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Modelling the Response of Catfish to Diesel and Water: The Probit Analysis Approach

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Abstract
This study investigated the histopathology of *Clariasgariepinus* after exposure to sublethal concentrations of water soluble fractions of diesel for a period of 30 days. The toxicological evaluations of water soluble fraction of diesel were carried out against juvenile stages of the African cat fish (*Clariasgariepinus*). On the basis of 96hr ED50 values, water soluble fraction of diesel was more toxic to the juvenile of *Clariasgariepinus* than the 24hr ED50, 48hr ED50 and 72hr ED50 values. The computed 96hr values were 8.58 ml/l, while the computed 24hr, 48hr and 72hr values were 2.45ml/l, 3.46ml/l and 5.67ml/l respectively. For 24hr (Figure 1), 48hr (fig 2), 72hr (fig3) and 96hr (fig 4). The graphs plotted indicates that the higher the concentration the higher the mortality experienced by the catfish (*Clariasgariepinus*). The chi-square results revealed that the Probit model adequately fit the data.

Keywords: Analysis, Approach, Catfish, Diesel, Modelling, Probit, Response, Water

Introduction
The idea of Probit analysis was originally published in *Science* by Chester Ittner Bliss in 1934. He worked as an entomologist for the Connecticut agricultural experiment station and was primarily concerned with finding an effective pesticide to control insects that fed on grape leaves (Greenberg 1980). By plotting the response of the insects to various concentrations of pesticides, he could visually see that each pesticide affected the insects at different concentrations, i.e. one was more effective than the other. However, he didn't have a statistically sound method to compare this difference. The most logical approach would be to fit a regression of the response versus the concentration, or dose and compare between the different pesticides. Yet, the relationship of response to dose was sigmoid in nature and at the time regression was only used on linear data.

Therefore, Bliss developed the idea of transforming the sigmoid dose-response curve to a straight line. In 1952, a professor of statistics at the University of Edinburgh by the name of David Finney took Bliss' idea and wrote a book called *Probit Analysis* (Finney 1952). Today, probit analysis is still the preferred statistical method in understanding dose-response relationships.

One type of assay which has been found valuable on many different fields, but especially in toxically studies is that dependent upon the quantal, on all or nothing, response. Though quantitative measurement of a response is always to be preferred when available. There are certain responses which permit of no graduation and which can only be expressed as occurring or not occurring. The most obvious of this kind of response is death. Although workers with insects have often found difficulty in deciding precisely when an insect is dead, in many investigations the only practical interest lies in whether or not it has reached a degree of inactivity such as is thought certain to be followed by early death. In fungicidal investigations, failure of a spore to germinate is a quantal response of similar importance. In studies of drug potency, response may be cure of some particular morbid condition. No possibility of partial cure being under considerations. The earlier attempts were made to characterize the effectiveness of a stimulus in relation to a quantal response referred to the minimal effective dose, for a more restricted class of stimuli, the minimal lethal dose, terms,

which failed to take account of the variation in tolerance within a population. The logical weakness of such concept is the assumption that there is a dose for any given chemical, which is only just sufficient to kill all or most of the animals of a given species, and that doses a bit lesser would not kill any animal of that species. It might be thought that the minimal lethal dose of a poison could instead be defined as the dose just sufficient to kill a member of the species with the least possible tolerance, and also a maximal non-lethal dose as the dose which will just fail to kill the most resistant member. Some doses are so low that no test subject will succumb to them and others so high as to prove fatal at all, but considerable difficulties attend determination of the end points of these ranges. The problem is, in fact, that of determining the dose at which the doses response curve for the whole population needs the 0% or 100% levels of kill and even a very large experiment could scarcely estimate these points with any accuracy. The median lethal dose, or as a more general term to include response other than death the median effective dose. This is the dose that will produce a response in half of the population. The median effective dose is commonly referred to as the ED50. The more restricted concept of median lethal dose as the LD50. The ED50 alternatively be regarded as the median of the tolerance distribution. That is to say the level of tolerance such that exactly half the subject lie on either side of it. This study is concerned with the statistical techniques needed in the analysis of quantal response data.

Fish has become increasingly important in the Nigerian diet since there is an increased awareness that regular red meat intake in adults above 40 years of age is not healthy. It is often imported into Nigeria and in resent time, has gained good consumer acceptance because of its economic availability. Nonetheless, fish processing methods, brings it in contact with water, smoke and high temperatures, which may interfere with the nutrients and are potential sources of Reactive Dicarbonyl Compounds (RDCs) and Polyaromatic Hydrocarbons (PAH) generation. Although these RDCs are responsible for the characteristic aromas of fish, high RDC levels are caused by thermal processes, moisture content and water activity.

In aquatic toxicity tests, fish are crucial test models (Harshbarger and Clark, 1990), not only due to their indispensable roles in aquatic food chains, but also because they are an important aquatic food source for humans (Lammer et al., 2009). Fish have been used in toxicity tests for more than 150 years (Penny and Adams, 1863, as cited in Hunn, 1989). Due to an increasing need for toxicant assessment and ecological study in recent decades, a number of valuable fish models, such as fathead minnow (Pimephalespromelas), zebrafish (Daniorerio), and Japanese medaka (Oryziaslatipes), have been established and widely used in toxicity or ecotoxicity investigations (Ankley and Johnson, 2004; Ankley and Villeneuve, 2006; T. Braunbeck et al., 2005; Hatanaka et al., 1982; Hill et al., 2005; McGrath and Li, 2008; Örn et al., 2003). Advantages of these fish models include small body size, well characterized development/growth process, short maturation/reproduction cycle, high fecundity, and high tolerance of different environmental stresses. Fathead minnow, a member of the demersal cyprinid family, is broadly distributed in temperate waters across North America (Divine, 1968; Eddy and Underhill, 1974; Held and Peterka, 1974; Isaak, 1961; Page and Burr, 1991; Zimmer et al., 2001), and plays a key role in the regulation of structure and function of aquatic ecosystems (Gingras and Paszkowski, 1999; Paszkowski et al., 2004; Scott and Crossman, 1973; Zimmer et al., 2002). As a native fish species, fathead minnow has become the most widely studied toxicological fish model in North America (Ankley and Villeneuve, 2006), owning one of the biggest aquatic toxicology databases (Ankley et al., 2001; Gray et al., 2002; Keddy et al., 1995; Miracle et al., 2003; Sinks and Schultz, 2001). Zebrafish (Daniorerio), another demersal cyprinid species, is originated from the Ganges River system, Burma, Malakka and Sumatra (Eaton and Farley, 1974; Engeszer et al., 2007; Talwar and Jhingran, 1991). Zebrafish has been proved to be a valuable fish model and used for decades in a variety of toxicity investigations, including chemical screening, water quality control, ecotoxicological assays, and neurotoxicology studies (Coverdale et al., 2004; Goldsmith, 2004; Goolish et al., 1999; Hill, et al., 2005; Hisaoka and Battle, 1958; Laale, 1977; Lele and Krone, 1996; Nagel, 2002; Parng et al., 2002).

Probit analysis is used to analyze many kinds of dose-response or binomial response experiments in a variety of fields. Probit Analysis is commonly used in toxicology to determine the relative toxicity of chemicals to living organisms. This is done by testing the response of an organism under various concentrations of each of the chemicals in question and then comparing the concentrations at which one encounters a response. As discussed above, the response is always binomial (e.g. death/no death) and the relationship between the response and the various concentrations is always sigmoid. Probit analysis acts as a transformation from sigmoid to linear and then runs a regression on the relationship. Once a regression is run, the researcher can use the output of the probit analysis to compare the amount of chemical required to create the same response in each of the various chemicals. There are many endpoints used to compare the differing toxicities of chemicals, but the LC50 (liquids) or LD50 (solids) are the most widely used outcomes of the modern dose-response experiments. The LC50/LD50 represent the concentration (LC50) or dose (LD50) at which 50% of the population responds.

Scope of the study

This work is concerned with the statistical techniques used for the estimation of dose-response relations. It will be assumed that the particular agent in question is known to be generally toxic and that the purpose of the statistical analyses is to obtain an indication of the relation between dose level and the toxic response.

Literature review

Dose-response can be defined as the change in effect caused by differing level of exposure to a stressor (Finney, 1971). Dose-response studies are important tools for investigating the existence, nature and extent of a dose effect on efficacy. Data from dose-response studies can either be independent or has some repeated measurement depending on the aims of the different studies being conducted, which in turn gives rise to different designs for data collection.

Different kinds of data collected from arthropod dose-

response studies will require different kinds of analysis depending on the nature of the data. Arthropod dose-response (mortality) data are usually analyzed to evaluate efficacy of insect control agents in terms of estimating lethal time (LT), lethal dose (LD) and lethal concentration (LC). These are usually estimated by different methods depending on the methodology and the assumptions made. The proportion of the estimates could be 50%, 90% or 95% and these are some of the standard measurements of efficacy.

Some of the commonly used methods in analyzing dose-response data (arthropod mortality data) include Probit analysis (Finney, 1971; Hubert, 1992), logistic regression analysis (Robertson and Preisler, 1992), serial-time-mortality model (Preisler and Robertson, 1989; Thorne et al. 1995), Life-table analysis, Kaplan-Meier Product Limit estimator, Time-dose-mortality model (Robertson and Preisler, 1992), Aalen-Nelson estimator, Cox Proportional hazard model and GEE for repeated measures logistic regression.

The Probit Analysis

Probit Analysis is a method of analyzing the relationship between a stimulus (dose) and the quantal (all or nothing) response. Quantitative responses are almost always preferred, but in many situations they are not practical. In these cases, it is only possible to determine if a certain response (such as death) has occurred. In a typical quantal response experiment, groups of animals are given different doses of a drug. The percent dying at each dose level is recorded. These data may then be analyzed using Probit Analysis.

The Probit Model assumes that the percent response is related to the log dose as the cumulative normal distribution. That is, the log doses may be used as variables to read the percent dying from the cumulative normal. Using the normal distribution, rather than other probability distributions, influences the predicted response rate at the high and low ends of possible doses, but has little influence near the middle. Hence, much of the comparison of different drugs is done using response rates of fifty percent. The probit model may be expressed mathematically as follows:

$$P = \alpha + \beta[log_{10}(dose)] \quad (1)$$

Where P is five plus the inverse normal transform of the response rate (called the Probit). The five is added to reduce the possibility of negative probits, a situation that caused confusion when solving the problem by hand.

The popularity of the method is due in large part to the work of Finney (1971), in his book Probit Analysis. He explains the proper use and analysis of quantal response data.

Probit analysis is a specialized regression model of binomial response variables used to analyze many kinds of dose-response or binomial response experiments in a variety of fields. It is commonly used in toxicology to determine the relative toxicity of chemicals to living organisms. This is done by testing the response of an organism under various concentrations of each of the chemicals in question and then comparing the concentrations at which one encounters a response. The response is always binomial (e.g. death/no death) and the relationship between the response and the various concentrations is always sigmoid. Probit analysis acts as a transformation from sigmoid to linear and then runs a regression on the relationship.

Once a regression is run, the output of the probit analysis is used to compare the amount of chemical required to create the same response in each of the various chemicals. There are many endpoints used to compare the differing toxicities of chemicals, but the LC50 (liquids) or LD50 (solids) are the most widely used outcomes of the modern dose-response experiments. The LC50/LD50 represent the concentration (LC50) or dose (LD50) at which 50% of the population responds.

Assumptions of Probit analysis

1. Probit analysis assumes that the relationship between number responding (not percent response) and concentration is normally distributed. If data are not normally distributed, logit is preferred.
2. Must correct data if there is more than 10% mortality in the control

One method is to use the Schneider-Orelli's (1947) formula:

$$Corrected = \frac{\%\ Responded - \%\ Responded\ in\ control}{100 - Responded\ \%\ in\ control} \text{ x } 100 \quad (2)$$

Limitations of Probit analysis

Probit Analysis is used to analyze data from bioassay experiments. Probit Analysis is the commonly used method of estimating lethal doses or lethal time or lethal concentration (Finney, 1971). When subjects are exposed to several concentrations of an agent one can determine the time taken by a particular dose to kill 50% of the insects (LT50). In probit analysis, different sets of insects are treated with varying amounts of insecticides and the insects are inspected for mortality at a single point in time or analyzed for a given dose or concentration separately. In this design, death of an individual is measured once and all observations on mortality are independent, an important assumption that must be met for probit or logit modeling as prescribed by Robertson and Preisler (1992). The independence assumption is violated if data are repeated measures. In many bioassay experiments (arthropod dose-response studies) investigators record at several points in time the number of subjects that have died giving rise to percentage insects dying. Probit analysis usually models the mortality data as a function of dose and hence it's ineffective when the data are repeatedly taken at several time points (Roberston and Preisler, 1992). Sahaf and Moharrmipour (2008) used probit analysis proposed by Finney (1971) to estimate the time required for 50% and 95% kill (LT50 and LT95 respectively) of extracts on cowpea beetle. Eaton and Kells (2009) used probit analysis to calculate LT50 and LT90 from the mortality curves (mortality versus time) which displayed sigmoidal curves for a given temperature on mortality of mold mites. Osbrinket al. (2001) used probit analysis described by Finney (1971) to determine LT50 and LT90 of insecticides subjected to termites.

Probit analysis has some limitations in that standard probit analysis techniques are not applicable on serial-time mortality data because observations made on the same group of organisms at different times are correlated and

ignoring the correlation aspects will lead to giving false estimates and conclusions (Thorne et al., 1995). Robertson and Preisler (1992) and Thorne et al. (1995) stated that alternatives to logit and probit analysis do exists, these are the ones that directly address the problem of correlation of serial-time mortality data. One of the approaches as described by Priesler and Robertson (1989) and Nowierskiet al. (1996) is to use complementary log-log model (time-dose-mortality model). Other approaches involve using survival analysis (Holbrook et al., 1999) and generalized estimating equations for repeated measures (Stokes et al., 2000).

The Chi-square Goodness-of-fit test

Chi –square (χ^2) is a special significance test which is used in a very large number of cases to test the accordance between fact and theory (or between observed valves and expected valves). As a general hypothesis testing procedure, Use of chi-square (χ^2) test always involves comparison of obtained sampled frequencies entered in defined data categories based on the assumption that the null hypothesis (H0) is true. It is possible to fit theoretical distribution of a population. A chi-square test (χ^2) is a test commonly used to determine whether there is a significant difference between observed and expected or theoretical frequencies obtained from a distribution (Quazim Oni, 2004).

Pearson's chi-square statistic is used to determine whether the population distribution estimated by a single random sample containing n independent observations is identical to some hypothesized or expected population distribution. Depending on the experimenter's interests, expectations may be based on one of the theoretical distributions (such as the normal curve) or on the results of an earlier empirical investigation. Pearson's chi-square statistic is used to test the hypothesis that the observed frequencies O1, O2,..., Ok in k mutually exclusive categories of a population are equal to a set of expected frequencies E1, E2, …, Ek.

The Goodness-of-fit test is concerned with testing a null hypothesis that the population distribution for a random variable follows a specified form. The null hypothesis could state that within the range of defined categories the distribution is uniform, and therefore the expected frequencies for the data categories are equal; or the null hypothesis could state that the population conforms to such a standard distribution as the binomial, Poisson, or normal probability distribution. On the other hand, the expected frequencies need not be based on a standard distribution but can be based on any specified distribution, such as, for instance, a distribution based on a historical pattern of frequencies.

For the null hypothesis to be accepted, the differences between the observed and the expected frequencies for the several data categories must be attributed to sampling variability at the designated level of significance.

The χ2 test statistic is based on the sum of the squared differences between the obtained and expected frequencies for each data category, relative to the expected frequency for each data category.

The Chi-square (Goodness-of-fit) test statistic is defined as

$$\chi^2 = \sum_{i=1}^{r}\sum_{j=1}^{c}\left[\frac{(O_{ij} - E_{ij})^2}{E_{ij}}\right] \qquad (3)$$

Where

r = the number of rows

c = the number of columns

Oij = the observed frequency.

Eij = the expected frequency

$$E_{ij} = \frac{T_{..}}{K} \qquad (4)$$

T...=Total number of observed frequencies or sample size *n*.

K = Number of frequency classifications.

If the conformity between the observed and expected frequencies is perfect, the computed χ2 test statistic is χ2 = 0. As the difference between the observed and expected frequencies becomes larger, so does the value of the test statistic. Therefore, the χ2 goodness-of-fit test is always a one-tail test, with the upper tail of the χ2 distribution representing the region of rejection.

The critical value of χ2 required to reject the null hypothesis depends on the degrees of freedom and the specified level of significance. In goodness-of-fit tests the degrees of freedom (df) are

$$df = k - m - 1 \qquad (5)$$

where k = number of data categories

m = Number of parameter values estimated on the basis of the sample data.

Research Methodology

The test reagents and organisms

The test compounds (diesel) were obtained from TOTAL filing station in Lagos, Nigeria. The juveniles of African catfish, Clariasgariepinuswere obtained from the catfish farm at the Department of Biological Science, Yaba College of Technology, Lagos, Nigeria and were brought to the laboratory for acclimatization. This organisms were selected for the test as a result of its sensitive nature and response to test substances. They were collected in the morning between the hours of 8.00a.m and 9.00a.m when the temperature was low enough to prevent heat stress. The number of animals collected at the sampling period ranged between 150 and 250.

Preparation of water soluble fraction of diesel

One litre of diesel fuel obtained from a filling station, was diluted with four litres of water with which the fingerlings were cultured, in a six litres flask in accordance with. The diesel – water mixture was stirred slowly for 24 hours with a Gallenkamp magnetic stirrer. This was to enhance the dissolution in the water of the water-soluble components of the fuel. The mixture was made to stand for 3 hours before it was poured into the separating funnel and allowed to stand overnight so as to obtain a clear oil-water interphase. The lower layer of water, containing the WSF (water soluble fraction) of diesel was decanted into a clean round bottom flask with stopper this process was repeated several times until sufficient quantity of the WSF was obtained to carry out the study.

Toxicity tests

Glass tanks of about 30 litrecapacity were used as the holding tank, and glass bowls of about 3 litres capacity

served as the bioassay containers. Healthy juveniles of similar sizes were taken from the holding tanks to bioassay containers using hand net. Variations in sizes were avoided in order to prevent variations to chemical reaction by the fishes as a result of concentration/body weight ratio. In each experiment, five juveniles were introduced to the following range of WSF concentration:
Diesel fuel: 60.0, 70.0, 80.0, 90.0, 100.0 ml/L
Mortality assessment was done once in every 24 hours over the 96 hours [4 days] period. The juveniles of Clariasgariepinus exposed during the bioassay were taken to be dead when there is no body movement. Fishes were also confirmed dead when there was no evidence of hopping or movement even when probed with glass rod. When fishes were about to die, their swimming rate reduces and after death, a yellowish coloration was noticed around the operculum. The numbers of death recorded during the bioassay period were recorded, against time. Toxicological dose-response data involving quantal response (mortality) were analysed by probit analysis using SPSS 20.0.

Analysis

The table below contains data on number of reported death of some catfish when exposed to several concentrations of an agent for 1 month and collected from School of Science, Department of Chemical Science, Yaba College of Technology, Yaba, and Lagos, Nigeria. This data was analyzed electronically using SPSS version 21. Graphs were plotted where necessary for clarifications.

Table 1: Reported number of death of some catfish.

Concentration (mg/l)	Exposed	Periods (hrs)	Mortality
60	20	24 Hours	5
60	15	48 Hours	1
60	14	72 Hours	1
60	13	96 Hours	2
70	20	24 Hours	6
70	14	48 Hours	2
70	12	72 Hours	2
70	11	96 Hours	2
80	20	24 Hours	7
80	15	48 Hours	3
80	11	72 Hours	2
80	10	96 Hours	2
90	20	24 Hours	8
90	12	48 Hours	3
90	9	72 Hours	2
90	19	96 Hours	4
100	20	24 Hours	10
100	9	48 Hours	3
100	6	72 Hours	2
100	9	96 Hours	2

Table 2: Parameter estimates for four 24hrs toxicity tests utilizing same toxicant and same species.

Parameter		Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Concentration (mg/L)	3.513	4.814	.730	.466	-5.923	12.948
	Intercept	-7.204	10.193	-.707	.480	-17.398	2.989

a. PROBIT model: PROBIT (p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

From table 2 above, the slope of the graph is 3.513 and the intercept is -7.204. The Probit model fit is:

$$\boldsymbol{Probit} = -7.204 + 3.513X \qquad (6)$$

Where X is the Log of concentration

Log ED50 is calculated when Probit = 5

That is, 5=-7.204+3.513X
5+7.204=3.513X
X≈3.474

Therefore, ED50 = Antilog (3.474) ≈ 2978.5

Table 3: *Natural Response Rate (24hrs)*

	Estimate	Std. Error
PROBIT	.091	.486

a. Control group is not provided.

The natural response rate is the probability that a fish will die after 24 hours if not given a dose. The value of 0.091 means that roughly 9.1% of all fishes would die after 24 hours without the dose.

Table 4: Cell Counts and Residuals (24hrs)

	Number	Concentration (mg/L)	Number of Subjects	Observed Responses	Expected Responses	% response	Probit	Probability
PROBIT	1	1.778	20	5	4.887	25	4.33	.244
	2	1.845	20	6	6.085	30	4.48	.304
	3	1.903	20	7	7.302	35	4.61	.365
	4	1.954	20	8	8.490	40	4.75	.424
	5	2.000	20	10	9.616	50	5.00	.481

The goodness of fit statistics are based on the cell counts and the residuals table. The concentration in mg/L shown are the natural logarithms of the actual values. The observed responses column reports the number of cases observed in the data file that are in the classification. The expected responses column reports the number of cases we would expect to see in the cell if the model is correct.

Table 5: Chi-Square Tests (24hrs)

		Chi- Square	df[b]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	.103	2	.950[a]

a. Since the significance level is greater than .150, no heterogeneity factor is used in the calculation of confidence limits.
b. Statistics based on individual cases differ from statistics based on aggregated cases.

The Pearson's goodness of fit chi-square statistic is used to test the null hypothesis that the model adequately fits the data. If the significance value of a given test is small (less than 0.05) then the model does not adequately fit the data. In Table 5 above, the significance value is 0.950 which is greater than 0.05, hence the model does adequately fit the data.

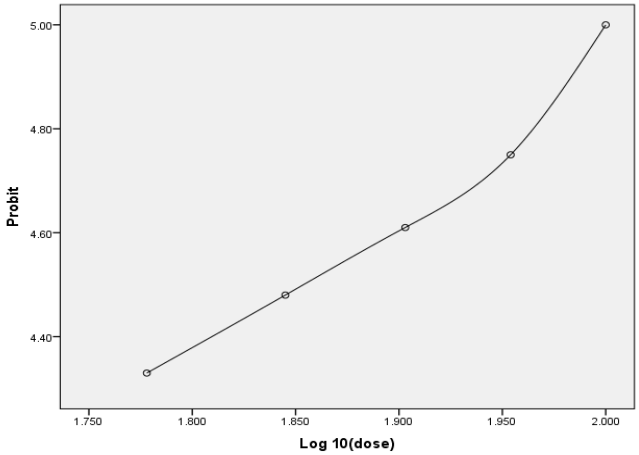

Fig. 1: Plot of Log10 (Dose) Vs Empirical Probit (24hrs)

Fig 1 above presents the Probit model. If the Probit model is to be a good approximation, this plot should show a linear relationship. Obviously, in Fig 1 above, the relationship is linear, indicating that the Probit model is of a good approximation.

Table 6: Parameter estimates for four 48hrs toxicity tests utilizing same toxicant and same species

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Concentration (mg/L)	2.060	2.728	.755	.450	-3.287	7.406
	Intercept	-4.835	4.773	-1.013	.311	-9.607	-.062

a. PROBIT model: PROBIT (p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

From table 6 above, the slope of the graph is 2.060 and the intercept is -4.835. The Probit model fit is:

$$\boldsymbol{Probit} = -4.835 + 2.060X \qquad (7)$$

Where X is the Log of concentration

Log ED50 is calculated when Probit = 5

That is, 5= -4.835+2.060X
5+4.835=2.060X
X≈4.474

Therefore, ED50 = Antilog (4.474) ≈ 29785.1

Table 7: Natural Response Rate (48hrs)

	Estimate	Std. Error
PROBIT	.004	.459

a. Control group is not provided.

The natural response rate is the probability that a fish will die after 48 hours if not given a dose. The value of 0.004 means that roughly 0.4% of all fishes would die after 48 hours without the dose.

Table 8: Cell Counts and Probits (48hrs)

	Number	Concentration (mg/L)	Number of Subjects	Observed Responses	Expected Responses	% response	Probits	Probability
PROBIT	1	1.778	15	1	1.854	7	3.52	.124
	2	1.845	14	2	2.148	14	3.92	.153
	3	1.903	15	3	2.744	20	4.16	.183
	4	1.954	12	3	2.542	25	4.33	.212
	5	2.000	9	3	2.158	33	4.56	.240

The goodness of fit statistics are based on the cell counts and the residuals table. The concentration in mg/L shown are the natural logarithms of the actual values. The observed responses column reports the number of cases observed in the data file that are in the classification. The expected responses column reports the number of cases we would expect to see in the cell if the model is correct.

Table 9: Chi-Square Tests (48hrs)

		Chi-Square	df[b]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	.738	3	.864[a]

a. Since the significance level is greater than .150, no heterogeneity factor is used in the calculation of confidence limits.
b. Statistics based on individual cases differ from statistics based on aggregated cases.

The Pearson's goodness of fit chi-square statistic is used to test the null hypothesis that the model adequately fits the data. If the significance value of a given test is small (less than 0.05) then the model does not adequately fit the data. In table 2c above, the significance value is 0.864 which is greater than 0.05, hence the model does adequately fit the data.

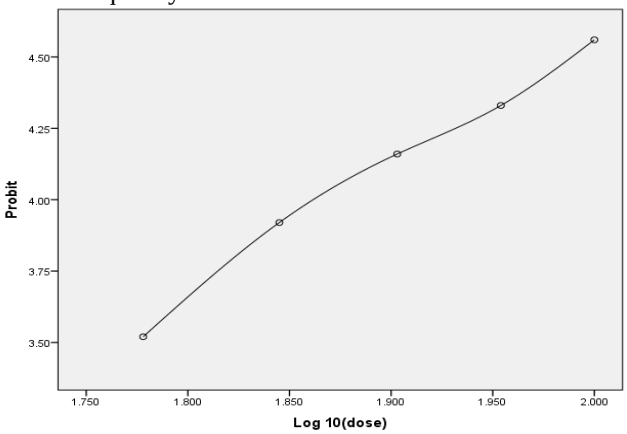

Fig 2: Plot of Log 10 (Dose) Vs Empirical Probit (48hrs)

Fig 2 above presents the Probit model. The Probit model is of a good approximation, since the plot shows a linear relationship between the Probit and Log 10(dose).

Table 10: Parameter estimates for four 72hrs toxicity tests utilizing same toxicant and same species

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Concentration (mg/l)	2.004	2.843	.705	.481	-3.568	7.577
	Intercept	-4.820	5.381	-.896	.370	-10.201	.561

a. PROBIT model: PROBIT (p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

From table 10 above, the slope of the graph is 2.004 and the intercept is -4.820. The Probit model fit is:

$$\boldsymbol{Probit} = -4.820 + 2.004X \qquad (8)$$

Where X is the Log of concentration

Log ED50 is calculated when Probit = 5
That is, 5= -4.820+2.004X
5+4.820=2.004X
X≈4.900

Therefore, ED50 = Antilog (4.900) ≈ 79432.8

Table 11: Cell Counts and Probits (72hrs)

	Number	Concentration (mg/l)	Number of Subjects	Observed Responses	Expected Responses	% response	Probits	Probability
PROBIT	1	1.778	14	1	1.465	7	3.52	.105
	2	1.845	9	1	1.180	11	3.77	.131
	3	1.903	11	2	1.732	18	4.08	.157
	4	1.954	9	2	1.650	22	4.23	.183
	5	2.000	6	2	1.252	33	4.56	.209

The goodness of fit statistics are based on the cell counts and the residuals table. The concentration in mg/L shown are the natural logarithms of the actual values. The observed responses column reports the number of cases observed in the data file that are in the classification. The expected responses column reports the number of cases we would expect to see in the cell if the model is correct.

Table 12: Chi-Square Tests (72hrs)

		Chi-Square	df[b]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	.901	3	.825[a]

a. Since the significance level is greater than .150, no heterogeneity factor is used in the calculation of confidence limits.
b. Statistics based on individual cases differ from statistics based on aggregated cases.

The Pearson's goodness of fit chi-square statistic is used to test the null hypothesis that the model adequately fits the data. If the significance value of a given test is small (less than 0.05) then the model does not adequately fit the data. In table 3c above, the significance value is 0.825 which is greater than 0.05, hence the model does adequately fit the data.

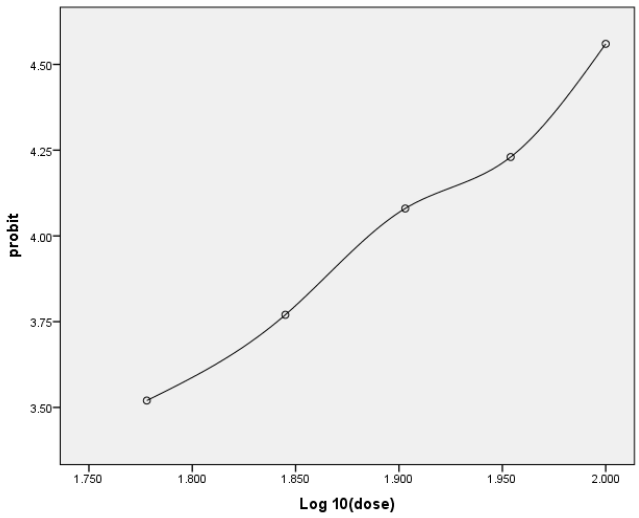

Fig. 3: Plot of Log 10 (Dose) Vs Empirical Probit (72hrs)

Fig 3 above presents the Probit model. The Probit model is of a good approximation, since the plot show a linear relationship between the probit and Log 10(dose).

Table 13: Parameter estimates for four 96hrs toxicity tests utilizing same toxicant and same species

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Concentration (mg/L)	1.676	16.146	.104	.917	-29.969	33.320
	Intercept	-4.336	37.246	-.116	.907	-41.582	32.911

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.)

From table 13 above, the slope of the graph is 1.676 and the intercept is -4.336. The Probit model fit is:

$$\boldsymbol{Probit} = -4.336 + 1.676X \qquad (9)$$

Where X is the Log of concentration

Log ED50 is calculated when Probit = 5

That is, 5= -4.336+1.676X
5+4.336=1.676X
X≈5.570

Therefore, ED50 = Antilog (5.570) ≈ 371535.2

Table 14: Natural Response Rate (96hrs)

	Estimate	Std. Error
PROBIT	.078	1.421

a. Control group is not provided.

The natural response rate is the probability that a fish will die after 96 hours if not given a dose. The value of 0.078 means that roughly 7.8% of all fishes would die after 96 hours without the dose.

Table 15: Cell Counts and Probits (96hrs)

	Number	Concentration (mg/L)	Number of Subjects	Observed Responses	Expected Responses	% response	Probit	Probability
PROBIT	1	1.778	13	2	2.064	15	3.96	.159
	2	1.845	11	2	1.941	18	4.08	.176
	3	1.903	10	2	1.940	20	4.16	.194
	4	1.954	19	4	4.012	21	4.19	.211
	5	2.000	9	2	2.051	22	4.23	.228

The goodness of fit statistics are based on the cell counts and the residuals table. The concentration in mg/L shown are the natural logarithms of the actual values. The observed responses column reports the number of cases observed in the data file that are in the classification. The expected responses column reports the number of cases we would expect to see in the cell if the model is correct.

Table 16: Chi-Square Tests (96hrs)

		Chi-Square	df[b]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	.008	2	.996[a]

a. Since the significance level is greater than .150, no heterogeneity factor is used in the calculation of confidence limits.

b. Statistics based on individual cases differ from statistics based on aggregated cases.

The pearson goodness of fit chi-square statistic is used to test the null hypothesis that the model adequately fits the data. If the significance value of a given test is small (less than 0.05) then the model does not adequately fit the data. In table 4d above, the significance value is 0.996 which is

greater than 0.05, hence the model does adequately fit the data.

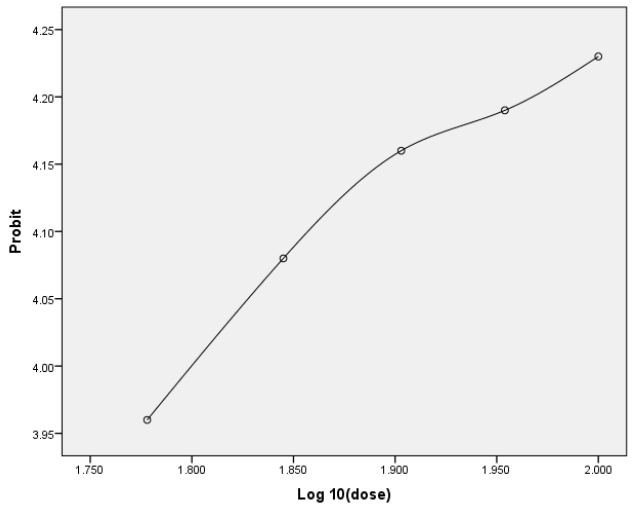

Fig. 4: Plot of Log 10 (Dose) Vs Empirical Probit (96hrs)

Fig 4 above presents the Probit model. The Probit model is of a good approximation, since the plot show a linear relationship between the Probit and Log 10(dose).

Discussion of results

Acute toxicity test of the toxicant; diesel showed that the Catfish (Clariasgariepinus) exhibits differential response upon single administration of the respective concentration of the compound. On the basis of 96hr ED50 values, water soluble fraction of diesel was more toxic to the juvenile of Clariasgariepinus than the 24hr ED50, 48hr ED50 and 72hr ED50 values.

The computed 96hr values were 8.58 ml/l, while the computed 24hr, 48hr and 72hr values were 2.45ml/l, 3.46ml/l and 5.67ml/l respectively.

For 24hr (Fig. 1), 48hr (Fig. 2), 72hr (Fig. 3) and 96hr (Fig. 4), the graphs plotted indicates that the higher the concentration the higher the mortality experienced by the catfish (Clariasgariepinus).

The Probit model is of a good approximation, since the plot show a linear relationship between the Pprobit and Log 10(dose). Also, the result of the chi-square for the 24hr, 48hr, 72hr and 96hr toxicity test indicates that the Probit model adequately fit the data.

The acute exposure of Clariasgariepinusto diesel exhibited a wide range of behavioural responses. These include pronounced gasping for breath, erratic swimming behaviour uncoordinated movement and occasional darting up and down the water column. This can be attributed to nervous reaction of the organism to the irritating effects of the toxicant and disturbance in physiological mechanism.

Conclusion

Measurement of biological effects of pollution on the organisms in the environment has become very important for the assessment of environmental quality. Result of the present study reflects the effects of WSF (water soluble fraction) of diesel through the use of juveniles of Clariasgariepinus as a sensitive indicator of environmental pollution in. Results obtained can provide a reasonable basis for comparison of the effects such pollutants can have on higher animals and human beings.

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