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Study on Tributyltin Chloride Accumulation Factors in Fish Tissue by Analysis of Variance

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ABSTRACT

In this article, statistical survey has been used to investigate effective parameters of tributyltin chloride bioaccumulation in food in one of the native fish species of Persian Gulf, Scat fish (Scatophagus argus), within a time period of 21 days at marine simulated aquarium bioassay. The Design Expert software (ver. 7) was applied for statistical analyzing the effects of biocide concentration in food and exposure time on compound concentration variations in fish tissues. Effects of concentration on the amount of absorption and accumulation of TBT were significant. The results show that there is relation between these factors. The results of the statistical analysis illustrate that exposure process is more sensible to the exposure time than to concentration of the biocide. The results present that body of fishes resist against biocide at limited period, and cellular metabolism controls the amount of bioaccumulation of biocide for a while, but bodies would lose their abilities and bioaccumulation would increase instantly and rapidly. The results have been improved by a modified cubic experimental model. Prog. Color Colorants Coat. 5(2012), 65-74. © Institute for Color Science and Technology.

1. Introduction

Butyltin compounds (BTs) are a group of man-made compounds that are being used in variety of industrial, agricultural and domestic products and are ubiquitous environmental contaminants [1]. Tributyltin (TBT) has been applied world-wide in antifouling paints and coatings. However, due to the gradual dissolution of this compound, it poses an environmental pollution danger to the oceans [2]. TBT is a hydrophobic substance with an octanol/water partition coefficient (log K_{ow}) varying according to the pH and salinity. Previous researches have indicated that the partition coefficient of Tributyltin chloride (TBTCl) varies from 3.21 to 3.85 with pH from 5.8 and 7.8, respectively. The lipophilic nature is conferred to nTBT by the alkyl groups to facilitate its bioaccumulation in living organisms [3].

The danger includes the risk of toxicity to marine organisms and fish after prolonged exposure and causes some severe environmental problems, such as

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neogastropod impo-sex, shell malformation of oysters and high accumulation by mussels [4]. Life forms from plankton to vertebrates are natural bioaccumulators of these compounds. Fishes live in close contact with their aquatic environment. This intimate contact facilitates the movement of chemicals into and through mucus, skin, gills, and other external layers and becomes a disadvantage when dangerous chemicals, pollutants, and contaminants enter the aquatic environment [5].

Experimental designs were first introduced by Fisher as an agricultural research tool in the 1920s [6, 7]. His primary aim was to obtain the most information about a process with the least number of experiments. Experimental designs and optimization methods for scientific and engineering branches were reviewed by Anderson and Whitcomb [7], Debets [8] and Adams et al. [9].

It should be emphasized that experimental design, factorial design and Taguchi method [6], are merely a tool and will not replace sound technical judgment or creativity in experimental work. In a broad way, the purpose of statistical experimental analysis is to investigate the significance of systematic effects. The application of this kind of experiments requires careful planning, prudent layout of experiments, and expert analysis of results. Generally, statistical project consists of five fundamental steps [6]:

- ♦ Problem definition
- ♦ System identification
- ♦ Statistical model formulation
- ♦ Data collection
- ♦ Statistical analysis and results

In this research, the experimental design methodology was used to schedule the operating variables for experimental runs, and the analysis of variance (ANOVA) was applied to discuss relationship between the response and the factors. The one-factor ANOVA is to study the effect of a single variable on response. Since the more reasonable and accurate experimental results were always accompanied with more cost and experimental runs, the two-level factorial experimental design methodology was adopted to schedule operating variables for experimental runs to reduce waste of resources. In addition to adopting the experimental design methodology, the method of ANOVA was used to analyze the effects of operating variables (factors) on mass transfer performance (response), to discuss the relationship between factorial interaction and mass transfer coefficient [10].

There are many studies about effects and bioaccumulation of TBTCl that the ANOVA method has been used for statistical analysis. In previous researches, the results have been summarized to interaction effect charts and statistical parameters, such as variance [1-5, 11-19]. In this study, the experimental results have been analyzed from different points of view by the Design Expert software (ver. 7). The software fitted experimental data to different equations, and it analyzed and selected the best equation. This equation has been extracted in this paper and modified for further applications.

2. Experimental

Tributyltin chloride (TBTCl), triphenyltin chloride (TPTCl) and other chemicals were supplied from Merck Chemical Company. Experiments were done in five glass aquaria (60 L) with artificial seawater at the same condition of Persian Gulf (pH=8 – 8.3, salinity= 26 – 27 ppt and dissolved oxygen= 3.43 -4.7 mg l^{-1}) and nine spotted scat (Scatophagus argus) fish with the body weight of 20 – 28 g were placed in each of them and maintained as a static system at 27 ± 1°C, and a light / dark regime of 16 h/8h during the experiment. For the oral – intake experiment, various tributyltin chloride dosages were made with different biocide concentrations (0; 0.00264; 0.0264; 0.0528 and 0.264 μg ml^{-1} in ethanol) and 0.5 ml of each dosage was added to 0.5 g of the commercial feed at room temperature. The feed containing TBTCl was given to the fishes at the rate of 0.5 g per fish twice a day during the experiments and three fishes were taken from each aquarium after 7, 14 and 21 days and freeze-preserved for the analysis. Fish samples were homogenized by meat grinder and extracted under the acidic condition of HCl, ethanol-ethyl acetate (1:1) and diethyl ether-hexane mixture (3:1). Then the organic layer was cleaned up with silica gel-sodium sulfate anhydrous, acetone-hexane (1:3), diethyl ether-hexane (1:3) and hexane-diethyl ether-acetic acid (75:25:1). The final acid portion was collected and used for organotin analysis. TPTCl was used as internal standard. Tributyltin chloride was detected by a Hewlett–Packard (Avondale, PA, USA 5890 series II) gas chromatograph equipped with an electron capture detector (ECD) [20].

An apolar fused-silica capillary column with a thin immobilized stationary phase of DB-1 (15m×0.25mm

I.D., 0.1μm film thickness, J&W Scientific, Rancho Cordova, CA, USA) with a dilute HBr-methanolic solution was utilized. The detection limit of $BuSnCl_3$ was 0.05 ng g^{-1} [21].

2.1. Statistical Analysis of Experiments

For this research, experimental analysis has been done by Design Expert Software (ver. 7) pack [22] and via 48 experimental data in Historical Data format. In these experiments, there were time and concentration, as 2 factors, and one response. The factors and the levels were considered for these experiments are shown in Table 1. Zero concentration has not been considered in this table.

Since the time's interval was constant, the time was considered as quantitative factor, and because the lack of this conditions, the concentration was considered as quantitative factor. Since every test is done in 3 times, 48 experiments were obtained. Table 2 shows experiment's conditions and responses and Figure 1 points out normal residuals distribution for which it was not necessary to apply response data converter because of its normal distribution.

To investigate factors behavior on the response of the tests, a model should be worked out. As mentioned before, it is a mathematical model which is used to forecast responses. In Table 3, the statistical modality survey is shown for different models.

Table 1: Factors and levels of statistical analyses of data.

Code	Factors	Unit	Levels			
A	Time	Day	0	7	14	21
B	Concentration in Food	$\mu g ml^{-1}$	0.00264	0.0264	0.0528	0.264

Table 3: Statistical qualitative study on various fitted models by software.

Source model	Std Deviation	R^2	Adjusted R^2	Predicted R^2	Square of sum error
Linear	0.320822	0.736653	0.712155	0.665005	5.629983
2FI	0.148831	0.94728	0.938054	0.924459	1.269549
Quadratic	0.139326	0.954953	0.945713	0.932367	1.136656
Cubic	0.070704	0.989589	0.98602	0.982192	0.29929

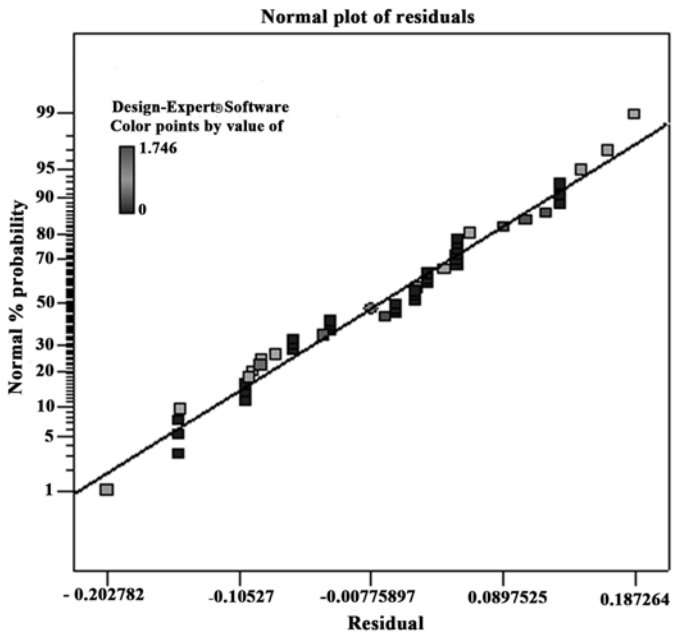

Figure 1: Normal distribution curve of residuals.

Table 2: Conditions of experiments and responses.

Std.	Run	Factor 1 A : Time, day	Factor 2 B :Concentration in Food, µg / ml	Response Y : Concentration in Fish, ng / g
1	12	0	0.00264	0
2	25	0	0.00264	0
3	40	0	0.00264	0
4	45	7	0.00264	0
5	23	7	0.00264	0
6	4	7	0.00264	0
7	32	14	0.00264	0
8	1	14	0.00264	0
9	15	14	0.00264	0
10	37	21	0.00264	0
11	27	21	0.00264	0
12	35	21	0.00264	0
13	3	0	0.0264	0
14	8	0	0.0264	0
15	24	0	0.0264	0
16	31	7	0.0264	0
17	36	7	0.0264	0
18	48	7	0.0264	0
19	6	14	0.0264	0
20	2	14	0.0264	0
21	22	14	0.0264	0
22	33	21	0.0264	0
23	11	21	0.0264	0
24	9	21	0.0264	0
25	47	0	0.0528	0
26	46	0	0.0528	0
27	16	0	0.0528	0
28	20	7	0.0528	0.376
29	44	7	0.0528	0.356
30	28	7	0.0528	0.395
31	13	14	0.0528	0.41
32	43	14	0.0528	0.356
33	39	14	0.0528	0.464
34	5	21	0.0528	0.94
35	34	21	0.0528	0.92
36	30	21	0.0528	0.9
37	42	0	0.264	0
38	29	0	0.264	0
39	19	0	0.264	0
40	26	7	0.264	0.93
41	14	7	0.264	0.95
42	10	7	0.264	0.94
43	17	14	0.264	1.724
44	41	14	0.264	1.708
45	38	14	0.264	1.739
46	7	21	0.264	1.7
47	18	21	0.264	1.746
48	21	21	0.264	1.654

According to Table 3 whatever the calculated R^2 are closer to 1 the fitted model situation would be suitable. For a model, the R^2 near 1 point out a proper model selection on the experiments. Last column shows square of sum error for each model. For last model which has better match to the experiments, the level of the PRESS column would be less.

According to the obtained consequences from table 3, the cubic model has been chosen as the best presented model to practice the tests by software.

The equation of cubic experimental model which estimates the response for coded amounts is:

$$Y = + 0.44 + 0.33A + 0.003B[1] - 0.005B[2] - 0.001B[3] + 0.003AB[1] + 0.014\ AB[2] + 0.0001AB[3] - 0.12A^2 - 0.0015A^2B[1] - 0.01\ A^2B[2] - 0.0003\ A^2B[3] - 0.0012\ A^3B[1] - 0.03\ A^3B[2] - 0.0018\ A^3B[3] \quad (1)$$

where A, B and Y are nomenclatures for time, concentration in Food, and concentration in Fish, respectively (Table 2). B[1], B[2] and B[3] are exposure concentrations (0.0264, 0.0528, 0.264 μg/ml, respectively) which have been chosen quantitatively according to their unequal intervals.

As it was pointed, the purpose of variance analysis is the recognition of effective factors and importance of their arrangement in response to tests by a series of mathematical functions. Table 4 indicates the response of the variance analysis.

It comes to consider that the suggested model is meaningful from statistical point of view and there is less than 0.01% probability that greater F-value is created by unanticipated agents. In addition, the depletion of selected model is not important from statistical point of view because P-value is more than 0.1. Both factors and their interactions have meaningful and important effect on response of experiments from the statistical point of view.

3. Results and discussion

With the described method of analysis and 0.1 ng/g detection limit, different concentrations of tributyltin chloride were detectable in experimented fish tissue after the period of the 7, 14 and 21 days.

Figure 2 indicates the effect of concentration on experimental response from the first day to 21th day. It means that effects of concentration on the absorption amount and accumulation of TBT are significant. The variation of TBT concentration has had different effects on the amount of its absorption into the fishes tissues.

Table 4: Variance analysis of software suggested response equation.

Source	Sum of squares	df*	Mean square	F Value	p-value, Prob > F
Model	16.79282	14	1.199487	2967.54	< 0.0001
A:Time	2.824038	1	2.824038	6986.691	< 0.0001
B: Concentration	9.556263	3	3.185421	7880.755	< 0.0001
AB	3.539837	3	1.179946	2919.194	< 0.0001
A^2	0.128961	1	0.128961	319.0513	< 0.0001
A^2B	0.581063	3	0.193688	479.1846	< 0.0001
A^3B	0.16266	3	0.05422	134.1408	< 0.0001
Residual	0.013339	33	0.000404		
Lack of Fit	0.001033	1	0.001033	2.687225	0.1110
Pure Error	0.012305	32	0.000385		
Cor Total	16.80616	47			

*df = Degree of Freedom

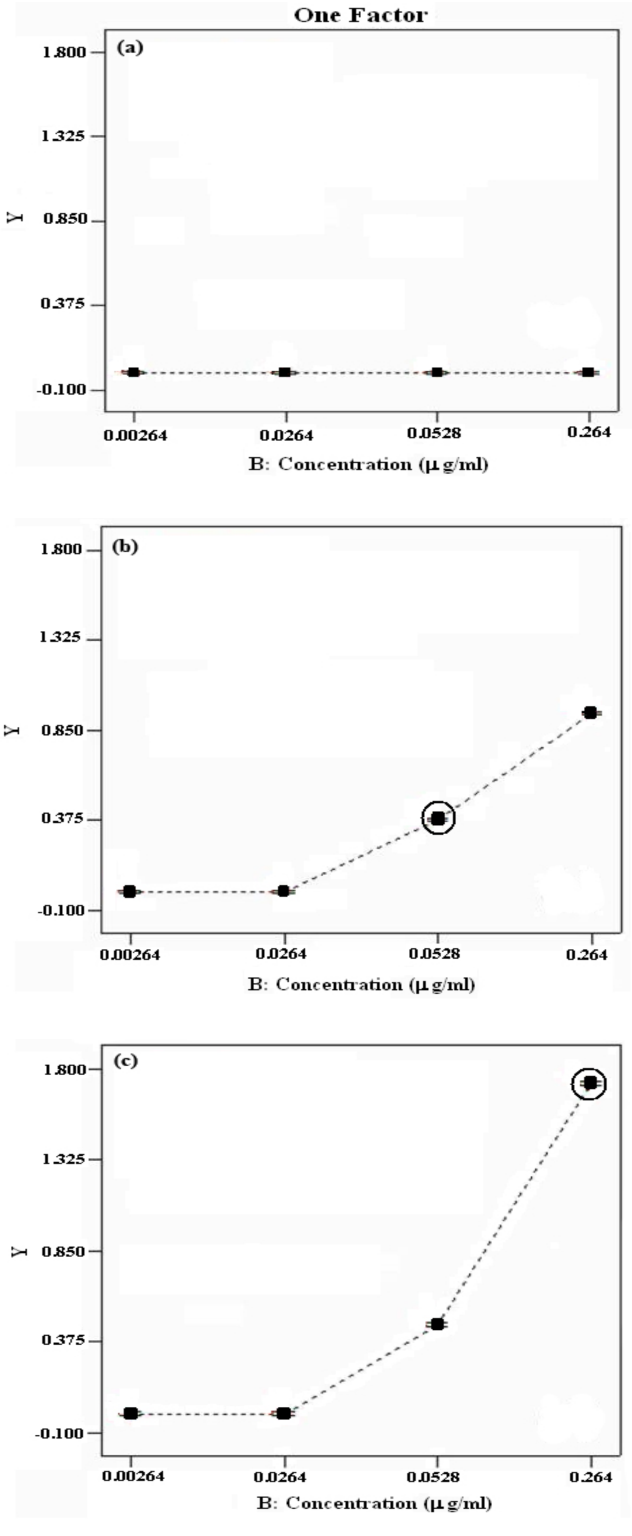

Figure 2: Concentration effect on response (O) after (a) 0 day, (b) 7 days, (c) 14 days and (d) 21 days.

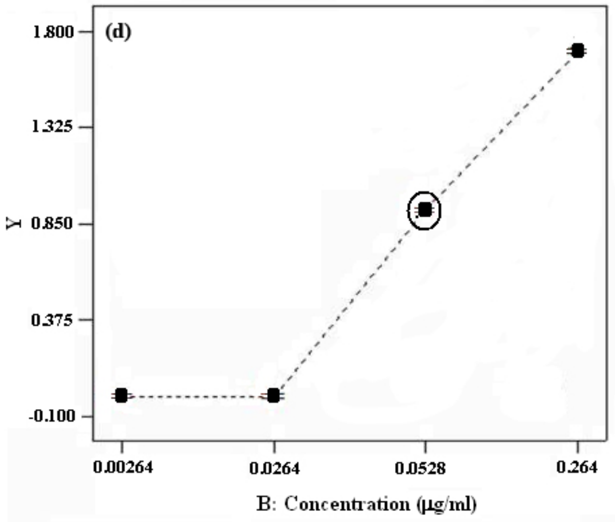

Figure 2: Continued.

Because of the low exposure concentration of fishes to tributyltin chloride through the food, the high level of excretion and rapid degradation of contaminated biocide, TBTCl cannot be measured in these low exposure concentrations. According to these experiments, it is probable that the cellular structures and metabolic mechanism have been changed in order to increase cellular absorption of TBTCl in higher exposure concentrations.

Figure 3 shows the effect of time on response when exposure concentration changes from its minimum (0.00264 µg/ml) up to its maximum (0.264 µg/ml) level.

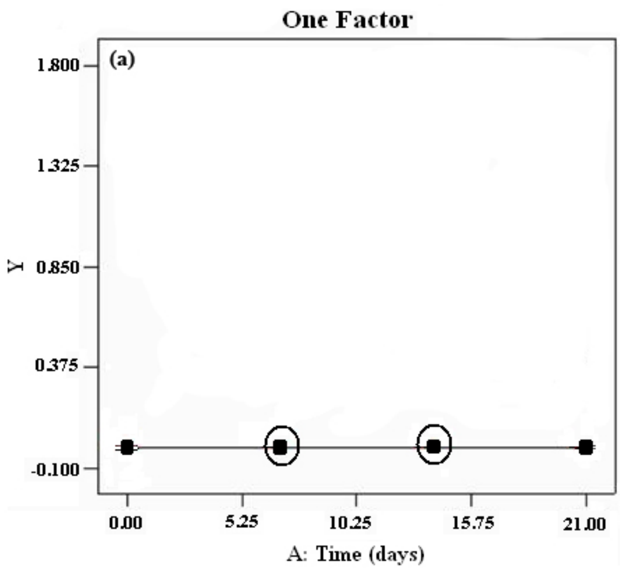

Figure 3: Time effect on response (○) in exposure concentration of (a) 0.00264 µg/ml, (b) 0.0264 µg/ml, (c) 0.0528 µg/ml and (d) 0.264 µg/ml.

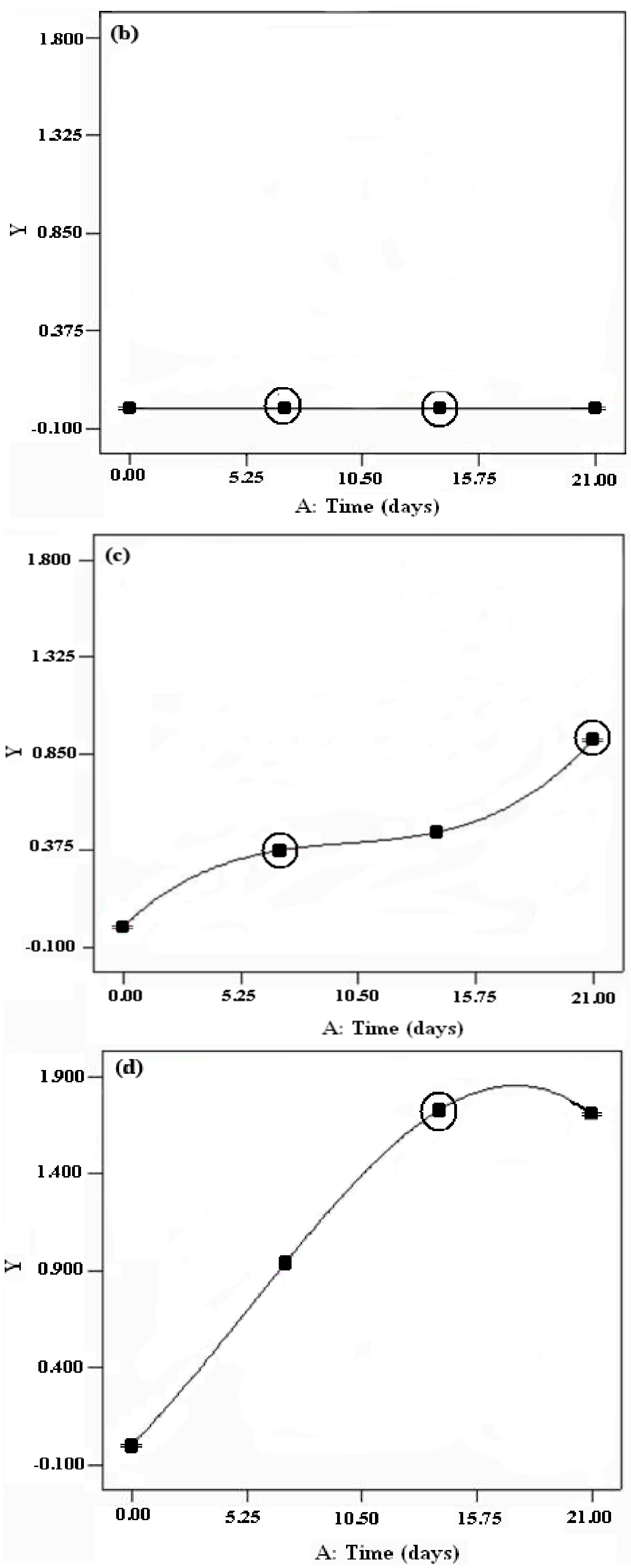

Figure 3: Continued.

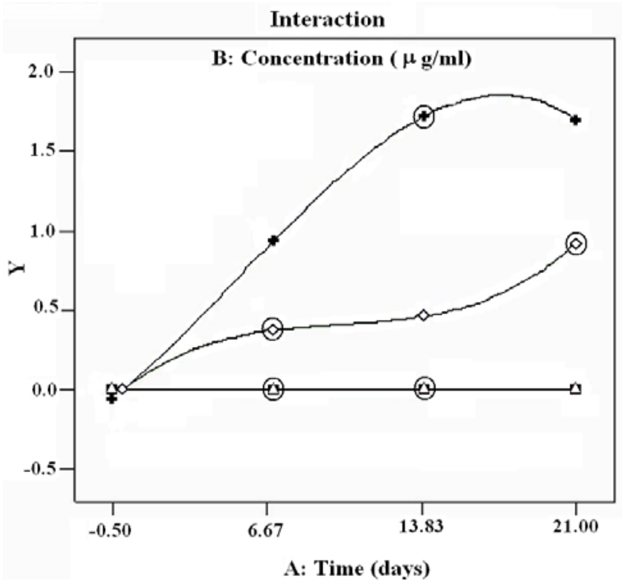

Figure 4: Interaction effect of time and concentration (■ 0.00264 μg/ml, △ 0.0264 μg/ml, ◊ 0.0528 μg/ml and ✚ 0.264 μg/ml) on the response (○).

Figures 3(a) and 3(b) point the effect of concentration of compound in environment on bioaccumulation, so that no TBTCl bioaccumulation was detected in the two low exposure levels. In figures 3(c) and 3(d), it is clear that there are significant differences in the amount of bioaccumulation by time.

High bioaccumulation rate of tributyltin chloride is observed in 0.0528 μg/ml (Figure 3 (c)) at the first time interval (from 0^{th} day to 7^{th} day), but the bioaccumulation does not show any growth during 7^{th} to 14^{th} day. Fish's body attains temporary stability in this exposure concentration and time range. Finally, the amount of accumulation would be increased after 14^{th} day.

In 0.264 μg/ml exposure concentration, the bioaccumulation of TBTCl would be raised from 0^{th} to 14^{th} day; it would not increase and stabilize in the period of 14th to 21st days. Non-linearity of the relation between bioaccumulation and time is obvious.

In addition to effects of main factors, the interaction effects were surveyed to get the better interpreting of the process and conditions. Figure 4 shows the interaction effect of the time and the concentration on the response.

As it is perceived in Figure 4, because the trend of changes of response is sensible to exposure concentration in constant time, there is interaction between main parameters.

But this model is unable to replicate properly to required estimations according to its quality part and merely its limitation to the three exposure concentrations. For this reason and according to the structure of equation 1, quantity terms were recommended for both time and exposure concentration and their validations were studied, and equation 2 showed the best match to experimental data. A simple tried and error method has been applied to find the best relation. The validity of the equation has been checked by curve fitting method. The R^2 of this model is 0.9946 that is more suitable than software's suggested model (0.9896).

$$Y = -0.07964396 - 0.035239771A + 2.396728175B + 0.54652438AB - 0.009466A^2 + 0.110115A^2B - 0.00211614A^3B - 0.845601161AB^2 - 0.22090057A^2B^2 + 0.000268974A^3 - 7.975110161B^2 \quad (2)$$

In this equation, terms such as AB, A^2B and so on are explanatory of interactions between time and exposure concentration as two independent variables. Since time has sentences with higher degree, it has more effect on this process.

4. Conclusion

Results of the statistical analysis showed that exposure process is more sensible to the exposure time than to concentration of the biocide. In addition, there are interactions between variables. This interaction has been shown by statistical analyses of experimental results obtained from poison exposure of fishes. Moreover, it has been shown that the body of fishes resisted against biocide at limited period and the cellular metabolism controls the amount of biocide bioaccumulation for a while. Besides, the experimental results and statistical analysis illustrate that the cells can not resist against poisoning with TBTCl for ever, and ability control of fishes would be reduced progressively. Raising the amount of TBTCl and exposure time would increase the bioaccumulation rate.

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