Reduced Life Expectancy Model Analyses of Exposure Time Effects of Endocrine Disruptors to Teleost Fishes Based on Effect Concentration of Hepatic Biomarkers

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Abstract

In this current paper, the exposure time effects on four endocrine disruptors and teleost fishes were evaluated using the reduced life expectancy (RLE) model based on the effect concentration (EC₅₀) of available literature published. The result on the regression analysis over different exposure times has demonstrated that the EC₅₀ of hepatic biomarkers falls with increasing exposure times in a predictable manner. The slopes of the regression equations reflect the strength of the toxic effects on the various teleost fish. The EC₅₀ reduction over time can be interpreted based on the bioconcentration process, which can be used to understand transfer routes of the compounds from water to fish body. RLE model also provides useful information in assessing the toxic effects on fish life expectancy as a result of the occurrence of compounds.

Keywords
Reduced Life Expectancy Model, Endocrine Disruptors, Effect Concentration, Hepatic Biomarkers, Teleost Fishes

1. Introduction

The endocrine disruptors are chemicals that may interfere with the body’s endocrine system and produce genotoxicity, reproductive toxicity, carcinogenic and metabolic disorders in both humans and wildlife [1] [2] [3] [4]. The chemicals that are known endocrine disruptors include 17α-ethynylestradiol (EE₃), bisphenol A (BPA) and nonylphenol (NP). Endocrine disruptors can be interfere or
block the way natural hormones (17β-estradiol, E2) binding to their receptors, or altering metabolism in the liver and vtg mRNA expression [4] [5]. Due to the low water solubilities and high octanol-water partition coefficients, endocrine disruptors are relatively stable in the environment [6] [7]. Therefore, it is important to study the estrogen effects of endocrine disruptors on organisms and to evaluate the potential risks of endocrine disruptors to the environment.

The teleost fishes also has about high genetic homology to humans, which are a popular model organism for studying molecular toxicology in vitro and in vivo of the endocrine disrupters [8] [9]. The vtg genes or VTG protein can be used as hepatic biomarkers to appraise the effect of endocrine disrupters [10] [11]. Many toxicological studies usually focus on concentration-response relationship based on biomarker in risk assessments. Relatively fewer studies exposure time has been studied as a quantifiable variable of toxic effects [12]. Reduced life expectancy (RLE) model which is based on the influence of exposure time has been developed to study time-response relationship [13]. Therefore, it is of significant for assessing the exposure time effect to hepatic biomarkers of teleost fishes due to the endocrine disrupters using the RLE model.

In previous study with zebrafish [5] was evaluated the relationship between exposure time and the EC₅₀ of hepatic biomarkers based on the analogy of RLE model. It was noted that the relationship between natural logarithm of exposure time (lnET₅₀) and EC₅₀ for zebrafish was linear. The RLE model allows the normal life expectancy (NLT) to be calculated from the toxicity data [12]. In previous study, the reported NLT and calculated NLT obtained were in general agreement [5]. The published literature has mainly focuses on the application of RLE model for the exposure time effect of a certain fish, but there are few studies on whether the relationship between EC₅₀ and exposure time of different types of fishes can be described using the RLE model.

The objectives of present study were to explore the RLE model for teleost fish based on the toxicological data of endocrine disruptors available in the literature published. The characteristics obtained from analogy of the RLE model would be used to analyze the relationship of exposure time with EC₅₀ of hepatic biomarkers and for estimation of effects and routes of the EC₅₀ reduction on fish life expectancy. The research results are of significance for the risk assessment of different types organisms as a result of endocrine disruptors in the environment.

2. Methodology

2.1. Organisms and Compounds Selected for Evaluations

Teleost fish were selected as organisms for this study since lots of toxic effects data related to this fish are available. Teleost fish are key components of both marine and freshwater food chains and serve as one of the important source of food for human [14] [15]. Thus teleost fish have a key role to play in the trophic structure of aquatic ecosystems, vital for energy transfer between the trophic levels [16]. It would be significant to evaluate the effects of exposure time on such
a key component of aquatic systems. Zebrafish, fathead minnow, brown trout, and rainbow trout are the organisms selected for study (Table 1). Toxicants having similar toxic mechanism are selected for this study and they included known endocrine disruptors (EE2, BPA and NP) and natural hormones (E2). Among these toxicants enter the environment through human activities [2] [17].

### 2.2. Sources and Collection of Data

Toxic effects data related to teleost fish with these particular endocrine disrupters were obtained from the literature published (Table 1). These data sets include EC50 for hepatic biomarkers (vtg genes or VTG protein) of teleost fish at different exposure times. The liver is an organ mainly characterized by metabolic function in the teleost fish [18] [19], so it is of significance to select hepatic biomarkers as research targets. The data obtained from the literature are in various units for concentration such as ng/L - μg/L. For consistency all units were converted into g/L. Similarly exposure time was also expressed in various units (hours and days) of time and all were converted into days (d). The NLT data of each organism was also obtained from literature published.

### 2.3. Reduction Life Expectancy (RLE) Model

The linear RLE model [20] was developed with the use of the concept of reduction in life expectancy and the model equation is given below:

| Compounds | Fish      | Observed EC50 | Exposure time (day) | Reference |
|-----------|-----------|----------------|---------------------|-----------|
| E2 (ng/L) | zebrafish | 41.2           | 8                   | [30]      |
|          | brown trout | 15.1           | 12                  | [31]      |
|          |           | 15             | 14                  | [32]      |
|          | fathead minnow | 25            | 14                  | [33]      |
|          |           | 60.7           | 5                   | [34]      |
|          | zebrafish | 30.46          | 5                   | [35]      |
| EE2 (ng/L)| zebrafish | 2.51           | 8                   | [30]      |
|          | brown trout | 5.2            | 12                  | [31]      |
|          | fathead minnow | 0.9          | 14                  | [33]      |
|          |           | 248.11         | 6                   |           |
|          | zebrafish | 193.88         | 9                   | [5]       |
| BPA (μg/L)| fathead minnow | 158     | 14                  | [33]      |
|          | zebrafish | 166.29         | 15                  | [5]       |
|          | rainbow trout | 14.14  | 8                   | [36]      |
| NP (μg/L) | brown trout | 6.9            | 12                  | [31]      |
|          | fathead minnow | 7.02     | 14                  | [33]      |
\[ LC_{50} = -a \ln LT_{50} + b \] (1)

Equation (1) is where \( LC_{50} \) is the lethal concentration, \( LT_{50} \) is the exposure time, \( NLT_{50} \) is the normal life expectancy of the organism, \( d \) is a constant, \( a \) is \( \frac{1}{d} \) and \( b \) is \( \ln NLT_{50}/d \).

Consistent with previous studies, \( LC_{50} \) and \( EC_{50} \) are frequently used in various tissues as toxicity endpoints, which is related to nominal concentration and \( \log Kow \) (octanol partition coefficient) [21]. Consequently, the relationship could be extended from the \( LC_{50} \) to the \( EC_{50} \) in the RLE model. Based on this extension a RLE model [5] for estrogenic effect was proposed and may be described by the equation given below:

\[ EC_{50} = -a \ln ET_{50} + b \] (2)

Equation (2) is where \( EC_{50} \) is the effective concentration, \( ET_{50} \) is the exposure time, \( NLT_{50} \) is the normal life expectancy of the organism, and \( a \) and \( b \) are constants as previously defined.

Equation (3) is when \( EC_{50} \) is zero the organism will have a normal life expectancy and it is related to the model constants as follows:

\[ \ln NLT_{50} = \frac{b}{a} \] (3)

2.4. Processing of Data

The data sets for each teleost fish were used to evaluate the relationship between \( EC_{50} \) and \( \ln ET_{50} \) with the RLE model expressed in Equation (2). Thus \( EC_{50} \) was plotted against \( \ln ET_{50} \) and linear regression analysis was used to obtain the regression equation and the correlation coefficient (\( R^2 \)) using Origin software (Microcal Software Inc., Northampton, Massachusetts, USA). The values of the slope (\( a \)) and intercept (\( b \)) were obtained from the regression equation (Table 2). These values were then used to obtain the calculated NLT of each organism by the use of Equation (3) (Table 3).

3. Results and Discussions

3.1. Relationship of Exposure Time with Toxic Effects Based on the \( EC_{50} \) Values

The plots of \( \ln ET_{50} \) against \( EC_{50} \) based on Equation (2) are shown in Figure 1.

Table 2. Characteristics of the regression equation relating \( EC_{50} \) to \( \ln ET_{50} \) for the endocrine disrupters.

| Compounds | Slope (\( a \)) | Intercept (\( b \)) | Regression coefficient (\( R^2 \)) |
|-----------|----------------|---------------------|----------------------------------|
| E2        | -3.91E-08      | 1.20E-07            | 0.5530                           |
| EE2       | -4.43E-08      | 1.12E-07            | 0.5735                           |
| BPA       | -9.18E-05      | 4.07E-04            | 0.9264                           |
| NP        | -1.37E-05      | 4.22E-05            | 0.8425                           |

The slope (\( a \)) and intercept (\( b \)) were obtained from the regression equations. \(^1\)For \( EC_{50} = -a \ln ET_{50} + b \). \(^2\)For \( \ln NLT_{50} = \frac{b}{a} \).
Table 3. Comparative analysis of calculated normal life expectancy and reported normal life expectancy.

| Fish       | Compounds | Calculated NLT (d) | Reported NLT (d) | Slope (a) | Regression coefficient (R^2) |
|------------|-----------|--------------------|------------------|-----------|-----------------------------|
| teleost fish | E2        | 22                 | Zebrafish 930 - 1350 [37] [38] [39] | 176.92    | 0.9202                      |
|            | EE2       | 13                 | Fathead minnow 540 - 1095 [40] | 177.67    | 0.9180                      |
|            | BPA       | 84                 | Brown trout 1000 - 1400 [41] | 171.75    | 0.9349                      |
|            | NP        | 22                 | Rainbow Trout 730 - 1095 [42] | 176.92    | 0.9202                      |

For lnNLT = b/a.

Figure 1. Plots of EC50 versus lnET50 for teleost fish with linear regression lines for E2 and EE2 data sets. Normal life expectancy (NLT) is indicated, where black dots on x-axis represents reported NLT range of different types of teleost fishes. The orange and green lines are displayed as regression lines for E2 and EE2. The horizontal and vertical dash lines indicate specific EC50 values and corresponding ET50 values.

Figure 2. These plots utilize data from Table 2 on teleost fish for EC50 due to short-term exposure to endocrine disruptors. Plots for the E2 and EE2 are shown in Figure 1 and the corresponding plots for BPA and NP are shown in Figure 2. The characteristics of the relationships established using the regression equation can be used to compare the toxic effects of the E2, EE2, BPA and NP to teleost fish based on EC50 of hepatic biomarkers (Table 2). Table 2 indicates that slopes of E2, EE2, BPA and NP between −9.18E−05 and −3.91E−08 and R^2 value between 0.5530 and 0.9264. The slopes of the regression equations reflect the strength of the toxic effects on the various teleost fish. Since the range of slopes obtained for the E2 and EE2 is from −4.43E−08 to −3.91E−08 while for the BPA...
Figure 2. Plots of EC$_{50}$ versus lnET$_{50}$ for teleost fish with linear regression lines for BPA and NP data sets. Normal life expectancy (NLT) is indicated, where black dots on x-axis represents reported NLT range of different types of teleost fishes. The magenta and blue lines are displayed as regression lines for BPA and NP. The horizontal and vertical dash lines indicate specific EC$_{50}$ values and corresponding ET$_{50}$ values.

and NP is from $-9.18E-05$ to $-1.37E-05$. The difference between the slopes is larger. Different toxic mechanisms involved with phenols and steroids may be responsible for this difference [22] [23]. Firstly, the observed EC$_{50}$ of E$_2$ and EE$_2$ are often at the level of ng/L, and the observed EC$_{50}$ of BPA and NP are often at the level of μg/L (Table 1). Secondly, the interaction potential of E$_2$ was the most potent, followed by BPA and NP [5]; the estrogenic activity of E$_2$ was much higher than that of BPA and NP [24]; the estrogenic potential of EE$_2$ was much higher than that of E$_2$, and the lowest observed effect concentration (LOEC) of E$_2$ and EE$_2$ is lower than BPA and NP [25]. And thus, the slopes is comparatively higher for the BPA and NP than for the E$_2$ and EE$_2$, and the slopes of E$_2$ and EE$_2$ is extremely small.

3.2. Interpretation of the EC$_{50}$ Reduction over Time Based on the Bioconcentration Process

All the regression relationships have negative slopes (Table 2) which indicates that the EC$_{50}$ of hepatic biomarkers is related to the exposure time and declines as the exposure time increases. These results are in accord which were studying the time dependent effects of E$_2$, BPA and NP to zebrafish hepatic vtg1 gene [5]. Other researchers also reached the same conclusion while studying the toxic effects of organic pollutants to fish [12]. The transfer of compounds from water to fish is the first step in the development of toxic effects [26]. In fish the routes of compounds uptake are from gills, food and outer body surface but uptake of compounds takes place mainly via gills [27].
can transfer from respiratory surfaces and gastrointestinal tract through circulatory fluid to liver in fish. In the process, some effect loss of the compounds, the compound continues to accumulate in the fish liver, and the increased metabolism of the hepatocytes and then the liver injury became exacerbated, these factors could be used to explain the decrease in the toxic effects over time. This was consistent with in previously studies [5] [28].

3.3. Correlation between the Toxic Effects and Fish Life Expectancy Based on the Reported and Calculated NLT

Calculated NLT is compared with reported NLT and calculated NLT differs from reported NLT (Table 3). Reported NLT and calculated NLT of teleost fish are in the range of 540 - 1400 d and 13 - 84 d respectively. The ratio of averages of reported NLT and calculated NLT is 1006/35 and the standard deviation is 311/33. The correlation coefficient (R²) obtained from the plots of reported NLT against calculated NLT was greater than 0.91 in a general accord (Table 3). The results show that the correlation between reported NLT and calculated NLT is better. The NLT introduces a fixed limiting point for a teleost fish and it is a reference point for the reduced life expectancy in fish exposed to the compound [12]. In Figure 1, Figure 2, from the cross point of regression line and x-axis to the reported NLT range, the trend changes of toxic effects based on the EC₅₀ of hepatic biomarkers can be predicted by the RLE model when no data available for these extended exposure times. In other words, the toxic effects of a compound can be described using the RLE model and be corrected the experimental data to a specific time point. It is noteworthy that the days from the each exposure time point to the reported NLT range is the days in which fish life expectancy reduced after exposure to the compound (Figure 1, Figure 2 x-axis). These analyses were also referred in the previously RLE model studies [13] [29].

4. Conclusion

This study investigated the RLE model for teleost fish based on the toxicological

Figure 3. Diagram of routes of endocrine disrupters in teleost fishes.
data of endocrine disruptors available in the literature. Analogy of the RLE model can be useful to analyze the exposure time effects of teleost fish by using EC50 as toxicity endpoint and to understand routes of the EC50 reduction over time and effects of the toxic effects on fish life expectancy. The study method used may be extended to other fish species and even other organisms as well.

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Conflicts of Interest
The authors declare no conflicts of interest regarding the publication of this paper.

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