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European Journal of Experimental Biology, 2013, 3(3):701-705

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Acute toxic effect of qua iboe light crude oil on tadpoles of frog, *Rana temporaria* from Eket, Akwa Ibom State, Nigeria

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ABSTRACT

The acute toxicity of Qua Iboe Light Crude oil on tadpole of frog, Rana temporaria was studied. A total of ten (10) tadpoles each were grouped into seven (7) test vessels and held for 24, 48, 72 and 96 hours in six (6) different concentrations of Qua Iboe Light Crude oil (0.313, 0.625, 1.25, 2.50, 5.00 and 10ppm) and was compared with control (0.00ppm). Toxicity measurements based on 96 hour-LC_{50} showed that the LC_{50} value with 95% confidence limits of Qua Iboe Light Crude oil on Rana temporaria is (1.637±0.105). The manifestation and survival time was found to be concentration dependent decreasing with increase in concentration. All the time-effect relationship was found to be concentration dependent. The concentration mortality relationship showed significantly ($p<0.05$) high coefficient of determination, R^2 of 0.96. Toxicant exposure induced behavioural changes such as abnormal and uncoordinated swimming movement. The relatively low LC_{50} determined from this work indicates that Qua Iboe Light Crude oil can have both lethal and Sub lethal effects on wide range of Organisms including frog and may pose some significant problems to the environment especially the aquatic environment. The highly active nature of Qua Iboe Light Crude oil and implication on aquatic life is discussed in this text.

Keywords: Acute Toxicity, Qua Iboe Light Crude Oil, *Rana temporaria*, Eket, Nigeria

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INTRODUCTION

The degree of hazardous effect of crude oil products is dependent on the degree of their concentration, chemical components and solubility in water. These products have been recognized as a potential environmental contaminant shortly after the beginning of twentieth century [Albers, 1995]. Researches carried out across the globe on the toxicity of Bonny Light crude oil (BLCO), Premium motor spirit (PMS), Qua Iboe Light crude oil (QILCO) and Dual purpose kerosene (DPK) on aquatic organisms have revealed their lethal, acute, short and long term effect [Baker, 1971]. It has been found that as little as 0.1ppm of oil can seriously affect fish, amphibians, crustaceans and plankton. “Oils” float and coats things and has the potential to kill quickly by coating aquatic lives, interfering with gas exchange necessary for life. When it sediments at the bottom, the contaminating impact has longer effect and benthic organisms become particularly susceptible [Baker, 1971].

Rana temporaria breed in shallow, still, fresh water such as ponds, with breeding commencing in March. The adults congregate in the ponds, where the males compete for females. The courtship ritual involves croaking, and a successful male grasps the female under the forelegs. During the mating season the males can be recognised by a darkened swelling, the *nuptial pad* on their 'thumbs'. The actual spawning typically takes place at night but the courtship rituals are also at daytime. The females, which are generally larger than the males, lay between 1,000 and 2,000 eggs [Eccleston, 2008]. Outside the breeding season, *Rana temporaria* live a solitary life in damp places near ponds or marshes or in long grass [Clive, 2006].

Adults *Rana temporaria* feed on any invertebrate of a suitable size, although they do not feed at all during the breeding season [Kuzmin, 2008]. Favourite foods include insects (especially flies), snails, slugs and worms. The frogs catch their prey on their long, sticky tongues. Their feeding habits change significantly throughout their lives; whereas older frogs will feed only on land, younger frogs will also feed in the water. Tadpoles are mostly herbivores, feeding on algae, detritus and some plants, although they will also eat other animals in small amounts [Kuzmin, 2008].

The concentrations of toxicants in the environment vary with duration of exposure, volume of discharge, as well as the nature of hydrocarbon. The acute toxicity of crude oil to aquatic organism like toads and frogs are assessed by the measurement of LC_{50} value, that is, the concentration of hydrocarbon mixture or specific hydrocarbon that results in 50% mortality of test organism during the designated exposure period, usually 72 or 96 hours.

In Nigerian waters, cases of oil spillage have been recorded between 1958 to date releasing about 2.4 million barrels of crude oil into coastal aquatic environment. Of importance are the Exxon Mobil spills, Idoho disaster, 1998, Ogoni oil spills disasters, 1958-2005 [Udo, 2007]. This poses a great risk to aquatic organisms like periwinkle, which is a source of protein for the coastal dwellers in Nigeria. Doertter [1992] reports that oil spills cause substantial mortality among fish, amphibians and invertebrates. Other effects include changes in species composition, low abundance, loss of species and tainting [Windows *et al* 1982]

This study therefore, concurrently evaluates the effects of Qua Iboe Light Crude Oil, at acute lethal doses on survival, morphology and behaviour of the tadpoles' frog *Rana temporaria* from Eket Local Government Area, Akwa Ibom State, Nigeria. This study is designed to identify the acute toxic effects of Qua Iboe Light Crude Oil widely used in Nigeria, and to contribute to the knowledge of the effects of this chemical substance on amphibian.

MATERIALS AND METHODS

Description of Study Area:- Eket Local Government Area is located in Akwa Ibom State Nigeria, West Africa. It lies Northwards between latitudes $4^0$33' and $4^0$45' and Eastwards between longitudes $7^0$52' and $5^0$02'. Eket is bounded on the North by Nsit Ubium Local Government Area, on the East by Esit Eket Local Government Area, on the west by Onna Local Government Area and on the South by Ibeno Local Government Area/ Bright of Bonny. The population is between 20,000 and 50,000. There is however rainfall throughout the year. Temperature rarely falls below 19^0C and averages 30^0C all year round. Seasonal variation occurs between the hottest month (February) and the Coolest Month (August). Relative humidity is usually high, between 80 and 100 percent with the air often saturated with water vapour resulting in precipitation in the morning during the rainy season [TCAW, 2007]. Most of the original vegetation in the study area has been replaced as a result of Agricultural, Industrial and residential activities. Human activities in the area include farming, hunting and sand mining.

Collection and Transportation of Crude Oil (Qua Iboe Light):- Qua Iboe Light Crude Oil was obtained from the Oil Company (Mobil Producing Unlimited), Eket, Akwa Ibom State in airtight plastic cans and transported to the research laboratory of the Institude of oceanography (IOC), University of Calabar, for subsequent use.

Collection and Transportation of Test Organism: - Six sexually mature adult frog, three males with pronounced vocal sac and a dark bluish-black nuptial pads, (swellings) on their first fingers as their secondary sexual characters and three females (larger in size than males and with orange underside as their secondary characters) of *Rana temporaria* were collected with a long handled scope net from stagnant pool in Eket, Akwa Ibom State. They were transported in plastic buckets in the late hours of the evening between 5-6pm to reduce heat shock during transportation to the research laboratory of the Institute of oceanography (IOC), University of Calabar, where they were cultured till the eggs were laid.

Acclimatization and Maintenance of Study Organism: - In laboratory, the frog tadpoles *Rana temporaria* were kept in two aquaria (30x60x30cm) for fourteen days in order to get them acclimatized to the prevailing laboratory condition using water that were obtained from the original habitat of the frogs, dead tree branches and cork floaters so as to create an aquatic/quasi terrestrial environment (mini-island) for these animals. The frog tadpoles were fed daily with dried ground maize powder, groundnut cake and termites.

Acute Toxicity Test: - Acute toxicity test were performed according to United State Environmental Protection Agency (USEPA) procedure for the static non-renewal test. Preliminary screening was carried out to determine the appropriate concentration range for the test chemical. The concentration was spaced at approximately logarithmic intervals and were arranged to allow for complete mortality within 24 hours at the highest concentration and no mortality within the 96hour test period at the lowest concentration, with partial mortality in at least two intermediate

concentrations. This method facilitates probit transformation of mortality data. The tests consisted of a control and at least six concentration groups, with ten frog tadpoles in each concentration solution. An average of ten tadpoles of *Rana temporaria* were siphoned (using 10.0mm rubber siphon) from the hatching tank into 54-1 glass aquarium each containing 1.0 litre of 0.00, 0.313, 0.625, 1.25, 2.50 and 10.00ppm of Qua Iboe Light Crude Oil respectively. The frog tadpole's survival was measured over a four day period after 24, 48, 72 and 96 hours exposure period in a modified flow-through system. Dead tadpoles and other debris were siphoned out of the test tanks every 12 hours. The median lethal concentration, 96 hours LC_{50} were computed using probit method of Finney, [1971]. Time – effect relationship such as manifestation time (the interval between exposure of the tadpole to the toxicant and the appearance of reaction), overturning time (the time between the introduction of the toxicant and the loss of equilibrium or balance by the tadpole) and survival time (the time between the introduction of the toxicant and the occurrence of mortality in a particular concentration) were observed in each concentration of the toxicant.

Statistical Analysis: - The mortality data were subjected to probit transformation using statistical software SPSS (Statistical package for social science, version 18.0). Regression analysis was performed using Microsoft excel and the LC_{50} values (the concentrations of test substances which were lethal to 50% of the tadpoles) were also computed. The 95% confidence limit was also being fixed (Mather, 1973; Finney, 1971). The significance of the slope was tested using Chi-square.

RESULTS

Manifestation time: - Manifestation time of the toxicity of Qua Iboe Light Crude Oil was demonstrated by response of the tadpoles in various concentrations of the toxicant as shown in Table 1. At this time the tadpoles showed weak movement after three hour (3hrs) of exposure in the highest concentration of the toxicant (5ppm) while the last time of effect was noted in the lowest concentration of toxicant (0.313ppm) at the 72 hours, the 3rd day of experiment.

Overturning time: - The overturning time was observed in various concentrations such as 5.00, 2.50, 1.25 and 0.625ppm of Qua Iboe Light Crude Oil respectively (Table 1). At this time the tadpole showed sign of abnormal and uncoordinated swimming (i.e. swimming with tail up and head down) as a result of equilibrium. The overturning time varied with time in each concentration with exception of the least concentration (0.313ppm) which showed no sign of overturning till the 96 hours when the experiment was over.

Survival time: - Survival time of the tadpoles of *Rana temporaria* exposed to toxicity of Qua Iboe Light Crude Oil was observed in various concentration of the toxicant (Table 1). Observation shows that death first occurred in the highest concentration (5.00ppm) at about six hours of the experiment while the last survival time was observed in the least concentration (0.313ppm) at about 96 hours, the last day of the experiment.

Table 1: Time-Effect Relationship Data for *Rana temporaria* tadpoles Exposed to Qua Iboe Light Crude Oil

Concentration (ppm)	Manifestation time (Hrs)	Over-turning time (Hrs)	Survival-time (Hrs)
0.313	72	-	96
0.625	48	24	48
1.25	12	12	24
2.50	9	6	12
5.00	3	3	6

Probit Analysis Result: - Table 2, 3 and 4 shows the results of probit analysis performed on mortality data. The trend in mortality data of tadpoles indicate that tadpole's mortality increased with increasing concentration of Qua Iboe Light Crude oil (Table 2). Probit transformation of mortality-log concentration relationship determined to be linear by regression analysis (Table 4). A log concentration probit regression analysis was highly significant ($p<0.05$) yield a co-efficient of determination, r^2 of 0.96 (Table 4), and the LC_{50} with 95% confidence limit was calculated to be 1.64±0.105 (Table 3).

Table 2: Probit Analysis of Mortality Data of *Rana temporaria* tadpoles exposed to concentration of Qua Iboe Light Crude Oil

Concentration (x 10) ppm	Log Concentration x	n	r	P	Empirical probit	Y	y	w	Calculated/Y
3.13	0.495	10	0	0.0	0	3.80	3.207	0.370	2.199
6.25	0.795	10	2	0.2	4.16	4.35	4.177	0.557	3.370
12.5	1.096	10	4	0.4	4.75	4.85	4.747	0.634	4.543
25.0	1.397	10	7	0.7	5.52	5.38	5.521	0.600	5.716
50.0	1.699	10	8	0.8	5.84	5.90	5.840	0.471	6.890
100.0	2.000	10	10	1.0	0	6.40	6.039	0.301	8.063

N= Number of tadpoles tested at each concentration, r = Number of tadpoles, P= response rate, r/n, Y= Expected probit from visual regression line, y = working probit, W= weighting coefficient, Calculated Y = Probit values from filtered regression line empirical probit from Fisher and Yates table.

Table 3: LC_{50} with 95% confidence limits of *Rana temporaria* exposed to concentration of Qua Iboe Light Crude Oil

LC_{50}±95%C.L (ppm)	Lower Limit	Upper Limit
1.637±0.105	1.532	1.742

Table 4: Result of regression analysis of Log concentration Probit (Mortality) relationship of *Rana temporaria* are exposed to Qua Iboe Light Crude Oil

Conc. (Log Units)	Responses rate, p	Equation Y= bX+a	Coefficient of determination, R^2	Significance Level , α
0.495	0.0	Y=3.89X+0.267	0.96	HS P<0.05
0.795	0.2			
1.096	0.4			
1.398	0.7			
1.699	0.8			
2.000	1.0			

DISCUSSION

One of the major problems of the Niger delta region of Nigeria is contamination of water and aquatic life by Crude oil. This contamination may not necessarily lead to outright mortality but may have significant effect, which can lead to physiological stress and dysfunction in animals [Oros and Werner, 2005].

Acute toxicity measures the damage potential of a substance when organisms are exposed to it in a short space of time (usually within 24 to 96hours). The result of the present study showed that the LC_{50} values of Qua Iboe Light Crude Oil exposed to *Rana temporaria* was different from the values reported by other researchers. [Ezemonye and Isioma, 2010] used a flow through system to test the acute toxic effect of bonny light crude oil on adult *Bufo regularis*. They reported that the 96hrs LC_{50} values of bonny light crude oil for *Bufo regularis* was 3.35±0.02 which is higher than 1.637±0.105 observed in the study. The differences in LC_{50} values of *Bufo regularis* and *Rana temporaria* may be based on different parameters which can affect the LC_{50} values and not necessarily depend on temperature. The parameters which affect the LC_{50} values may be due to variety of species, age, habitat, duration of exposure, bioassay system, type of crude oil used in the test, and the analytical methods applied in the assay of the exposure of crude oil.

Manifestation time, overturning time and survival time of toxicity of Qua Iboe crude oil exposure to *Rana temporaria* tadpoles were observed to be concentration dependent, decreasing with increase in concentration. Shortly after the introduction of tadpoles into the test vessels a condition developed in the tadpole. The tadpole showed signs of restlessness and active swimming. The tadpoles swim faster than normal and resting at one minute interval. The initial excitation and fast erratic swimming displayed by tadpole in the test vessels was accompanied by increase in the general weakness and most probably available of the tadpole following increase in ventilation aimed at compensating for reduced ability for cutaneous gaseous exchange. Hence, nostril seems to be the highest at the point overturning where accumulation of the toxicants in the blood exceeds the level the tadpole can tolerate and still maintain co-ordination and swimming balance. Similar finding was observed by [Oros and Werner, 2005].

Dede and Kaglo [2001] reported that the dissolved oxygen tension in aquatic organisms in water contaminated with organic pollutants has been shown to be due to the diversion of the dissolved oxygen meant for respiration to the oxidation of organic pollutant. The extent of depletion of oxygen in water is often a function of the concentration of the organic pollutants in it [Horsfall and Spiff, 1998]. This report is in line with the present study. The oxygen stress encountered by the tadpole, which is responsible for the respiratory distress and death, was due to their inability to withstand the oxygen depletion of the water induced by the organic compounds in the Qua Iboe crude oil. Similar oxygen stress imparted by this toxicant had been studied in the *Bufo regularis* [Igloh *et al* 2001].

Exposure of *Rana temporaria* to Qua Iboe Light crude oil showed mortality even at low concentration, as low as 0.313ppm while other researchers reported that exposure of *Bufo regularis* to water soluble fraction of crude oil, showed mortality at low concentration, even as low as 1.6ppm [Igloh *et al* 2001]. This study reveal that Qua Iboe Light Crude Oil is more toxic than water soluble fraction and that *Rana temporaria* tadpole are very sensitive to Qua Iboe crude oil.

Fading of the skin colour of tadpole exposed to toxicants as observed in the study has been reported in other researches [Ezemonye and Tongo, 2009], this could however be related to the observed loss of mucus membrane from skin which is due to the scratching effect of the toxicants [Ezemonye and Tango, 2009].

The general weakness, which follows the short burst of activities, could probably be attributed to the suppression of breathing by the toxicant hypoxia. The effects were more pronounced in higher concentrations of the toxicant. Toxic effect may also result from disruption of the mitochondria membrane and inhibition of energy metabolism [Berrill *et al* 1998].

The assimilation of Qua Iboe Crude oil by tadpole from water is directly passive. The nostrils and the surrounding are the primary site of absorption. Passive uptake of Qua Iboe Light Crude oil continues until an equilibrium level is attained. This level depends on the concentration of Qua Iboe Crude oil spilling the surrounding water, the composition of the Qua Iboe Light Crude oil affect the physiological condition of individual tadpoles.

CONCLUSION

The result of the experiment indicates that Qua Iboe Light Crude oil is quite toxic to *Rana temporaria* tadpoles even at low concentrations. Therefore it could be recommended that the level of this toxicant in aquatic environment should not exceed the 10% of their 96 hours LC_{50}. This 10% LC_{50} value should correspond to the maximum allowable toxicant concentration (MATC) [APHA, 1998] used in setting environmental quality standards for chemicals and effluents. Qua Iboe Light Crude oil therefore should definitely no longer be disposed into water streams or landscape, not even at sub-lethal concentrations; because of the inherent toxicity of Qua Iboe Light Crude oil and the associated danger of bioaccumulation.

The result generated from this study is suggestive of the fact that Qua Iboe Light Crude oil is toxic which causes loss of co-ordination, equilibrium and respiratory paralysis to *Rana temporaria* tadpoles exposed to it and may finally result in death. Thus it could be concluded that Qua Iboe Light crude oil has serious consequences on the tadpole of frog, *Rana temporaria*.

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