 mg/L and subadult 57,000 mg/L [20]; >10,000 mg/L [21]; 72,860 mg/L [22] and Bluegill Sunfish (*Lepomis macrochirus*) >111,300 mg/L [18,23].

In some studies, the formulated mixtures are reported to be substantially more toxic than pure glycol liquids. For example, the 48-hour LC_{50} for the water flea (*Ceriodaphnia dubia*) was 13,140 mg/L and 1,020 mg/L, using the formulated EG (Ethylene glycol) and PG (Propylene glycol) and 34,400 mg/L and 18,340 mg/L. EG and PG respectively. The 96-hour LC_{50} for Fathead minnow (*Pimephales promelas*) was 8,750 mg/L and 710 mg/L using EG and PG respectively, 72,860 mg/L and 55,770 mg/L using pure EG and PG. However, if different compounds are added to the mixtures, the toxicity of the glycol fluids either increases significantly or may decrease in some cases [22].

On the other hand, some studies suggest that glycols have low toxicity in water and that algae are more sensitive than vertebrates or invertebrates. In the results of this study the most sensitive response recorded for ethylene glycol exposure was observed in green algae *Selenastrum capricornutum* for 96-h LOEC 1923.4 mg/L. Thus, although ethylene glycol has low toxicity for aquatic organisms, the recommended water quality criterion for ethylene glycol in Canada is around 192 mg/L [4]. In the present study, ethylene glycol was also found low toxic to rainbow trout. These results are similar findings as the other reports.

In order to reduce the environmental risks of the chemicals such as ethylene glycol and propylene, which prevent icing, it is planted with alfalfa (Medicago sativa) and Kentucky bluegrass (*Poa pratensis*) species and only ethylene glycol decomposition occurs in the soil. With this method, it was reported that ethylene glycol in the conditions of 0 °C provided conversion of carbon dioxide to 60.4%, 49.6%, and 24.4% [24]. Therefore, vegetation and mineralization of these types of chemicals in terrestrial environments without reaching the aquatic environment can be recommended as a valid measure for the protection of all environmental mediums.

V. Acknowledgements

Thanks to valuable contributions of Dr. Ertuğrul Terzi for this work. This research has been presented as an oral presentation in First International Black Sea Congress on Environmental Sciences (IBCESS-2016) and its abstract was published in IBCESS16 Abstract Book.

References

[1] Bolis, C.L. Piccolella, M. Dalla Valle, A.Z. and Rankin, J.C, 2001. Fish as Model in Pharmacological and Biological Research. *Pharmacological Research*, 44, pp. 265-280.

[2] Haygarth, P.M., Jarvis, S.C., 2002. *Agriculture, Hydrology and Water Quality*. CABI, Wallingford, Oxfordshire, UK, pp. 8–25.

[3] Svensson, B.G., Hallberg, T., Nilson, A., 1994. Parameters of immunological competence subjects with high consumption of fish contaminated with persistent organochlorine compounds. *Int. Arch. Occup. Environ. Health 65, 351–358*.
[4] Kent, R.A., Andersen, D., Caux, P.Y., Teed, S., 1999. Canadian Water Quality Guidelines for glycols - An ecotoxicological review of glycols and associated aircraft anti-icing and deicing fluids, *Environmental Toxicology, Vol.14, No:5, 481-522pp.*
[5] EGNC, 1999. *Guideline: General Management of Hazardous Waste, Guideline for the Management of Waste Antifreeze*, Department of Environment Government of Nunavut P.O. Box 1000, Station 1360, Iqaluit, NU, X0A 0H0.
[6] WHO, 2000. World Health Organization, *Concise International Chemical Assessment Document 22*, ETHYLENE GLYCOL: Environmental aspects, Geneva, 27 p.
[7] APHA, AWWA, WEF,1985. *Standard Methods for the Examination of Water and Wastewater*. APHA, AWWA, WEF, 16th ed. APHA, Washington, DC.
[8] Molony, B., 2001. Environmental requirements and tolerances of Rainbow trout (Oncorhynchus mykiss) and Brown trout (Salmo trutta) with special reference to Western Australia:A review, Government of Western Australia, Department of Fisheries, Fisheries Research Division, WA Fisheries Research Laboratories, *Fisheries Research Report, No:13*, North Beach, Western Australia.
[9] ATSDR, 1993. *Agency for Toxic Substances and Disease Registry, Draft Technical Report for Ethylene Glycol and Propylene Glycol*. Prepared for U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.
[10] AFS–FHS, 2003. *Suggested procedures for the detection and identification of certain finfish and shellfish pathogens, fifth ed.* Fish Health Section. American Fisheries Society, Bethesda, MD, USA.
[11] SPSS, 2002. *SPSS Inc*., Chicago, Illinois, USA.
[12] USEPA, 1999. *EPA Probit Analysis Program Used for Calculating LC/EC Values, Version 1.5*, Ecological Monitoring Research Division, Environmental Monitoring Systems Laboratory, US Environmental Protection Agency, Cincinnati, OHIO, 45628.
[13] Novak, L.J., Holtze, K., Kent, R.A., Jefferson, C., Andersen, D., 2000. Acute toxicity of storm water associated with de-icing/anti-icing activities at Canadian airports, *Environmental Toxicology and Chemistry, Vol. 19, No. 7, pp. 1846–1855*.
[14] Hymel, M.K., Baltz, D.M., Chesney, E.J., Tarr, M.A., and Kolok, A.S., 2002. Swimming Performance of Juvenile Florida Pompano Exposed to Ethylene Glycol, *Transactions of the American Fisheries Society 131:1152–1163, 2002*.
[15] Bridie, A., Wolff, C.J.M., and Winter, M., 1979. The acute toxicity of some petrochemicals to gold fish, *Water Research, 13:623-626*.
[16] Finley, M.T. and Johnson, W.W. 1980. *Handbook of Acute Toxicity Of Chemicals To Fish And Aquatic Invertebrates*, Summaries of Toxicity Tests Conducted at Columbia National Fisheries Research Laboratory, United States Department of the Interior Fish And Wildlife Service, Resource Publication 137 Washington, D.C.
[17] Jank B.E., Guo H.M., Cairns V.W., 1974. Activated sludge treatment of airport wastewater containing de-icing fluids. *Water Research*, 8:875–880.
[18] Mayer, F,L, Ellersieck, M.R., 1986. *Manual of acute toxicity: interpretation and database for 410 chemicals and 66 species of freshwater animals*. Washington, DC, US Department of the Interior, Fish and Wildlife Service (Resource Publication No. 160).
[19] Konnemann, H., 1981. Quantitative structure–activity relationships in fish toxicity studies. I. Relationship for 50 industrial pollutants. *Toxicology*, 19:209–221.
[20] Mayes, M.A., Alexander, H.C., Dill, D.C., 1983. A study to assess the influence of age on the response of fathead minnows in static acute toxicity tests. *Bulletin of environmental contamination and toxicology, 31:139–147*.
[21] Conway, R.A., Waggy, G.T., Spiegel, M.H., Berglund, R.L., 1983. Environmental fate and effects of ethylene oxide. *Environmental science and technology, 17(2):107–112*.
[22] Pillard, D.A., 1995. Comparative toxicity of formulated glycol deicers and pure ethylene and propylene glycolto Ceriodaphnia dubia and Pimephales promelas, *Environmental Toxicology and Chemistry, Vol. 14, No. 2, 311-315*.
[23] Khoury, G.A., Abdelghani, A.A., Anderson, A.C., Monkiedje, A., 1990. Acute toxicity of ethylene glycol to crayfish, bluegill sunfish and soil micro-organisms. *Trace substances in environmental health, 23:371–378*.
[24] Rice, P.J., Anderson, T.A., Coats, J.R., 1997. Evaluation of the Use of Vegetation for Reducing the Environmental Impact of Deicing Agents, Phytoremediation of Soil and Water Contaminants, *ACS Symposium Series, Vol. 664, Chapter 12, pp 162–176*.