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ECOTOXICOLOGICAL ASSAYS OF DIETHYLTOLUAMIDE AND LEMONGRASS ESSENCIAL OIL IN IRRADIATED AND NON-IRRADIATED AQUATIC ORGANISMS

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ABSTRACT

Aquatic invertebrates can be potentially exposed to nonradioactive contaminants in conjunction with ionizing radiation, especially in highly industrialized areas surrounding nuclear facilities, where radionuclides can accidentally be discharged in the aquatic environment containing stable chemicals. The aquatic organisms have continually been exposed to chemical contaminants like personal care products (PCPs) which have been found in various environmental matrices and may cause adverse effects to aquatic life and human health as radioactive products. In this study was used C. silvestrii as bioindicator organism in chronic ecotoxicity assays with lemongrass essencial oil (LEO) and Diethyltoluamide (DEET), both are insect repellent. In addition to exposition of the compounds, the organisms were irradiated with gamma rays from Co-60 source. Thus, the aim of this study was to evaluate the possible synergistic effect of gamma radiation and mosquito repellent products in the reproduction of Ceriodaphnia silvestrii utilizing standardized ecotoxicological tests. The *C. silvestrii* inhibition concentration (IC25; 7 days) result after DEET exposition was 16.4 ± 1.4 mg L^{-1} and for LEO was 3.1 ± 1.4 mg L^{-1}. In the irradiated (25 Gy) *C. silvestrii* exposed to DEET and LEO, the concentration that inhibited reproduction was 16.1 ± 0.9 mg L^{-1} and 2.4 ± 0.3 mg L^{-1} respectively. The results showed that the reproduction of irradiated *C. silvestrii* was not significantly affected when compared with non-irradiated organisms when exposed to DEET or LEO.

Keywords: Ionizing radiation, Diethyltoluamide, Ceriodaphnia silvestrii, Lemongrass essential oil

1. INTRODUCTION

Radioactive material could be release to the environment by nuclear power industry, military establishments, research organizations, hospitals and general industry. Discharges of any significance should be subject to statutory control; authorized and monitored by some regulatory agencies as International Atomic Energy Agency (IAEA) [1].

The Fukushima Daiichi accident has raised public concern about health and environmental monitoring. The World Health Organization (WHO) reported that the contamination was reaching dozens of kilometers away from Fukushima Daiichi nuclear power plant [2]. The AIEA [1] and the United States Environmental Protection Agency (USEPA) [3] reported that since the occurrence of the accident were found radionuclides in some foods and soil samples, drinking water, rainwater, groundwater, seawater, marine sediment and aquatic organisms approximately the nuclear station.

Aquatic invertebrates potentially can be exposed to some contaminants in conjunction with ionizing radiation, especially in highly industrialized areas surrounding nuclear facilities, where radionuclides can accidentally reach the aquatic environment containing stable

chemicals. According to *Dallas et al.*, there are no laboratory studies investigating the effects of ionizing radiation on aquatic invertebrates with other known pollutants [4].

Once personal care products (PCPs) like soaps, lotions, toothpaste, fragrances, sunscreens, insect repellents etc. are widely used all over the world, are found in the aquatic environment unaltered and can cause adverse ecological or human health effects. Recent studies have indicated that many of them are environmentally persistent, bioactive, and have the potential for bioaccumulation [5].

In the last 20 years, insect-borne diseases have dramatically affected the health of the world population. The main strategy suggested for individual protection against bites of vectors is the use of repellents. The compound commonly known as "DEET" (*N*,*N*-diethyl-m-toluamide) is considered to be the most effective topical repellent currently available [6]. The U.S. Army developed DEET in 1946 for military personnel insect-infested areas use, and registered in 1957 by US for general public use [7].

The DEET has been detected in aquatic water samples around the world indicating that it is mobile and persistent and the DEET registration category does not require an ecological risk assessment, thus information on the ecological toxicity of DEET is sparse [8].

The frequent and repeated use of synthetic repellents has resulted in resistance, affection of the ecosystem and toxic effects on humans and other organisms. Therefore, there is a priority to find alternative insecticides, environmentally safe, biodegradable and specific against some insect. A large number of plant essential oils has been used against diverse insect pests. Unlike conventional pesticides, usually these natural products present less risk to humans and the environment. The most significant specie of lemongrass essential oil (LEO) is *Cymbopogon flexuosus* [9].

In the literature, there are no published data about natural repellent toxicity on aquatic organisms. Although there are many data on the acute toxicity of DEET, there is no knowledge of chronic toxicity studies on aquatic organisms [10].

The absence of data about dose limits and reference dose of radiation many authors have developed studies about irradiation of organisms to determine doses that cause interference in their life cycle.

The aim of this study was to evaluate possible synergic effect of DEET and lemongrass essential oil on reproduction of *C. silvestrii* (*Ceriodaphnia silvestrii*) previously irradiated with gamma radiation utilizing ecotoxicological assays.

2. METHODOLOGY

The organism used to test was *C. silvestrii*. The chemical compounds used as insect repellent were DEET from Sigma – Aldrich and lemongrass essential oil (LEO) from Ferquimica, São Paulo, Brazil and the ionizing radiation was gamma rays from Co-60 source.

The organisms were cultured in the Ecotoxicology Laboratory at Energy and Nuclear Research Institute, São Paulo, Brazil and they were maintained in natural freshwater as

culture medium under controlled conditions of temperature (25±2ºC), light and dark cycle (12:12h) and the culture medium was changed every day. The feeding was an algal suspension of *Pseudokirchneriella subcapitata* at the concentration of 10^5 cells mL^{-1} added to yeast and fish chow [11].

Natural water was collect from monitored area by CETESB, localized at Ribeirão do Piraí Reservoir, Salto, São Paulo, Brazil. The filtered water was maintained under aeration after the physical-chemical parameters fixed (pH 7.0, conductivity 120 µS cm^{-1} and hardness 44 mg L^{-1} of $CaCO_3$). The culture medium physical-chemical parameters were measure at the beginning and the end of the tests.

The test solutions were prepared as described:
i) DEET Stock solution (300 mg L^{-1}) in distilled water. From this stock solution, five concentrations were prepared: 3.7; 7.5; 15; 30 and 60 mg L^{-1} with fresh water.

ii) LEO solution (10^5 mg L^{-1}) in dimethyl sulfoxide. This solution diluted in distilled water to 100 mg L^{-1} and used as stock solution. From this stock solution were prepared the concentrations of 1; 2; 4; 8 and 16 mg L^{-1} with fresh water. The highest DMSO concentration used in each experiment was simultaneously test as the solvent control. This control and fresh water was performed under the same test conditions.

Ten replicates were used for each concentration and for water control.

Chronic ecotoxicity assays: Ten neonates (less than 24 h old) of *C. silvestrii* were individually seed in 10 glass beakers with 15 mL of the diluted test solution and stayed under the same conditions of laboratory culture. The test control was the neonate in fresh water in the same conditions of the assay. During 7 days of experiments, the daily number of released neonates for each female was register and these neonates were removed after counting.

Chronic ecotoxicity assays with irradiated organisms: About 80 neonates of *C. silvestrii* were placed in polystyrene tube containing freshwater and were *ex*posed to Co-60 gamma radiation from Gammacell 200 at 25 Gy dose and 1.48 to 1.50 kGy h^{-1} dose rates. After exposure to gamma radiation, one of test organisms was transferred to each glass beaker with 15 mL of the test solution (10 replicates and 5 concentrations). This experiment was carried out under the same conditions of ecotoxicity assays. During the assays, the daily number of released neonates was registered for each female. The radiation control was by the distribution of the organisms to fresh water instead test solution. Test endpoint, related to reproduction and mean number of neonates per female were evaluated.

The results were evaluated according to the linear interpolation statistical method for reproduction Inhibition Concentration (IC_{25}) calculation. IC_{25} is the test solution concentration that reduces 25 % of the *C. silvestrii* reproduction during 7 days. All assays were performed in triplicate.

3. RESULTS AND DISCUSSION

The results of the chronic ecotoxicity assays (n=3) of *C. silvestrii* organisms exposed to lemongrass essential oil and DEET are presented in the Table 1.

Table. 1. Results of *C. silvestrii* reproduction (mean of neonates per female) submitted to DEET and LEO

Lemongrass essential oil		Diethyltoluamide	
Concentration (mg L^{-1})	Reproduction	Concentration (mg L^{-1})	Reproduction
Control	18.6 ± 2.7	Control	19.1 ± 4.2
1.0	19.2 ± 3.4	3.75	21.0 ± 2.3
2.0	16.5 ± 4.3	7.5	18.3 ± 4
4.0	13.2 ± 5.2	15.0	17.0 ± 1.8
8.0	5.1 ± 3.4	30	6.2 ± 10.3
16.0	0.05 ± 0.1	60	1.4 ± 2.4

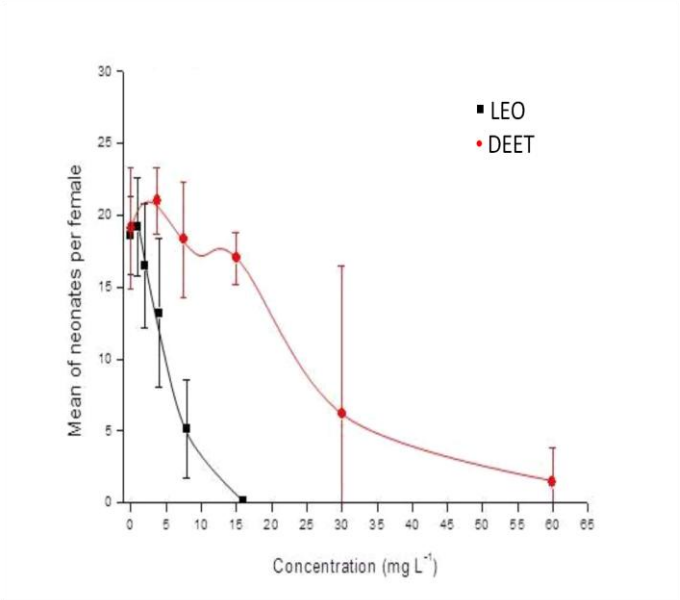

Figure 1. *C. silvestrii* reproduction (mean of neonates per female) in relation to LEO and DEET concentration

The reproduction Inhibition Concentration in *C. silvestrii* (IC_{25}; 7 days) showed by DEET was in the range of 15 mg L^{-1} to 17.8 mg L^{-1} with mean value of 16.4 ± 1.4 mg L^{-1} and for the LEO ranged from 1.7 mg L^{-1} to 4.5 mg L^{-1} with mean value of 3.1 ± 1.4 mg L^{-1}.

The tested LEO solvent control (DMSO) at the highest concentration showed no statistically significant difference (Student's *t*-test p = 0.05) compared with freshwater (control).
The essential oil showed greater toxicity than DEET in the reproduction of *C. silvestrii* in this assay but in the aquatic environment, this natural compound has physicochemical properties that facilitate its degradation as well as photodegradation and volatility [9].

It was expected greater toxicity of DEET due to its mobility and persistence in the aquatic environment, which explains the presence in environmental matrices from different countries. According to several authors the DEET concentrations about ng L^{-1} to µg L^{-1} have been

found in various environmental compartments as well as surface water, groundwater, seawater and sewage treatment plants (Table 2) [12-16].

Table 2. Concentration of DEET in different environmental matrices

Water type	Country	Max. conc. ($ng\ L^{-1}$)	Source
Drinking water	USA	0.07	[12]
WWTPs effluents	Europe	15800	[13]
Raw water	USA	<500	[14]
Wastewater	USA	410	[15]
Groundwater	England	300	[16]

WWTPs = Wastewater treatment plants

The DEET IC_{25} found in this study for the *C. silvestrii* was 16.4 ± 1.4 mg L^{-1} ($16.4x10^6$ ng L^{-1}) about 1,000 times higher than the maximum concentration presented in Europe WWTPs effluents [30]. The DEET concentration in the aquatic environment all over the world could not cause acute toxicological effect to aquatic organisms.

In the chronic ecotoxicity assays with irradiated organisms, the concentrations of LEO that inhibited the reproduction of *C. silvestrii* (IC_{25}; 7 days) irradiated at 25 Gy dose ranged from 2.1 mg L^{-1} to 2.6 mg L^{-1} with mean value of 2.4 ± 0.3 mg L^{-1}.

In the Table 3 and Fig. 2 are presented the results of the chronic ecotoxicity assays (n = 3) of *C. silvestrii* organisms unirradiated and irradiated when exposed to different LEO concentrations.

Table 3. Reproduction of *C. silvestrii* unirradiated and irradiated (25 Gy) and exposed to different LEO concentration

LEO Concentration ($mg\ L^{-1}$)	Reproduction (%) ± CV	
	Unirradiated	Irradiated
Control	100 ± 17.7	100 ± 11.7
1.0	96.7 ± 17.7	98.7 ± 11.0
2.0	82.0 ± 17.3	88.3 ± 12.7
4.0	82.0 ± 16.3	73.7 ± 17.7
8.0	28.3 ± 16.7	22.0 ± 18.7
16.0	0	2.3 ± 13.3

CV = Coefficient of variation

The exposure of organisms at mixture of pollutants may lead to a different biological response from the expected by the action of each contaminant alone, but analyzing the results presented in the Table 3 and Fig. 2, there was no statistically significant difference (Student's *t*-test p = 0.05). These results indicate that the dose of 25 Gy caused no significant effect on

reproduction of *C. silvestrii* when exposed at those different concentrations of essential oil in the assay.

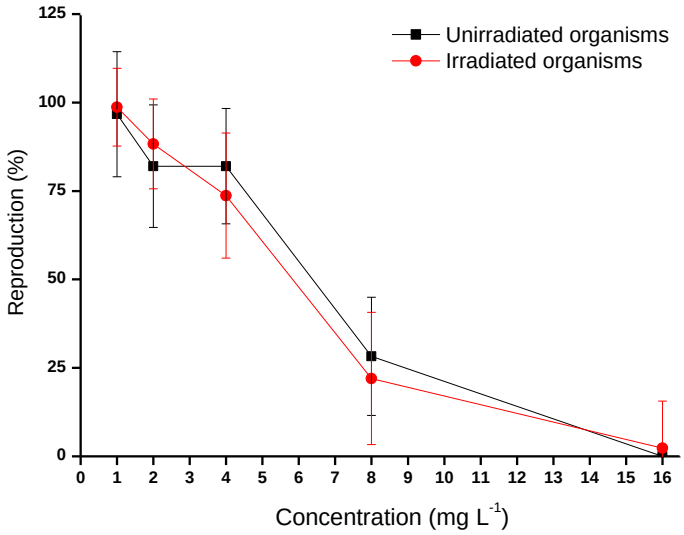

Figure 2. Reproduction assay curves of *C. silvestrii* unirradiated and irradiated and exposed to different concentrations of LEO

The results of the chronic ecotoxicity assays (n = 3) of *C. silvestrii* organisms unirradiated and irradiated and exposed at different DEET concentrations are presented in the Table 4 and Fig. 3.

Table 4. Reproduction of *C. silvestrii* unirradiated and irradiated (25 Gy) and exposed to different DEET concentration

DEET Concentration (mg L^{-1})	**Reproduction (%)**	
	Unirradiated	Irradiated
Control	100 ± 16.7	100 ± 13.3
3.7	99.7 ± 18.0	96.7 ± 14.7
7.5	98.7 ± 16.3	88.7 ± 12.0
15.0	96.0 ± 17.7	88.3 ± 18.3
30.0	0.7 ± 0.2	0
60.0	0	0

CV = Coefficient of variation

DEET showed 25 % reproduction Inhibition Concentration (IC_{25}; 7 days) in the irradiated organisms (25 Gy) at 15.4 mg L^{-1} to 17.1 mg L^{-1} with mean value of 16.1 ± 0.9 mg L^{-1}.

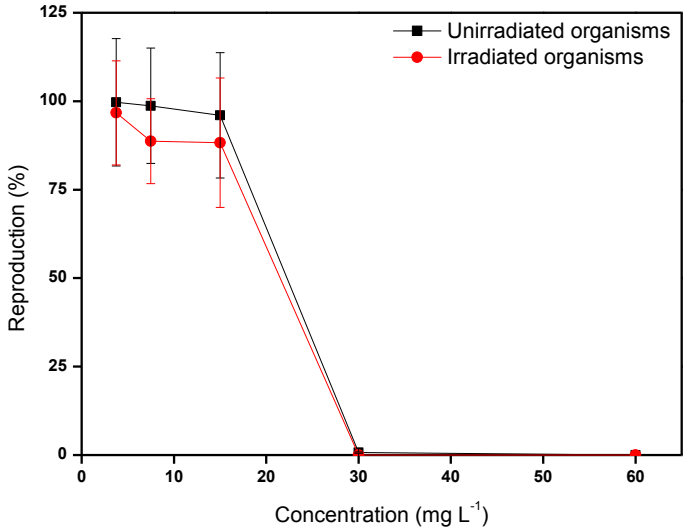

Figure 3. Results of the reproduction assay of *C. silvestrii* unirradiated and irradiated, exposed to different concentrations of DEET.

The reproduction rate of the irradiated organisms was more sensible in the first concentrations than to unirradiated organisms, but according to Student's *t*-test ($p = 0.05$) analysis, there was no statistically significant difference between the reproduction of the unirradiated and irradiated organisms.

It was expected greater toxicity of DEET in the *C. silvestrii* reproduction compared to LEO. The physicochemical properties of these chemicals compounds may have provide the difference in the toxicity to organisms.

4. CONCLUSIONS

The results found in this study were similar in the groups of irradiated and unirradiated organisms. There was no statistically significant difference in the tested groups meaning that there was no synergistic effect between the reproductions of irradiated and unirradiated organisms when exposed to the DEET and LEO.

Studies of the synergistic effect between ionizing radiation and chemicals have to be continued utilizing other different aquatic organisms to obtain results for possible comparison and verify suitable organism to this kind of ecotoxicological assay.

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