Evaluation of effects of exposure time on aquatic toxicity with zooplanktons using a reduced life expectancy model

Vibha Verma ^{a,*}, Qiming J. Yu ^a, Des W. Connell ^b

^aGriffith School of Engineering, Griffith University, Nathan Campus, Brisbane, Queensland 4111, Australia

* Corresponding author. Tel: +617 3735 7186 Email address: v.verma@griffith.edu.au

^bGriffith School of Environment, Griffith University, Nathan Campus, Brisbane, Queensland 4111, Australia

Abstract

Traditionally in toxicological studies time is not studied as quantifiable variable but as a fixed endpoint. The Reduced Life Expectancy (RLE) model which relates exposure time and exposure concentration with lethal toxic effects was tested previously using fish data. In this current paper the effects of exposure time on aquatic toxicity with zooplanktons and various toxicants were evaluated using the RLE model based on ambient exposure concentration. The model was evaluated by plotting $\ln LT_{50}$ against LC_{50} using toxicity data with zooplanktons from the literature for metal, metalloid and organic compounds. Most of the experimental data sets can be satisfactorily correlated by use of the RLE model, but deviations occurred for some data sets. Those data sets were satisfactorily fitted by a two stage RLE model. This model was based on two phases: one in the peripheral system and other in the central system. Both the single and two stage RLE model support the hypothesis that toxicity is time dependent and decreases in a systematic way with increasing exposure time. A calculated normal life expectancy (NLT) can be obtained from the single stage model and is in accord with reported NLT but those obtained from the two stage RLE model are in excellent agreement.

Key Words: Aquatic Toxicity, Effects of Exposure Time, Nonlinear Relationship, Zooplankton Toxicity, Two Stage Reduced Life Expectancy Model, Uptake Route

1. Introduction

During his work on acute toxicity of gases used in warfare, Haber (1924) discovered that the multiple of the exposure concentration and time produces the same biological effect e.g. lethality. Thus $C \times t = \text{constant}$ where C is lethal exposure concentration and t is exposure time. This came to be known as Haber's rule. Because of its simplicity, the relationship became a popular tool for toxicological evaluations, initially among entomologists (Miller et al., 2000). Interestingly deviations from Haber's Rule were observed, specifically noting that exposure concentration is more important than exposure duration for toxic effects to take place (Flury and Wirth, 1934). Therefore use of the rule beyond certain limits would give an incorrect relationship (Weller et al., 1999). However this simple approach has been effectively used by regulatory agencies such as USEPA as the foundation for setting the exposure limits (Atherely, 1985; Henschler, 1984).

Understanding the effects of exposure time on toxicity is important for the assessment of risks associated with the long term exposure time of humans but at a low level. This usually occurs with environmental contaminants associated with human health risks. Recently Rozman (1998) have argued that dose alone is insufficient because exposure time is an equally important factor for the occurrence of toxic effects. The fundamental significance of exposure time as a variable in toxicology has been recognised but less well understood (Witschi, 1999). Even a low level of a chemical can be toxic if the exposure time is relatively long (Yu et al., 1999). Work performed by various other researchers also indicates the importance of exposure time in toxicological studies (Zhao and Newman, 2004, 2006).

Conventionally time is not studied as a quantifiable variable in standard toxicity testing (Rozman and Doull, 2000, 2001; Sanchez-Bayo, 2009). The traditional approach is to evaluate dose-response relationship with time taken as a fixed endpoint only (Cairns and Mount, 1990; Forbes and Forbes, 1994; Ashauer and Escher, 2010). Chronology of the response intensity at the various doses or concentrations is seldom taken into consideration in these studies. But in the last few decades there have been several studies where exposure time is taken as quantifiable variable (Pascoe and Shazili, 1986; Hickie et al., 1995; Chaisuksaint et al., 1997; Hoang et al., 2007; Sanchez-Bayo and Goka, 2007; Beketov and Liess, 2008). By using time as a quantifiable variable, information can be obtained not only for the particular toxic dose levels but also for the exposure time (Newman and McCloskey, 1996).

In our previous study with fish (Verma et al., 2011) the RLE model was evaluated using the relationship between exposure time and the exposure lethal concentration. It was noted that irrespective of toxicant and the toxic mechanism involved the relationship between exposure time as $\ln LT_{50}$ and exposure lethal concentration LC_{50} for fish was always linear. It was suggested that possibly the relationship is not a reflection of the toxic mechanism involved but more the route of toxicant uptake. However, with increasing exposure time a systematic decrease in exposure concentration was recorded. The RLE model allows the NLT to be calculated from the toxicity data. In previous work the reported NLT and calculated NLT obtained were in general accordance.

The objectives of present study were designed to evaluate the RLE Model with zooplanktons based on the toxic effects of exposure time available in the scientific literature. The characteristics obtained from evaluation of the RLE model would be used to validate the relationship of exposure time with toxicity and for estimation of effects of exposure time on life expectancy.

2. Theory

2.1 RLE Model

The RLE model is based on a linear relationship between the internal lethal concentration of a toxicant within the organism and the corresponding exposure time. The model was developed with the use of the concept of reduction in life expectancy (Yu et al., 1999) and the model equation is given below,

$$ILC_{50} = \frac{(\ln NLT_{50} - \ln LT_{50})}{d} \quad (1)$$

Where ILC_{50} is the internal lethal concentration, LT_{50} is the exposure time, NLT_{50} is the normal life expectancy of the organism and d is a constant.

But due to the limited availability of ILC_{50} data in the scientific literature, relationship was extended from using the internal concentration to using the external concentration. Based on this extension a new model was proposed (Connell and Yu, 2008) which can be described by the equation given below:

$$LC_{50} = \frac{(\ln NLT_{50} - \ln LT_{50})}{d} \quad (2)$$

or,

$$LC_{50} = -a \ln LT_{50} + b \quad (3)$$

where LC_{50} is external lethal concentration, a is $-1/d$ and b is $\ln NLT_{50}/d$.

According to the RLE model, when LC_{50} is zero the organism will have a normal life expectancy and it is related to the model constants as follows,

$$NLT_{50} = \frac{b}{a} \quad (4)$$

2.2 A Two Stage RLE Model

The RLE model is based on the concept that there is a linear relationship between exposure time period ($\ln LT_{50}$) and concentration (LC_{50}). However, it was observed in this study that some data sets exhibit nonlinear characteristics. Nonlinear relationships between $\log LT_{50}$ and $\log LC_{50}$ have also been noticed (Bliss 1940; Wuhrmann, 1952; Herbert, 1965; Zhao and Newman, 2006) at low concentrations and longer exposure durations. At higher concentrations and shorter exposure durations, this relationship is usually linear (Naddy et al., 2000). In order to correlate this nonlinearity observed in some data set, here it is proposed that the data set is divided into two segments at different exposure time periods, and each of the segments can be correlated with the linear RLE model. The original RLE model is referred as the single stage RLE model and the new model as the two stage RLE model. The equation of the single stage RLE model (Equation 3) is applied individually for each segment of the two stage RLE model. This can be illustrated with a conceptual diagram (Figure 1) where the solid line represents the original RLE model (single stage RLE model) and the two broken lines represent the two stage RLE model. These two broken lines converge at a common end point where a change in slope takes place. The first stage in the diagram has a higher slope and the second stage has a relatively lower slope.

The concept underlying the development of the single stage and the two stage model is the single compartment approach where a single compartment is used to represent an organism (Moriarty, 1975; Connell, 1990). When the organism is exposed to a toxicant, it penetrates the organism body, enters into the peripheral system and then gets distributed in the entire body of the organism. Exchange processes like elimination and degradation are the dominant processes in the peripheral system. Because of these dilution processes and movement of toxicant as it is

distributed in the entire body of organism towards the target site, the concentration of the toxicant cannot increase beyond a certain level. Thus toxicant accumulation in the peripheral system does not reach a threshold level. The toxicant resulting from the dilution processes in the peripheral system of the organism body reaches to the central system and it starts accumulating there. Once the concentration of the toxicant in the central system reaches to a threshold level, toxic effects (mortality or some other toxicity endpoint) are observed (Mancini, 1983).

The first stage of the two stage model corresponds to the processes in the peripheral system at the earlier phase of the exposure to toxicant and the second stage of model represents the second phase of the exposure processes. The processes in the first phase occur at faster paces while the processes taking place in the second phase occur at considerably slower paces. In general when the overall relationship obtained is linear, first and second stages cannot be distinguished from each other. In this case a single stage model can be used to describe the toxicity. However in the situation where relationship observed is nonlinear, both first and second stages can be distinguished due to a changing slope at the common end point.

3. Methodology

3.1 Organisms and Toxicants Selected for Evaluations

Zooplanktons were selected as organisms for this study since plenty of toxicity data related to this group are available. Zooplanktons are key components of both marine and freshwater food web as they are the primary grazers in several ecosystems and serve as a main source of food for developing larvae and fish (Hook and Fisher, 2001). Thus zooplanktons have a key role to play in the trophic structure of aquatic ecosystems, vital for energy transfer at secondary level between the autotrophs and the heterotrophs (Deivanai et al., 2004; Kanagasabapathi and Rajan, 2010). It would be interesting to evaluate the effects of exposure time on such a key component of aquatic

systems. Microscopic crustacean, ostracods (*Cypridopsis vidua* and *Stenocyris major*), copepods (*Arctodiaptomus salinus* and *Mesocyclops lueckarti*), cladoceran (*Daphnia magna*) and small millimetresized amphipods (*Gammarus pulex*, *Gammarus lacustris* and *Gammarus psuedolimnaeus*) are the organisms selected for study.

Toxicants having significantly different toxic mechanism are selected for this study and they included toxic metals (Cu, Zn, Pb, Ni, Fe, Mn, Al and Cd), a metalloid (Se) and organic compounds (imidacloprid, thialcoprid, lindane, fenvalerate, flucythrinate, BHC, fudaran and dimethoate). Among these toxicants, Pb, Cd, Ni has no known biological significance while Cu, Zn, Fe, Mn and Se are biologically important. Organic compounds selected for study are mainly pesticides and those have no known biological significance. Among these toxicants metals and metalloids enter the environment through both natural and human activities but presence of organic compounds is entirely consequences of human activities.

3.2 Sources and Collection of Data

Toxicity data related to zooplanktons with these particular groups of toxicants were obtained from the scientific literature. These data sets include information about change in LC_{50} against varying exposure durations with zooplanktons. The data obtained from the literature are in various units for concentration such as mg/L, g/L, ppm or ppb. For consistency all units were converted into $\mu\text{g/L}$. Similarly exposure time period was also expressed in various units (hours, minutes and seconds) of time and all were converted into days (d). The NLT data of each organism was also obtained from scientific literature. But the NLT of ostracod *S.major* and copepod *A.salinus* is not available in the literature. The temperature of the ambient water used as the medium to conduct the experiments from where toxicity data are obtained ranged between 13 °C to 30 °C.

3.3 Processing of Data

The data sets for each zooplankton species were used to evaluate the relationship between LC_{50} and $\ln LT_{50}$ with the RLE Model expressed in Equation 3. Thus LC_{50} was plotted against $\ln LT_{50}$ and linear regression analysis was used to obtain the regression equation and the correlation coefficient (R^2) using Excel. The values of the slope (a) and intercept (b) were obtained from the regression equation. These values were then used to obtain the calculated NLT of each organism by the use of Equation 4.

All the data sets were correlated by using the single stage RLE model first. It was noted that most of the plots followed the single stage RLE model well. However some plots had regression coefficient value less than 0.8. For those, plots based on the two stage model were obtained. Firstly, each data set was divided into two subsets, in such a way that two separate linear segments appear converging at a common endpoint where the slope of the relationship changes sharply. These plots resemble the conceptual diagram in Figure 1. In the situation when numbers of data points in a data set are limited, a few points were used as common points in both segments, such that there was enough data to obtain two linear regression lines. The regression line with the higher slope corresponds to the peripheral system and it was referred as Stage-1 (S-1). The other line with a lower slope corresponds to the central system and referred as Stage-2 (S-2). The values of a and b were obtained from the regression equations for each segment and then NLT_{50} was calculated from S-2 using Equation 4.

4. Results and Discussions

4.1 Relationship of Exposure Time to Toxicity

The plots of $\ln LT_{50}$ against LC_{50} based on Equation 3 are shown in Figures 2- 4. These plots utilise data from the literature (Table1) on zooplanktons for lethal effects as a result of exposure to

toxicants over time. Plots for the metalloid and metals are shown in Figure 2 a-e and the corresponding plots for pesticides are shown in Figure 3 a-d. With data sets where correlation coefficient was less than 0.8, two stage plots were obtained (see Section 3.3). Plots corresponding to the two stage model are shown in Figure 4 a- d. This occurred with four data sets out of a total of twenty five sets.

The characteristics of the relationships established using the regression equation can be used to compare the toxic effects of the metals, the metalloid and the pesticides (Table 1). The slopes of the regression equations reflect the strength of the toxic effects on the various zooplanktons. Though on average the slope is approximately same for both groups, the metals exposure differs to that observed with the pesticides. Since the range of slopes obtained for the metals and metalloid is from -0.08 to -25,000 while for the pesticides the range is from -0.07 to -11,000. The range is comparatively wider for the group of metals and the metalloid than for pesticides. This is also apparent from the ratio of the average of the slopes with pesticides to metals which is -2,000/-2,800 while the ratio of the standard deviations with pesticides to metals is 3,300/6,500. Different toxic mechanisms involved with metals and pesticides may be responsible for this difference.

A comparison of these results with those obtained from previous paper with fish listed in Table 3 (Verma et al., 2011) indicates that though slopes obtained from regression analysis are negative but fish has much wider range (-0.420 to -210,000) with excellent R^2 (0.830 to 0.999) than zooplanktons having R^2 value between 0.571 to 0.994 and slope range between -0.07 to -25,000. When slope obtained are compared for individual groups of toxicants, ratio of average for fish to metals and zooplanktons to metals is -13,300/-2,800 and ratio of standard deviation for these groups is -13,700/-6,500 respectively. For fish with metals, both average and standard deviation of slope is much higher than zooplanktons with metal. Similar pattern is repeated when ratio of

average for fish to pesticides and zooplanktons to pesticides are compared which is -33,000/-2,900 and standard deviation ratio is 68,000/4,000 respectively. Here over all ratio of the average of the slopes obtained for fish to all toxicants with zooplanktons to all toxicant is -21,000/-5,500 with standard deviation ratio of 44,000/2,800 respectively. Overall trend obtained for all toxicants with fish and zooplanktons is that the ratio of average and standard deviations for fish is higher than zooplanktons.

All the regression relationships have negative slopes (see Table 1) ranging from -0.07 to -25,000 which indicates that the toxicity is related to the exposure time and declines as the exposure time increases. These results are in accord with the results obtained by Landrum et al. (2005) who were studying the time dependent toxicity of dichlorodiphenyldichloroethylene to *Hyalea azteca*. Also Kurve et al., (2010) reported that toxicity is inversely proportional to exposure time when comparing the toxicity of pesticides, BHC with fudaran. Various other researchers also reached the same conclusion while studying the toxicity of fenvalerate to amphipod (*G.pseudolimaneus*) and various metals to copepod (*S.major*) respectively (Anderson, 1982; Shuhaimi-Othman et al., 2011).

In zooplanktons the routes of toxicants uptake are from outer body surface, gills and food. But due to smaller body size the most effective route of toxicant uptake is diffusion through outer body surface which is thin (Pirow et al., 2004). Uptake of toxicant takes place in fish principally through diffusion via gills but in the relatively small sized zooplanktons, diffusion is through outer body surface. In fact diffusion is effectively operational for only a short distance, thus it can be related to transport through relatively thin physical barriers such as respiratory surfaces and internal tissues (Pirow et al., 2004). Relatively very small zooplanktons with smaller volume and a

larger surface area are ideal for this process to be operational. But in larger aquatic animals such as fish, ventilatory and circulatory convection is the effective process (Shelton, 1992).

The relationship of LC_{50} and $\ln LT_{50}$ is linear for most of the zooplankton plots obtained, with high correlation coefficients ranging from 0.815 to 0.994 (Figures 2 a- e and Figures 3 a-d) irrespective of whether the particular toxicant is a metal or a pesticide. But some of the plots have nonlinear relationship with correlation coefficients ranging from 0.571 to 0.758, belonging to both groups of toxicants. Thus a linear or nonlinear relationship between LC_{50} and $\ln LT_{50}$ does not characterise to any particular group of toxicants. Previous work also supports the occurrence of both linear and nonlinear relationship among zooplanktons e.g. Gause (1932) obtained nonlinear relationship on logarithmic scale using the data published by Hartmann (1918) who studied toxicity of mercuric chloride on crustaceans (*Belonostomus longirostris*). Interestingly a study on toxicity of mercuric chloride with crustacean (*Simocephalus*) also reached to same conclusion (Breukelman, 1932). Bliss (1940) worked on the data obtained from Alison (1928) based on toxicity of hydrocyanic acid with cockroach and supported the occurrence of a nonlinear relationship. Though, he stated that the nonlinear relationship is not very common. While Yu et al. (1999) observed linear relationship from their study using the data obtained from the literature on chlorobenzenes with crabs (*Portunus pelagicus*).

4.2 Nonlinear Relationship: Application of Two Stage Model

Data sets which do not have a linear relationship i.e. $R^2 < 0.8$ were replotted in Figure 4, using two stage model. The total number of plots of toxicity data made in this work was 25 and from this 14 are with metal data, 1 metalloid data and 10 pesticide data. However there are only four plots out of this 25 which had a nonlinear relationship i.e. $R^2 < 0.8$. Interestingly out of these four plots only 1 belongs to the metal group though it is a comparatively larger group with 14 data sets in total.

The remaining 3 plots are of pesticides data having 10 datasets in total. This makes the two stage relationship more common among the organism exposed to pesticides than metals and metalloid.

The characteristics of the relationship obtained using the regression equations obtained from two stage RLE model can be used to compare the toxic effects of datasets having nonlinear relationships (Table 2). It is suggested that S-1 takes place in peripheral system at faster rate while S-2 takes place in central system at a slower rate depending upon the mode of toxic action. When only one mode of action dominates then S-1 and S-2 cannot be differentiated and a linear relationship is observed. But when a particular toxicant has more than one mode of toxic action, one in the peripheral system and other in the central system then a nonlinear relationship is observed.

According to Gause (1932) and Bliss (1940), it is not usual for a toxicant to act upon an organism in several different modes. These authors concluded that up to a certain range of toxicant concentration one mode of action is operational but replaced by another with an increase in concentration. Later on Miller et al., (2000) suggested a nonlinear relationship was a result of involvement of more than one mode of toxic action which depends upon length of exposure duration and exposure concentration.

The correlation coefficients obtained for S-1 are in the range of 0.727 - 0.999 and for S-2 in the range of 0.843 - 0.999 indicating an excellent linear relationship with S-2 which corresponds to the central system. A negative linear relationship in this stage indicates that toxicity is related to exposure time and decreases with increasing exposure time and is independent of a change in mode of toxic action.

The slopes obtained from the two stage model for S-1 range from -700 to -28,000 while S-2 is much less at -40 to -600. Ratio of the average of slopes obtained for S-1 to S-2 is $-8500/-280$ and ratio of the standard deviation obtained is $13,000/280$. This confirms that the processes in the first phase occur at faster rate and slow down after entering the second phase.

4.3 Comparison of the Reported NLT with Calculated NLT

Several sources of error have been recognised in carrying out a comparison of the reported NLT with that calculated from the relationship of Equation 4 to derive the NLT. The most important source of error is the age of the zooplankton before exposure to toxicant since it is usually unknown. In addition another factor influencing these results is the procedure for making a decision on death during the toxicity test. Normally zooplanktons exposed to toxicant are considered dead during the experiment, once they are immobile but sometimes immobility can be due to stress or unconsciousness while animal is still physiologically alive (Seepersad et al., 2004). These facts can lead to errors in the toxicity data which can flow onto errors in the NLT calculated by extrapolation of this toxicity data.

The reported NLT was compared with calculated NLT and in most cases the calculated NLT differs from the reported NLT (Table 1). Interestingly calculated NLT of *D. magna* with Zn, Cu, Cd and imidacloprid is longer than reported NLT.

In comparison of the reported NLT with calculated NLT the characteristics of the relationship established using the regression equations have been used (Table 1). The reported NLT and calculated NLT are in the range of 30 - 500 d and 2 - 260 d respectively. The ratio of averages of the reported NLT and the calculated NLT is $240/50$ and the ratio of the standard deviation is $200/80$. On a theoretical basis these ratios should be approximately the same, but the results are in

general accordance. The coefficient obtained from the plots of reported NLT against calculated NLT is low (0.430) and the P-value obtained from the Student's t-test, is 0.017 which also denotes that the results are in a general accord.

The ratios of averages and standard deviations calculated for both reported NLT and calculated NLT with fish from previous paper are compared with those obtained with zooplanktons from the present work. The ratio of average reported NLT for all toxicants with fish to zooplanktons is - 1300/240 and standard deviation is 800/200 respectively. While the ratio of average calculated NLT for all toxicants with fish to zooplanktons is 260/50 and standard deviation is 1100/80 respectively. The ratio of R^2 obtained from plotting reported NLT against calculated NLT with fish to zooplanktons is 0.262/0.431 and the P-value obtained from the Student's t-test is 0.003/0.017 respectively.

The calculated NLT obtained for S-1 and S-2 ranges from 1 to 25 and 5 to 620 respectively. But the ratio of the average obtained for reported and calculated NLT from S-1 is 290/10 and standard deviation is 210/10, having approximately 20 to 30-fold difference. The poor correlation coefficient (0.431) obtained for the ratio between two is an indication that the relationship is not in harmony with the RLE model. On the other hand the ratio of the average obtained for reported and calculated NLT from S-2 is 290/280 and the standard deviation is 210/290 with a correlation coefficient of 0.925 and P-value obtained from Student's t test of 0.954. Thus this indicates an excellent agreement between reported and calculated NLT for S-2.

Several discrepancies regarding the reporting of the NLT of zooplankton were also noticed. For example the reported NLT for *D. magna* (cladoceran) is on average 60 d (Van Leeuwen et al., 1987) but according to Euent et al., (2008) NLT of the female is 105 d and the male is 48 d. It is

important to note that some have reported that the NLT of *D. magna* varies with temperature e.g. 40 d at 25 °C, 60 d at 20 °C (Jonczyk and Gilron, 2005) and 80 d at 14 °C (Korpelainen, 1986). Hoang et al. (2007) and Sanchez-Bayo (2009) have mentioned that *D. magna* exposed to toxicants lived longer than its reported NLT during the experiment. Particularly Hoang et al., (2007) reported LT_{50} for *D. magna* exposed to zinc (59 µg/L) at 160 d and Sanchez-Bayo (2009) reported LT_{50} for *D. magna* exposed to imidacloprid (250 µg/L) was 380 d.

Interestingly the NLT of *M. leuckarti* (copepod) also changes with temperature in similar pattern which is 120 d at 10 °C, 58 d at 15 °C, 45 d at 20 °C and 32 d at 25 °C (Vijvenberg, 1980). While Gophen (1976) reported the NLT of male and female are different at different temperatures so that at 27 °C the male NLT is 8 ± 4 d while the female NLT is 42 ± 19 d. *G. pulex* has a reported NLT of 12 months (Nilsson, 1977; Sutcliffe et al., 1981), while according to Mortensen (1982) the NLT is 2 yrs. Welton and Clark (1980) have reported that female and male *G. pulex* have different NLT which are 17 - 23 months and 2 - 5 yrs respectively.

This reflects the difficulties in establishing NLT which is normally not the primary objective of the various research group but rather a secondary objective. Apparently the NLT of these zooplanktons is temperature dependent. In general reduced temperature leads to a reduced metabolic rate, oxygen consumption, respiration and growth rate, resulting in an increased NLT (Korpelaianen; 1986; Paul et al., 1997). Later supported by Lampert et al. (2010) who argued longevity is enhanced at low temperature due to reduced metabolism. Thus a decrease in temperature of the ambient water decreases the metabolism of organism which results in longer NLT. The toxicity data used in this study are obtained from various sources from experiments carried out in water with temperatures ranging from 13 - 30 °C. Thus the NLT of zooplankton at 13 °C temperature can increase test organism's NLT while 30 °C can reduce the NLT.

5.0 Conclusions

The relationship between $\ln LT_{50}$ and LC_{50} using the single stage RLE model with 21 sets of the zooplankton data is linear but nonlinear with four sets. Thus with most species and toxicants it has the competence to effectively relate effects of exposure time and exposure concentration to toxic effects using a linear RLE model. Additionally, the two stage RLE model has been applied and gives an approximation of the relationship of exposure time with exposure concentration to describe the toxicity when the relationship is nonlinear.

Thus zooplanktons exhibit both linear and nonlinear relationships. In both situations slopes obtained are negative, similar to the slopes obtained with fish. Thus toxicity is function of exposure time and decreases in an orderly pattern with increasing exposure time. There are differences in the slopes of these relationships which suggests that groups of toxicants chosen for study ranging from metal ions to organic compounds have different toxic mechanisms. These conclusions are in excellent harmony with the results obtained for fish. Interestingly the calculated NLT obtained from RLE model is found to be different from reported NLT in most cases. However the calculated NLT obtained from the two stage model for S-2 which is the dominant phase of nonlinear relationship, is in excellent agreement with reported NLT.

The results obtained from evaluation of toxicity data with the zooplankton are in excellent agreement with those obtained for fish described in our previous paper but with one exception. The relationship is linear with excellent correlation coefficient for fish while both linear and nonlinear relationship is observed with zooplanktons.

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Table 1: Regression analysis of zooplanktons toxicity data for metals, metalloid and pesticides from the literature

Zooplanktons & Toxicants	Reported* NLT(days)	Calculated NLT (days)	Slope (a)	Regression Coefficient (R ²)
Single Stage Model				
Zooplanktons - Metals				
<i>D.magna</i> – Zn	Avg. 60 (Korpelianen, 1986)	160	-110	0.704
<i>D.magna</i> – Cu (Hoang et al., 2007)		260	-10	0.862
<i>D.magna</i> – Cd as CdCl ₂ (Kooijman (1981)		160	-10	0.840
<i>G.pulex</i> – Cd as CdCl ₂ .2½H ₂ O (I)	400 (Nilsson, 1977)	4	-200	0.873
<i>G.pulex</i> – Cd as CdCl ₂ .2½H ₂ O (P) (McCohan & Pascoe, 1988)		4	-80	0.931
<i>A.salinus</i> – Cu** (Parra et al., 2005)		4	-0.8	0.994
<i>S.major</i> – Cu as CuSO ₄ .5H ₂ O**		6	-40	0.926
<i>S.major</i> – Cd as CdCl ₂ .2.5H ₂ O		4	-80	0.969
<i>S.major</i> – Zn as ZnSO ₄ .7H ₂ O		12	-1,000	0.966
<i>S.major</i> – Pb as Pb(NO ₃) ₂		4	-4,400	0.986
<i>S.major</i> – Fe as FeCl ₃		9	-430	0.964
<i>S.major</i> – Al as Al ₂ (SO ₄) ₃ .18H ₂ O		6	-6,700	0.990
<i>S.major</i> – Mn as MnSO ₄ .5H ₂ O		4	-2,600	0.962
<i>S.major</i> – Ni NiSO ₄ .6H ₂ O (Shuhaimi_Othman et al., 2011)		10	-25,000	0.949
Zooplanktons - Metalloid				
<i>D.magna</i> - Se (Hoang et al., 2007)	60	3	-700	0.934
Range			-0.8 to -25,000	
Average			-2,800	
Standard Deviation			6,500	
Zooplanktons - Pesticide				
<i>C.vidua</i> – Imidacloprid (Sanchez-Bayo & Goka, 2007)	30	4	-2,400	0.815
<i>D.magna</i> – Imidacloprid (Sanchez-Bayo, 2009)	60	110	-2,100	0.571
<i>G.pulex</i> – Thialcoprid (Beketov & Liess, 2008)	400	30	-11,000	0.748
<i>G.pulex</i> – Lindane (Abel, 1980)		2	-550	0.653
<i>G.psuedolimaneus</i> –Fenvalerate(A)	500	6	-0.07	0.983
<i>G.psuedolimaneus</i> –Fenvalerate (J) (Anderson R.L., 1982)	(Bousfield, 1958)	4	-0.24	0.928
<i>G.lacustris</i> - Flucythrinate (Anderson R.L., 1984)	500 (Wudkevich et al., 1997)	5	-0.3	0.991

<i>A.salinus</i> – Dimethoate** (Parra et al., 2005)		5	-9,500	0.994
<i>M.leuckarti</i> – BHC <i>M.leuckarti</i> – Fudaran (Kurve et al., 2010)	75 (Vijvenberg, 1980)	8 43	-2,500 -1,200	0.883 0.819
Range Average Standard Deviation	30 – 500 240 200	2 to 260 50 80	-0.07 to -11,000 -2,900 4,000	
Total Slope Range Average Standard Deviation			-0.07 to -11,000 5,500 2,800	

*calculated from $\ln \text{NLT}_{50} = b/a$, Equation 4. **Lifespan not available in literature.

Table 2: Regression analysis of zooplankton toxicity data with a nonlinear relationship*

Zooplanktons &Toxicants	Reported NLT (d)	Calculated NLT** (d)		Slope (a)		Regression Coefficient (R ²)	
<u>Two Stage Model</u>		S-1	S-2	S-1	S-2	S-1	S-2
Zooplanktons - Metals <i>D.magna</i> – Zn	60	3	620	-700	-40	0.999	0.999
Zooplanktons - Pesticide <i>G. pulex</i> – Thialcoprid	400	8	70	-4300	-400	0.986	0.896
<i>G. pulex</i> – Lindane	400	1	5	-800	-70	0.727	0.843
<i>D. magna</i> – Imidacloprid	60	25	400	-28000	-600	0.892	0.882
Range	60 to 400	1 to 25	5 to 620	-700 to -28000	-40 to -600		
Average	290	10	280	-8500	-280		
Standard deviation	210	10	290	13000	280		

*nonlinear correlation coefficient (R^2) < 0.8**calculated from $\ln \text{NLT}_{50} = b/a$, Equation 4. *** Term S-1 and S-2 are used for Stage-1 and Stage-2 of the two stage model respectively.

Table 3 Regression analysis results obtained for fish with metals and pesticides (Verma et al., 2011).

Fish	Range	Average	STD
Slope - Organic compounds	-0.42 to -210,000	-33,000	68,000
Slope – Metals	-34 to -42,000	-13,300	13,700
Slope Total	-0.42 to -210,000	-21,000	44,000
Reported NLT	360 to 2,600	1,300	800
Calculated NLT	3.6 to 5,000	300	1,100
R ²	0.834 to 0.999

Figure 1: Model showing conceptual relationship between LC_{50} and exposure time for a single stage and a two stage RLE model with central and peripheral system.

Figure 2: Plots of LC_{50} versus $\ln LT_{50}$ for zooplanktons with linear regression lines for metalloid (a) and metal (b, c, d, e) data sets. Normal life expectancy (NLT) is indicated, where one cross on the x-axis represents average of reported NLT values when only one species of zooplankton is involved. When more than one species are involved two crosses represent range of NLT between lowest and highest average NLT. **S.major* and *A.salinus* lifespan is not available in literature.

Figure 3: Plots of LC_{50} versus $\ln LT_{50}$ for zooplanktons with linear regression lines with pesticides with normal life expectancy shown. One cross on x-axis represents average reported NLT where only one species of zooplanktons is involved. When more than one species are involved two crosses represent range of NLT between lowest and highest average NLT.

Figure 4: Plots of LC_{50} versus $\ln LT_{50}$ for zooplanktons with linear regression lines using the two stage RLE model. One cross on the x-axis represents average reported NLT where only one species of zooplankton is involved. When more than one species are involved two crosses represent range of NLT between lowest and highest average NLT.