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ARAŞTIRMA MAKALESİ

RESEARCH PAPER

Occurrence and Environmental Risks Assessment of DEET (N,N-diethyl-m-toluamide) Pesticide in Seyhan River, Turkey

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*Corresponding author's: Evsen YAVUZ GÜZEL Cukurova University, Faculty of Fisheries, Department of Basic Sciences, Adana, Turkey ⊠: eguzel@cu.edu.tr **Abstract:** The insect repellent DEET (*N*,*N*-diethyl-*m*-toluamide) is one of the most common organic chemical pollutants in water in a wide range of countries around the World. In this study, surface water samples were collected seasonally, from 7 stations in Seyhan River, following a period between December 2016 and November 2017. The DEET concentrations in Seyhan River were detected between 18.55-334.71 ng/L. DEET was detected in all seven sampling stations and detection frequency was 100%. Results obtained in autumn were higher than in summer. According to the environmental risk assessment, DEET in the Seyhan River, has no environmental risk (all measured RQ values were lower than 0.01).

Keywords: Environmental risk assessment, insect repellent, pesticide, seyhan river, water pollution.

Seyhan Nehri'nde DEET (N, N-dietil-m-toluamid) Pestisitinin Bulunurluğu ve Çevresel Risk Değerlendirmesi

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Anahtar kelimeler: Çevresel risk değerlendirmesi, böcek kovucu, pestisit, Seyhan Nehri, su kirliliği.

INTRODUCTION

Personal care products (PCPs) are compounds used in lotions, toothpastes, soaps, fragrances and sunscreen products. The classes of PCPs include insect repellents, preservatives, disinfectants, fragrances, and UV filters. PCPs are products for external use on the human

body and are therefore not subject to metabolic changes. Consequently, large amounts of PCPs enter the environment through regular use (Brausch & Rand, 2011). PCPs are considered as environmental emerging pollutants due to their sustained release into the aquatic environment

and the persistence of ecotoxicological impacts (Calza, Medana, Raso, Giancotti, & Minero, 2011).

Insect repellents are organic synthetic chemicals that can be applied on garments, skin or other surfaces to prevent the effects of insects and insect bites. Mosquito, fly, fleas and spiders are the most common insect bites. Bites can produce many side effects such as skin irritation, allergic reactions, infections, fever or encephalitis. And even western Nile fever, malaria, dang can cause diseases such as spread (Molins-Delgado, García-Sillero, Díaz-Cruz, & Barceló, 2018). DEET (N,N-diethyl-*m*-toluamide or N,N-diethyl-3-methylbenzamide) is the most widely used insect repellent for the prevention of bites from insects (Calza et al., 2011; Guzel, Cevik, & Daglioglu, 2017).

DEET was developed in 1946 by the US military to protect against mosquito bites related diseases during World War II. In 1957, DEET was made available for public use. EPA has declared this product safe for normal use. However, there is still a debate on whether DEET constitutes a significant risk for people, animals and the environment (Campos et al., 2016; Merel, Nikiforov, & Snyder, 2015).

DEET is sold in a wide variety of products including lotions, sprays, and wristbands. The formulations used can vary from 4% to 95% of the active ingredient. Agricultural use of DEET has also been reported (Aronson, Weeks, Meylan, Guiney, & Howard, 2012). As of 2017, 27 companies in the United States reported that about 119 consumer products containing DEET were in use (Keith et al., 2017). However, such data could not be found for Turkey.

The presence of DEET in the aqueous environment contains many and potentially complex pathways. DEET can be released directly or indirectly to the environment by its presence in commercial products. There is no known natural source of DEET known to be environmentally important. Due to recreational activities such as swimming (via skin or clothing of swimmers or indirectly over spraying), DEET can directly enter surface waters. Although often neglected, domestic waters (through human activities such as showering and bathing) are now expected to be the major source of DEET in the aquatic environment (Keith et al., 2017; Merel & Snyder, 2016). These aquatic environments being often used as drinking or tap water sources. So DEET can be detected in water for human consumption (Merel et al., 2015; Merel & Snyder, 2016).

The insect repellent DEET is one of the most common organic chemical pollutants in water in a wide range of countries from all around the world (Merel & Snyder, 2016).

The first studies about the occurrence of DEET in the aquatic environment was in samples of Swedish municipal landfill leachate and in Mississippi river (USA) were published in 1993 (Öman & Hynning, 1993; Pereira & Hostettler, 1993). DEET has been identified and quantified in different aquatic environments, such as surface water (Bartelt-Hunt, Snow, Damon, Shockley, & Hoagland, 2009; Calza et al., 2011; Costanzo, Watkinson, Murby, Kolpin, & Sandstrom, 2007; Guzel, Cevik, & Daglioglu, 2018b; Ma et al., 2016; Yoon, Ryu, Oh, Choi, & Snyder, 2010), ground water (Sorensen et al., 2015; Stuart, Lapworth, Crane, & Hart, 2012), drinking water (Benotti et al., 2009), seawater (Dsikowitzky et al., 2014) and wastewater treatment plants (Bartelt-Hunt et al., 2009; Costanzo et al., 2007; Glassmeyer et al., 2005). Studies on the presence of DEET in surface waters have increased significantly since 2000. The reason for this is that the sensitivity of the analytical methods used to detect DEET has increased over the last two decades (Aronson et al., 2012). With the present methodology, DEET can detect even at concentrations of <0.1 ng/L. European studies about the occurrence of DEET are limited to Western countries. And Asian studies are mostly dominated by China but highest DEET concentrations were also reported for Indonesia (Dsikowitzky et al., 2014; Merel & Snyder, 2016).

Numerous reviews and researches have been published investigating toxicity (Legeay, Clere, Apaire-Marchais, Faure, & Lapied, 2018; Martinez, Vélez, Mayo, & Sastre, 2016; Swale, Sun, Tong, & Bloomquist, 2014; Weeks, Guiney, & Nikiforovz, 2012) and occurrence of DEET in aquatic environments (Bartelt-Hunt et al., 2009; Benotti et al., 2009; Guzel et al., 2018b; Sandstrom, Kolpin, Thurman, & Zaugg, 2005). But less attention has been paid to identify the environmental risk assessment of PCPs especially insect repellents release on aquatic environments (Aronson et al., 2012; Sun et al., 2016).

In the Seyhan River Basin, there are freshwater marshes, salt marshes and reed marshes suitable for proleferiation of mosquito species (TUBITAK MAM, n.d.). Although the municipalities in the basin apply pesticides to combat mosquitoes, mosquito populations can be quite intense in some areas close to wetlands.

The present study aims to investigate the occurrence of DEET pesticide in Seyhan River surface water samples for assessing seasonal and spatial variations (seasonal sampling over one year) in seven stations. And the other aim of this study is to calculate the risk quotient (RQ) with the measured concentrations of DEET obtained from Seyhan River and to determine the environmental risks. To our knowledge this is the first study about the occurrence and environmental risk assessment of DEET in Seyhan River, Turkey.

MATERIALS AND METHODS

Chemicals and materials: Methanol (MeOH), methyl tert-butyl ether (MTBE) and water were gradient grade for liquid chromatography and purchased from Merck Millipore, Germany. Solid-phase extraction (SPE) cartridges (Oasis HLB, 500 mg, 6 cm3), SPE extraction manifold (20-positions) which combined with vacuum pomp were purchased from Waters (Millford, MA, USA). DEET (<98%) was purchased from Sigma-Aldrich (Steinheim, Germany). Internal standard diazepam-d5 (99%) was obtained from Lipomed (Switzerland). Glass fibre filter disks (GF/C, pore size 1.2 µm) were purchased (VWR, Belgium). Allure® from Whatmann Pentafluorophenylpropyl (PFPP) column (50 mm x 2.1 mm, 5 µm) was purchased from Restek (Bellefonte, PA, ABD).



Figure 1. Geographic location of Seyhan River in Turkey and sampling stations.

Sampling site and sample collection: The Seyhan River is the longest river in Turkey that flows into the Mediterranean Sea. It is located in the Seyhan Basin, south Turkey, and this basin has the total drainage area of 20.600 km². The total length of the Seyhan River is approximately 560 km. According to population and area information, population density is 83 person/km². Surface water samples were collected seasonally, from 7 stations in Seyhan River, following a period between December 2016 and November 2017. First two stations (SEY-01 (37°) 1'59.94"N, 35°20'29.66"E) and SEY-02 (36°58'45.92"N, 35°20'5.90"E) are dominated by a large city centre (Adana) million inhabitants living. 1.7 (36°55'6.59"N, 35°21'16.66"E), SEY-04 (36°47'4.66"N, 3'47.58"E), and SEY-06 (36°45'39.10"N, 34°57'20.16"E) surrounded by a variety of land uses including agriculture and industry. SEY-05 (36°45'29.03"N, 34°58'36.87"E) is located on a drainage canal near Baharli Village that carries waste water. SEY-07 is located in the region where Seyhan River and drainage channel are mixed and poured into the Mediterranean Sea. Surface waters were sampled from a depth of approximately 25 cm. Water samples were transferred in a cooler bag to the laboratory. In the autumn season sampling, no sample was taken at SEY-03 station, as there was no water.

Sample preparation and extraction: Water samples were filtered through glass fibre filters (GF/C) and H_2SO_4 was added for pH adjustment (pH=2±0.1). Solid phase extraction (SPE) was used for sample preparation before instrumental analysis. 1 L filtered surface water samples were loaded through preconditioned (Oasis HLB cartridges) SPE cartridges at a flow rate of 15 mL/min using a vacuum extraction manifold (Waters 20-Position extraction manifold (Milford, MA, USA)).

Instrumental analysis: Chromatographic analyses were carried out with ultra-fast liquid chromatography (Shimadzu CBM-20A), automatic sampling system (Shimadzu SIL-20A/HT) and mass spectrometry (Shimadzu 8030, Kyoto, Japan). DEET was separated chromatographically on PFPP column (at 40 °C, flow rate of 0.4 mL/min) using ultra-pure water with 10 mM ammonium formate (eluent A) and methanol (eluent B), with the gradient program started with 10% eluent B and was held for 10 min at a flow rate of 0.4 mL/min. Multiple reactions monitoring (MRM) mode was used for the determination of DEET (precursor: 192.2, products 91.1 and 119.1). Total method run time was 18 minutes. The retention time was 7.78 minute. The area ratio of the ions and retention time were compared by using the standard solutions under identical conditions. The limit of quantification (LOQ) was 0.3 and the limit of detection (LOD) was 0.2 ng/L for the DEET method. The correlation coefficients for calibration curve was higher than 0.99.

Environmental risk assessment calculations: Risk quotient (RQ) was calculated for the environmental risk assessment. The hazard and environmental risk level of DEET compound in the receiving environment can be determined by calculating the RQ value. PNEC value is calculated by using the smallest NOEC value calculated for many different species in ecotoxicology studies and the assessment factor (AF) suitable for the recipient environment (Aronson et al., 2012; Guzel, Cevik, & Daglioglu, 2018a). RQ formula is:

RQ = MEC/PNEC

In this formula, MEC is the measured environmental concentrations and PNEC is the no effect concentration in the surface water. If RQ values; < 0.1 means insignificant risk (no adverse effect expected), = 0.1-1 means low risk (potential adverse effects), = 1-10 means moderate risk (probable adverse effect) and > 10 means high risk (Ma et al., 2016). The PNEC value for DEET is 47000 (ECOSAR (Ecological Structure Activity

Relationship) class program Version 1.11 was used to obtain the pNEC value).

RESULTS

DEET was detected in all sampling stations, concentrations in Seyhan River were between 18.55 ng/L and 334.71 ng/L and detection frequency was 100%. Mean,

median, maximum and minimum concentrations were listed in Table 1.

The lowest DEET concentration was found as 18.55 ng/L in winter and highest concentration was found as 334.71 ng/L in autumn. The mean concentrations of DEET were found as 32.58, 78.31, 81.46 and 171.66 ng/L in winter, spring, summer and autumn respectively (Fig.2).

Table 1. DEET concentrations in surface waters of Turkey and other countries.

Study Area	Mean	Median	Min.	Max.	References	
•	(ng/L)	(ng/L)	(ng/L)	(ng/L)		
Seyhan River, Turkey	88.01	75.89	18.55	334.71	*This study	
Ceyhan River, Turkey	12.45	4.41	0.57	40.41	(Guzel et al., 2018b)	
Konya Basin Lakes, Turkey	3.92	3.57	3.41	4.79	(Guzel et al., 2018b)	
Antalya Basin Lakes, Turkey	3.43	2.73	1.54	7.77	(Guzel et al., 2018b)	
Ceyhan Basin Lakes, Turkey	3.45	2.56	0.64	14.22	(Guzel et al., 2018b)	
Seyhan Dam Lake, Turkey	5.50	1.78	1.01	17.43	(Guzel et al., 2018b)	
USA	-	55	13	660	(Brausch & Rand, 2011)	
The Northern River, Germany	-	-	0.11	1.09	(Weigel et al., 2002)	
Po River, Italy	-	-	0.6	155.55	(Calza et al., 2011)	
Schwarzbach, Modau, Winkelbach, and	124	-	<lod< td=""><td>1292</td><td>(Quednow & Püttmann, 2009)</td></lod<>	1292	(Quednow & Püttmann, 2009)	
Weschnitz Rivers, Germany						
Zhujiang, Shijing Rivers, China	-	-	0.2	107	(Yang et al., 2013)	
Jakarda River, Indonesia	-	-	30	24000	(Dsikowitzky et al., 2014)	
Han River, South Korea	160	190	120	-	(Yoon et al., 2010)	
5 stream sites, USA	-	-	30	180	(Mottaleb, Usenko, Brooks, & Chambliss, 2009)	
Assunpink Creek, USA	-	-	45	340	(Alvarez et al., 2005)	
139 stream sites, USA	-	60	-	1100	(Kolpin et al., 2002)	

<LOD: lower than the limit of detection

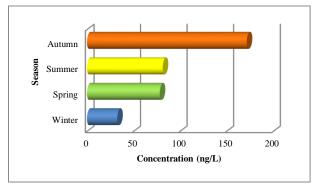


Figure. 2. Seasonal comparison of DEET concentrations (ng/L) in Seyhan River

In Figure 3, the cumulative distribution of DEET concentrations measured in Seyhan River by stations was shown. DEET detected higher where there was the possibility of wastewater contamination, via human, industrial, and agricultural sources, entering the streams and near urban areas during summer and late winter (autumn). The station where the highest DEET concentration measured was SEY-06 was under the influence of the influent wastewaters of Seyhan wastewater treatment plant (WWTP), which is the largest WWTP in Adana Province, and during the field studies, it was observed that the mosquito population around the station was very high in autumn. Recent studies indicate that the highest DEET concentration in aquatic environments correlates with its increased application during the summer months and late winter (Keith et al., 2017; Merel et al., 2015; Sandstrom et al., 2005).

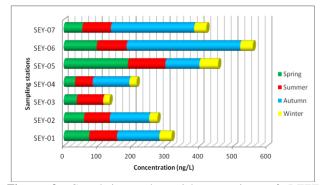


Figure 3. Cumulative and spatial comparison of DEET concentrations (ng/L) in Seyhan River

A study which was a summary of latest studies in surface water samples published in 2011 reported concentrations ranging from 13 to 660 ng/L for DEET throughout the USA (Brausch & Rand, 2011). In another review which reported worldwide DEET concentrations in water samples ranging from 40–3000 ng/L (Costanzo et al., 2007). Along the northern River, Germany DEET concentrations was between 0.11 to 1.09 ng/L (Weigel, Kuhlmann, & Huhnerfuss, 2002). DEET concentrations in Po River water samples were between 0.6 to 155.55 ng/L (Calza et al., 2011). Water samples taken from the Zhujiang and Shijing Rivers, concentrations of DEET were minimum 0.2 ng/L and maximum 107 ng/L. And in Assunpink Creek in Trenton, New Jersey DEET was detected at levels of 45-340 ng/L (Alvarez et al., 2005). DEET was detected in 74.1% of the water samples analyzed at a maximum concentration of 1100 ng/L (Kolpin et al., 2002). In Table 1, DEET concentrations in Seyhan River compared with Ceyhan River (Turkey), Konya Basin Lakes (Turkey), Antalya Basin Lakes (Turkey), Ceyhan Basin Lakes (Turkey), Seyhan Dam Lake (Turkey), The Northern River (Germany), Po River (Italy), Schwarzbach, Modau, Winkelbach, and Weschnitz Rivers (Germany), Zhujiang and Shijing Rivers (China), Jakarda River (Indonesia), Han River (South Korea), 5 stream sites, Assunpink Creek and 139 stream sites (USA). Highest DEET concentrations in Turkey were detected for Seyhan River. Among the studies in the world, the highest DEET concentration was measured in Jakarda River, Indonesia (max: 24000 ng/L) (Dsikowitzky et al., 2014), followed by the results of Winkelbach, and Weschnitz Rivers (1292 ng/L) in Germany (Quednow & Püttmann, 2009), and the results of 139 stream waters (1100 ng/L) in the United States (Kolpin et al., 2002).

Risk assessment calculations were made for each station and season using DEET concentrations measured in the Seyhan River. According to the environmental risk assessment, DEET in the Seyhan River, pose no environmental risk (all measured RQ values were lower than 0.01, means no environmental risk) (Table 2). However, toxic effects may be higher when all pollutants behave together in natural environment.

As a result, DEET pesticide has been detected in all surface waters collected seasonally over a year from the Seyhan River, with concentrations varying between 18.55-334.71 ng/L. Aquatic organisms are thought to be affected as result of chronic exposure to DEET. The results obtained for Seyhan River were found lower than in USA (4700 ng L⁻¹ in surface waters) and Europe. According to the risk assessment calculations, it was determined that the current concentrations of DEET pesticide do not pose an environmental risk.

Table 2. Measured environmental concentrations (MECs) and risk quotient values (RQs) of DEET detected in Seyhan River.

		MECs	(ng/L)		RQs				
	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	
SEY-01	74.749	82.096	124.964	36.125	0.002	0.002	0.003	0.001	
SEY-02	59.99	75.886	116.257	24.185	0.001	0.002	0.002	0.001	
SEY-03	38.491	77.952	-	18.547	0.001	0.002	-	0.000	
SEY-04	33.388	52.065	108.089	21.457	0.001	0.001	0.002	0.000	
SEY-05	189.121	110.308	100.505	54.985	0.004	0.002	0.002	0.001	
SEY-06	97.414	87.849	334.713	36.898	0.002	0.002	0.007	0.001	
SEY-07	54.985	84.047	245.442	35.894	0.001	0.002	0.005	0.001	

CONCLUSION

This study was one of the first and most comprehensive studies in the region. The results obtained in autumn were found higher than in summer. It was unexpected, but it provides clue about the season that mosquitos are found more. The results obtained for Seyhan River were found lower than in USA and Europe. In future studies, bioaccumulation of DEET should be added to such research. Because DEET can accumulate in aquatic organisms and damage as a result of continuous exposure.

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