2015 International Nuclear Atlantic Conference - INAC 2015
São Paulo, SP, Brazil, October 4-9, 2015
ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR - ABEN
***ISBN:* 978-85-99141-06-9**

CITOTOXIC EFFECTS OF GAMMA RADIATION AND DEET ON *Perna perna* MUSSELS

Gisela A. Martini[1], Sizue O. Rogero[1], Marina V. Silva[1] and José Roberto Rogero[1]

[1] Instituto de Pesquisas Energéticas e Nucleares (IPEN / CNEN - SP)
Av. Professor Lineu Prestes 2242
05508-000 São Paulo, SP
gisela.martini@usp.br

ABSTRACT

Aiming at the protection of aquatic biota becomes desirable progress in identifying and quantifying the presence of chemical contaminants to an evaluation of its bioavailability and harmful effects to the aquatic ecosystem. Diethyltoluamide (DEET) is one of the most commonly used active ingredients in insect repellents and is considered an emergent contaminant. In addition the environment in regions near nuclear facilities (mostly coastal) and in regions with higher level of background radiation, many aquatic organisms could be exposed to radiation, becoming more susceptible to other contaminants. Therefore, the purpose of this study was the investigation of the biological effects of ionizing radiation in combination with the exposure of *Perna perna* mussels to DEET. Previously it was determined the doses of ^{60}Co gamma radiation (3 and 107 Gy) and the DEET concentrations (0.02 and 0.001mg L^{-1}) which causes cytotoxicity in lysosomes of *Perna perna* mussels hemocytes. The effects were evaluated by the neutral red retention time assay in irradiated and non-irradiated organisms. The assays were performed in 72 hours, with readings on each 24 hours. The endpoint was the lysosome membrane disruption causing the decrease of the neutral red retention time observed . The results showed that was no statistic difference of irradiated organisms with 3 Gy and exposed to different concentrations of DEET in comparison with non-irradiated organisms, the mean neutral red retention time in assays was around 40 minutes. The organisms that were exposed to DEET after 107 Gy irradiation dose showed cell lysis and neutral red retention time less than 30 minutes, this suggests that synergistic effect can be observed depending on the irradiated dose and the DEET concentration.

1. INTRODUCTION

Each year new chemicals are synthesized and most directly or indirectly affects ecosystems, the introduction of these compounds can cause changes in the environment, and can configure contamination events. Aiming at the protection of aquatic biota, becomes desirable progress in identifying and quantifying the presence of these contaminants to an evaluation of its bioavailability and harmful effects to the aquatic ecosystem. Among the worldwide compounds the DEET (*N,N-diethyl-m-toluamide*)is used in formulating insect repellents, and is commercially available for more than 50 years [1], has already been evidenced the presence of DEET in environmental matrices, showing that this compound is persistent feature [2]. DEET is one of the most commonly used active ingredients in insect repellents, and because of its effectiveness, it is produced in relatively large volumes and used globally [3]. European Union (EU) assessments conducted prior to 2010 classified DEET, which required the labeling of the active ingredient (but not products containing <25% DEET) as ‘‘harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment’’[4]. In recent years, considerable research has been conducted to define the

most important physicochemical properties and fate characteristics of DEET, as well as the toxicity to aquatic and terrestrial organisms [3]. In addition the environment in regions near nuclear facilities (mostly coastal) and in regions with higher level of background radiation, many aquatic organisms may be exposed to high levels of radiation, becoming more susceptible to other contaminants. Therefore, the purpose of this study was the investigation of the biological effects of ionizing radiation in combination of the exposure of DEET on marine aquatic biota. The aquatic environment is often the ultimate recipient of a wide range of contaminants including chemical and radioactive wastes, a large proportion of which could be potentially genotoxic and carcinogenic [5,6]. Therefore in our study, in order to simulate a scenario where radiation is a potential hazard to the environment, it was determined the dose of ^{60}Co gamma radiation and the DEET concentration that causes cytotoxicity in lysosomes of *Perna perna* mussel hemocytes. The effects were evaluated by the neutral red retention time assay on irradiated and non-irradiated organisms.

2. METODOLOGY

2.1. Organism Test and Sea Water

The organisms as well as the water used in the cytotoxicity tests were collected from an unpolluted area at Cocanha Beach – Caraguatatuba, SP. The water was packed in barrels of 50L, transported to the Ecotoxicology Laboratory at IPEN, USP and maintained at a temperature of 22±2°C. The test organisms were *Perna perna* mussels, with 4-5cm long, and were kept at laboratory for 1 to 3 days in sea water tank for the process of acclimatization. Marine bivalves have been employed as biomonitor in marine pollution assessments all around the world because of their sessile habits, broad distribution, and economic importance, which make them suitable species to be employed in ecotoxicological studies [7].

2.2. Neutral Red Retention Time Assay (NRRA)

The mussel hemolymph was extracted from the posterior adductor muscle. An aliquot of 40µL of sample containing hemocytes was placed carefully on top of the histological slide prepared in advance with poly-L-lysine covering. Slides were left on a rack in a light-proof humidity chamber during the assay. After 15min, the excess solution was carefully tipped off and 40µL of neutral red working solution were added. Slides were thereafter examined systematically under an optical microscope at 15min intervals to determine the evidence of dye loss from the lysosomes to the cytosol of the hemocytes. It was observed a decrease in the neutral red retention time in comparison to the control. The citotoxicity tests with haemocytes of mussels has been an important source to knowing about quality of the environment. The bioassays using mussels cells provide a significant result on biomonitoring programs. Further, the mussels and other aquatic invertebrates, are frequently used as model organisms for toxicological tests [8]. The neutral red retention assay was used to verify the ionizing radiation and DEET exposure effect on the lysosomal membrane of hemocytes of *Perna perna* mussels, and followed the protocol proposed by Lowe and Fossato [9]. The test is performed by the evaluation of lysosome membrane integrity in the hemocytes. NRRA was evaluated in non-irradiated organisms exposed to different concentrations of DEET in order to evaluated the difference that radiation causes on lysosomal membrane mussels. And for

irradiated tests the organisms were irradiated with 3, and 107 Gy and the results were obtained 24, 48 and 72h after irradiation. The test endpoint was achieved when was observed the evident dye loss in at least 50% of the hemocytes. The mussels (n=15) were placed in polypropylene bottles containing 750 mL marine water and were irradiated by ^{60}Co gamma rays at 3 and 107 Gy. The NRRA technique was applied after 24, 48 and 72h of radiation exposure.

3. RESULTS AND DISCUSSION

In non-irradiated mussels was observed statistic significance at concentrations of 0.001 and 0.02 mg L-1 for all exposure times (24, 48 and 72 h) comparing to the control (Fig. 1a). In this assay the NOEC (no observed effect level) was 0.0001 mg L^{-1} and the 0.001 mg L^{-1} the LOEC (lowest observed effect level). In mussels irradiated with 3 Gy of ^{60}Co it was observed that the retention time was not significantly different from trials in which the organisms were not irradiated (Fig. 1b), suggesting that this dose has not been sufficient to promote damage observable within the period in which the experiment was conducted. The dose of 107 Gy caused some adverse effects to organisms (Fig. 1c). It was observed a significant reduction in the number of cells compared with the other doses that were tested. In addition, cell lysis occurs in most of analyzed slides (>80%). There was also a decrease in retention time of dyestuff in 48 hours for concentrations of 0.001 and 0.02 mg L^{-1} with increase in 72 hours.
Table 1 summarize the average time (minutes) of the NRRA for non-irradiated organisms, and organisms that were exposed to 3 Gy and 107 Gy, respectively. Also, mortality occurred in some organisms that were irradiated with 107 Gy of ^{60}Co and exposed to 0.001 and 0.02 mg L^{-1} of DEET, we also observed an intense release of gametes in mussels those received this dose of radiation. The mussels that were exposed to 107 Gy ^{60}Co gamma radiation had a shorter retention time of neutral red dye in the lysosomes of hemocytes, in this case it is possible that the radiation has caused a potentiation effect with DEET, suggesting that the toxicity increases when organisms are irradiated and exposed to the compound. In recent years, the contamination of DEET has been widely disseminated, and the compound has been detected in several aquatic environmental matrices, including rivers, groundwater, ocean, wastewater and even in water treated by conventional water treatment systems [10, 11, 12].

Table 1 – Average time (minutes) of NRRA

DEET (mg L^{-1})	0 Gy			3 Gy			107 Gy		
	24h	48h	72h	24 h	48 h	72 h	24 h	48 h	72 h
Control	66.6	63	64.8	64	61	50	64	57	51
Solvent	64.8	60	64.8	62	58	49	57	56	50
0.0001	55	55	47	54	50	40	53	49	43
0.001	35.2	36	24.7	28	28	19	29	11	25.7
0.02	25.8	27	16.2	19	22	15	18	8	16

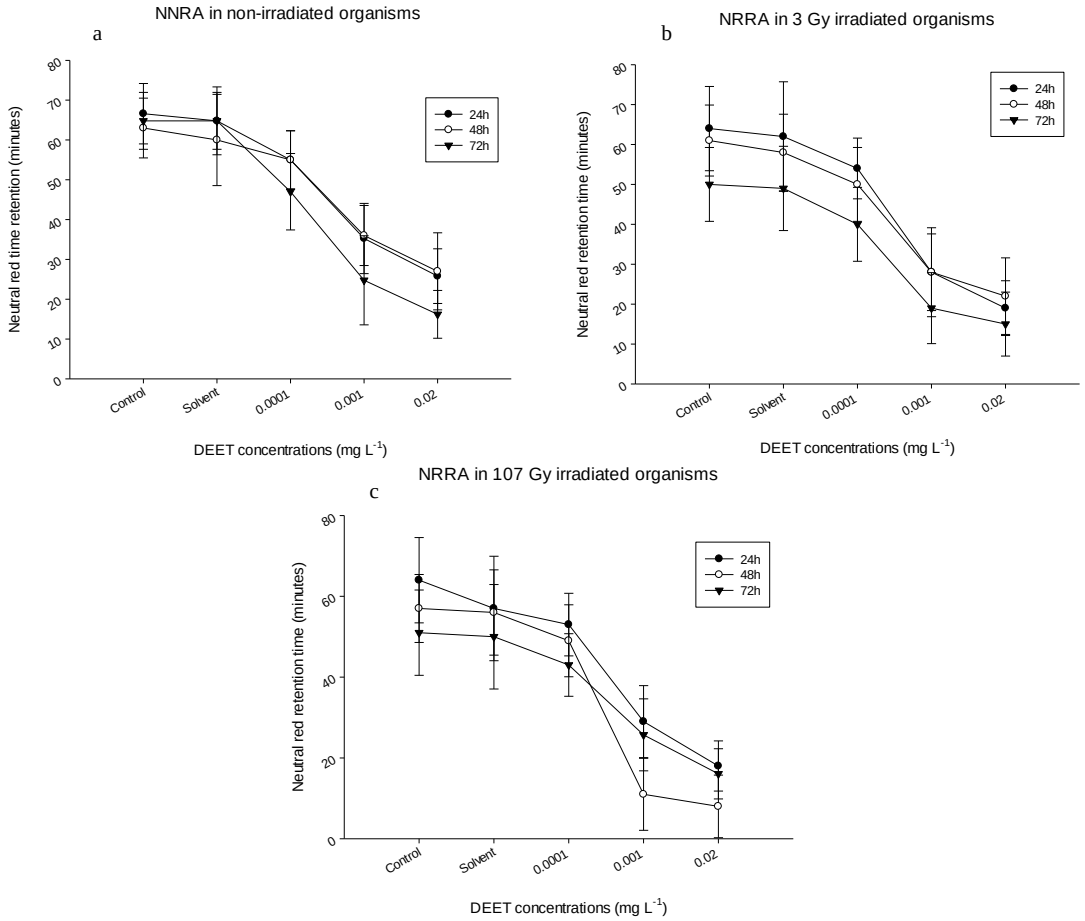

Figure 1 – Neutral red retention time for non-irradiated organisms exposed to DEET concentrations in 3 times of exposure: (a) non-irradiated organisms, (b) 3 Gy ^{60}Co irradiated organisms, (c) 107 Gy ^{60}Co irradiated organisms

4. CONCLUSION

In the present study the radiation served as a stressor to the mussels, causing a decrease in the retention time of neutral red dye when compared with non-irradiated organisms, but not enough for there to be a synergism between some concentrations of the compound (0.002, 0.001 and 0.0001 mg L^{-1}) and the action caused by a few doses of ionizing radiation (3 and 107 Gy). With several studies identifying concentrations of DEET in the order of µg and ng L^{-1} in various matrices show the importance of evaluating the toxicity of this compound in non-target organisms, adverse effects caused on the biota and its possible potential for synergistic effect in combination with other substances. The present study found cytotoxic effect of DEET for *Perna perna* mussels at concentrations above 0.1 µg L^{-1} a value very close to the concentrations identified in the environment, which highlights the need for more studies on the toxicity, persistence and degradation of this compound in the aquatic environment and in biota.

ACKNOWLEDGMENTS

The main author would like to thank CNEN for financial support. We are grateful to Elizabeth S. R. Somessari, Carlos G. Silveira and Hélio A. Paes for helping the irradiation of samples at Radiation Technology Center - IPEN.

REFERENCES

[1] USEPA - United States Environmental Protection Agency. Reregistration Eligibility Decision (RED) DEET. Prevention, pesticides and toxic substances., p.134. 1998

[2] Singh, S. P. A. A.; Chaudhary, A.; Khan, S.; Willett, K.L.; Gardinali, P.R. Occurrence and distribution of steroids, hormones and selected pharmaceuticals in South Florida coastal environments. Ecotoxicology., n. 19, p. 338-350., 2010.

[3] Weeks, J. A. G., P. D.; Nikiforov, A. I., Assessment of the Environmental Fate and Ecotoxicity of N,N-Diethyl-m-toluamide (DEET). Integrated Environmental Assessment and Management. **,** v. **8,** n. 1, p. 120-134., 2011.

[4] EC: European Commission. 2008. EU Regulation No. 1272/2008, Table 3.2. Official Journal of the European Union. 31 December 2008.

[5] Moore, M.N., Depledge, M.H., Readman, J.W., Paul Leonard, D.R., An integratedbiomarker based strategy for ecotoxicological evaluation of risk in environmen-tal management. Mutation Research, Fundamental and Molecular Mechanismsof Mutagenesis 552 (1–2), 247 268. 2004.

[6] Jha, A.N., Cheung, V.V., Foulkes, M.E., Hill, S.J., Depledge, M.H., Detection ofgenotoxins in the marine environment: adoption and evaluation of an integratedapproach using the embryo larval stages of the marine mussel, Mytilus edulis.Mutation Research 464, 213–228. 2000.

[7] Farrington, J.W., Tripp, B.W., International Mussel Watch Project: Initial Implementation Phase. Final Report. International Mussel Watch Committee. National Centers for Coastal Ocean Science, Silver Spring, MD, USA. 1995.

[8] Depledge, M. H. Nondestructive Biomarkers in Vertebrates (M. C. Fossi & C. Leonzio, eds.) Boca Raton, FL, USA. : Lewis Publishers, 271-285. 1993.

[9] Lowe, D.M. and V.U. Fossato, The influence of environmental contaminants on lysosomal activity in the digestive cells of mussels (Mytilus galloprovincialis) from the Venice Lagoon. Aquatic Toxicology, **48**(2–3): p. 75-85. 2000.

[10] Sandstrom, M. W. K., D.W.; Thurman, E.M.; Zaugg, S.D. Widespread detection of N,N-diethyl-m-toluamide in U.S. Streams: Comparison with concentrations of pesticides, personal care products, and other organic wastewater compounds. Environ. Toxicol. Chem., v. **24**, p. 1029–1034., 2005.

[11] Tay, K. S. R., N.A.; Abas, M.R. Degradation of DEET by ozonation in aqueous solution. Chemosphere., v. **76**, p. 1296-302., 2009.

[12] Calza, P. M., C.; Raso, E.; Giancotti, V.; Minero, C. N,N-diethyl-m-toluamide transformation in river waters. Sci. Total Environ., v. **409**, p. 3894-3901., 2011.