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Assessment of Acute Toxicity of Tributyltin on *Gammarus fossarum* using the Multispecies Freshwater Biomonitor©

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ABSTRACT: *Gammarids were exposed to different concentrations of Tributyltin - TBT (0.5µl/l, 1µl/l, 2.5µl/l) for 24 hours. Tests were conducted to identify the toxic range of TBT for Gammarids. Using Multispecies Freshwater Biomonitor (MFB), the normal behaviour of 8 Gammarids were recorded as control before exposing them to different concentrations of TBT for 24 hours. Stress ventilation (2.5 – 5 Hz, band 2) was observed in TBT concentration of 0.5µl/l (at low concentration), while at high concentrations (1µl/l, 2.5µl/l) toxicity increased the morbidity with a decreased locomotion (between 0 – 2Hz, summarised in band 1). 50% and 100% mortality was observed visually while exposed to 1µl/l and 2.5µl/l concentrations of TBT respectively. Test results leads to the conclusion that TBT concentration of 1µl/l was 24-hr LC_{50} in G.fossarum.*

Keywords: *Gammarids, Multispecies Freshwater Biomonitor, TBT, toxicity, locomotion, stress ventilation*

I. INTRODUCTION

According to European water and wastewater laws, biological and chemical water quality of surface water bodies should reach and maintain a "good" quality status [EU-Water framework Directive, 2000]. In order to achieve and monitor the success of different measures and improvements on both the technological and management levels, environmental legislation clearly defines the need for regular monitoring of organic substances down to nano gram per litre levels. Since many of the so called priority substances to be monitored in water were rather difficult to analyze, European expert groups have developed a unique biological - ecotoxicological online biomonitoring system (Multispecies Freshwater Biomonitor© (MFB) (Gerhardt et al., 1994).

Tributyltin is an organotin compound consisting of one to four organic components attached to a tin atom via carbon-tin covalent bonds. The most common use of TBTs is as marine anti-fouling agents, to prevent the growth of algae and barnacles on the hull of ships and on fishing nets. They are also used as fungicides in preservatives for wood, textiles, paper, plastics etc. (Dobson and Cabridenc, 1990). The most significant releases of TBTs are likely to occur as gradual leaching from products treated with pesticides containing them. TBTs are toxic to aquatic life because it is extremely toxic to non-target organisms (eg: *Gammarus fossarum*) . TBTs can bind strongly to particles in water bodies and to sediments and can then persist for a considerable time (EPA, 2003). According to the Environmental quality standards applicable to surface water (by Environmental Quality Standards Directive, 2008), TBT should be below 0.0002µg Sn/l in surface water. The purpose of this study was to monitor the behaviour of *G.fossarum* while exposed to various TBT concentrations using MFB and also to find the 24 hour LC_{50} value of TBT on *G.fossarum*.

II. MATERIALS AND METHODS

Multispecies Freshwater Biomonitor© (MFB) :

The Multispecies Freshwater Biomonitor© (MFB) (typical type of automated biomonitoring system available in European countries) based on quadrapole impedance conversion technique is a "biological early warning system" (BEWS) for online water quality biomonitoring (Gerhardt et al., 1998). Impedance conversion is an appropriate method for detecting stress responses to metals and can be used in "early warning" biomonitoring systems as well as for acute and chronic behavioural toxicity testing (Gerhardt, 1995). MFB uses all aquatic and sediment invertebrate and vertebrate species for biological early warning of pollution pulses in surface, waste and drinking water (marine, freshwater) and sediments for continuous biomonitoring in laboratory as well as directly in situ.

The non-optical recording principle allows for recording in raw unfiltered turbid water as well as in sediments, where no video-based technology works. The MFB is a modular system and can be equipped with 8 to 96 recording channels where each channel is connected to a sensor with one test animal inside. Therefore high numbers of replication as well as many species can be managed simultaneously for a reliable biomonitoring, e.g. one sediment species (chironomids), one epibenthic species (*Gammarus sp*.) and one predator (e.g. fish).

The MFB comprises an exposure chamber for housing an aquatic organism having ventilatory behaviour and body movement sensitive to water quality. Electrodes sense electrical signals produced by the organism during its ventilatory behaviour and body movement, and a controller responsive to signals from the electrodes determines a plurality of ventilatory parameters based on the signals. The ventilatory parameters are compared with corresponding thresholds to determine when the water to which the organism is exposed has caused physiological stress to the organism (Gerhardt et al., 1994; 2006).

***Gammarus fossarum* :**

The genus *Gammarus* is most commonly used in experiments in Europe (Schmidlin et al., 2015) *Gammarus fossarum* (Crustacea; Amphipoda) is a relatively robust and abundantly occurring member of the macrozoobenthos of European springs (Kunz et al., 2010). *G.fossarum* is a key species in the stream food web (Gerhardt et al., 2012; Kienle et al., 2013) they feed on small invertebrates, broken down leaves and organic matter. In the saprobic system they indicate 'good' water quality. However they are sensitive towards many anthropogenic chemical substances released into surface water bodies (Gerhardt et al., 2007; Kienle et al., 2013). Due to this fact they are increasingly being used as test species in ecotoxicity testing and biomonitoring (Kunz et al., 2010). In various tests for studying survival and behaviour of *G.fossarum* with different toxicants, *G.*fossarum proved more sensitive towards toxicants than other species (Taylor et al., 1991; Rinderhagen et al., 2000; Lukancic et al., 2009; Alonso et al., 2010; Gerhardt, 2011; Schmidlin et al., 2015). For these reasons *G.fossarum* can be used as a suitable organism for finding the impacts of pollution and it is readily used in ecotoxicological assays (Maltby et al., 2002; Gerhardt, 2011).

Acute Range Finding Tox-Tests using TBT :

The acute toxicity tests were conducted as described in the Ecological Effects Test Guidelines by the US Environmental Protection Agency (*Gammarids* Acute Toxicity Test) (EPA, 1996) with adjustments to perform a realistic test set-up under laboratory conditions. The purpose of the tests was to determine the 24 hour LC_{50} values of TBT on *Gammarids*.

Tox-test done by making them exposed to three different concentrations of TBT (0.5µl/l - 0.215mg Sn/l, 1µl/l - 0.4mg Sn/l, 2.5µl/l - 1.075mg Sn/l) (20µl/L TBT = 8.6mg Sn/L - Blum, personal communication) with eight *Gammarids* in each for 24 hours. The used concentrations of TBT was chosen by the aid of pre-trial. Both normal behaviour (movement activity : locomotion) and stress behaviour were recorded for each concentration

Tests were conducted with TBT concentrations of 0.5µl/l - 0.215mg Sn/l, 1µl/l - 0.43mg Sn/l and 2.5µl/l - 1.075mg Sn/l. First the normal behaviour of 8 *Gammarids* in lake water were recorded (which is considered as control) and then the organisms were exposed to TBT concentrations. The behaviour of *Gammarids* were recorded for 24 hours.

III. STATISTICAL ANALYSIS

Behaviour measurements of movement activity of 8 *Gammarids* in various hours and various TBT concentrations (0.5, 1 and 2.5µl/l) was statistically analysed using Friedman Repeated Measures Analysis of Variance on Ranks. For all statistical analysis and graphs, Sigma Plot 13.0 (Systat Inc.) was used. Normality Test (Shapiro-Wilk) : Passed ($P = 0.059$)/ Equal Variance Test (Brown-Forsythe): Failed ($P < 0.050$) was followed by Tukey test for non-parametric data. There is a statistically significant difference ($P =< 0.001$) in the median values among the treatment groups (Control vs. 0.5, 1 and 2.5µL/L, also 0.5µL/L vs. 1µL/L) were found, which is plotted in the graph (figure 1).

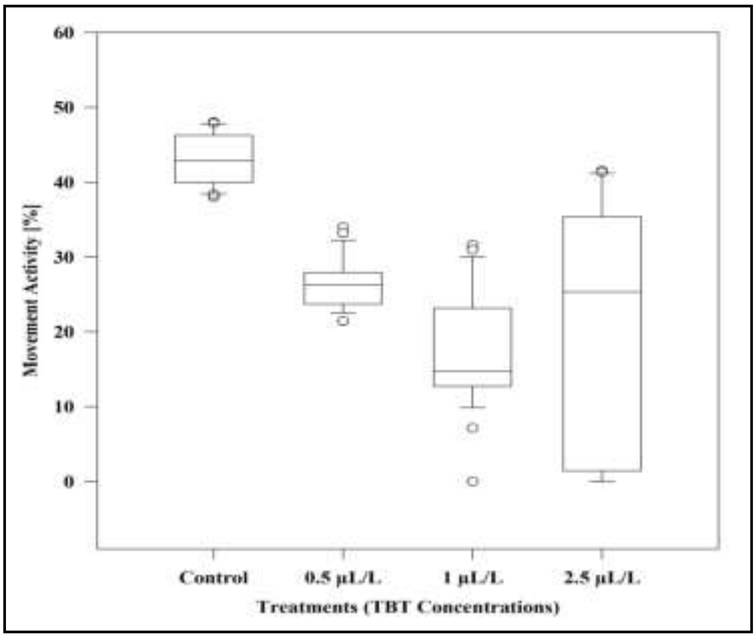

Figure 1: Movement activity of *G.fossarum* in control and various concentrations of TBT

IV. RESULTS AND DISCUSSIONS

Differences in movement activity (percentage of frequencies between 0.5 and 2 Hz) of 8 *Gammarids* in control and in various TBT concentrations (0.5µl/l, 1µl/l and 2.5 µl/l) were shown in figure 2. TBT concentration of 0.5µl/l caused 12.5% mortality and 1µl/l concentration of TBT caused 50% mortality (LC_{50} : lethal concentration of 50 % lethality). TBT concentration of 2.5µl/l, caused 100% mortality after a twenty hours exposure which was observed visually. The data obtained from MFB software, showed stress ventilations (band 2 : between 2.5 – 5Hz,) in TBT concentration of 0.5µl/l (at low concentration), while in high concentrations toxicity increased morbidity with a decreased locomotion (between 0 – 2Hz, summarised in band 1 in 1µl/l TBT) and increasing locomotion (a sign of avoidance behaviour in 2.5µl/l TBT).

96hr LC_{50} of TBT on *Nitocra spinipes* were 1.88 and 1.96µg/L for male and female respectively (Linden et al., 1979) where in another study, 96hr LC_{50} of TBT on *Nitocra spinipes* was 12.7µg/L (Karlsson et al., 2006). 72hr LC_{50} of TBT on *Eurytemora affints* (copepod) was 0.6µg/L and 48hr LC_{50} of TBT on *Acartia tonsa* (copepod) was 1.1 µg/L (Bushong et al., 1988). While, 6 days LC_{50} of TBT on juvenile *Acartia tonsa* was calculated as 0.24µg/L and 8 days Mortality: lowest observed effect concentration (LOEC) of TBT on *Acartia tonsa* was 3ng/L (Kusk and Petersen, 1997).

In a studied conducted with the marine harpacticoid copepod *Tigriopus japonicus* to find the acute and subchronic toxicity of tributyltin chloride (TBTCl), the 48-hr LC_{50} adult females were 0.85µg/L, whereas this value for adult males were 0.51µg/L, respectively (Ara et al., 2010). These results suggested that the concentrations of current ambient TBT (tributyltin) compounds in coastal waters can be assumed as the safety range for the survival, but are unlikely to cause a reduction in the number of *T. japonicus*. Where, 96hr LC_{50} of TBT on *T. japonicus* was reported as 18µg/L, 50µg/L and 0.15µg/L (Kwok and Leung, 2005; Lee et al., 2007 and Boa et al., 2011) respectively.

24hr LC_{50} of TBT on copepod species *Tisbe biminiensis* was observed as 36µg/L (Costa et al., 2014) where this value for *Tisbe battagitai* (Copepodite) was 17µg/L (Macken et al., 2008). In the chronic toxicity tests conducted to find the biological effects of bis (tributyltin) oxide (TBTO) on the calanoid copepod *Pseudodiaptomus marinus* over two generations (F1 & F0)it was observed that F1 copepods were more vulnerable than F0 copepods and a drastic increase in mortality was observed as the TBTO concentration become higher. Exposure of copepods to 60 ng/L TBTO concentration reduced the fecundity and resulted in some females being infecund (in the F0 generation). The time to the first egg sac for females in the F1 generation exposed to 6ng/L TBTO concentration was significantly reduced, and the fecundity of this generation was increased. 72hr Mortality: LOEC of Bis(tributyltin) oxide (TBTO) on *Pseudodiaptomus marinus* F0 was observed as 60ng/L and for *Pseudodiaptomus marinus* F1 was 6ng/L. 72hr Fecundity: LOEC of Bis(tributyltin) oxide (TBTO) on *Pseudodiaptomus marinus* Female F0 was 60ng/L and for *Pseudodiaptomus marinus* Female F1 was 20ng/L (Huang et al., 2006).

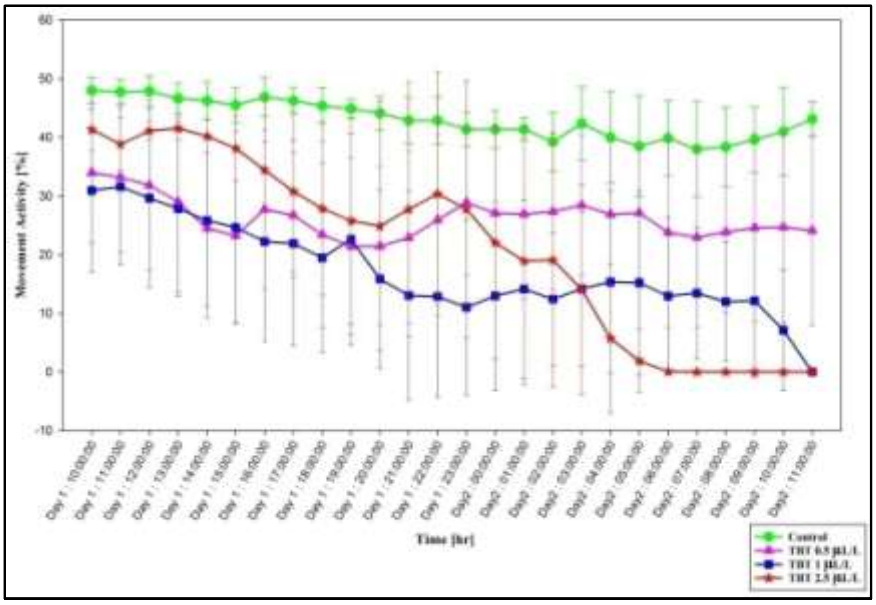

Figure 2 : Movement activity of *G.fossarum* during 24hr exposure in control and various TBT concentrations

The lethal concentrations of TBTO to Daphnia magna over 24 and 72hr were 0.12mg/litre and 0.06mg/litre, respectively. Another aquatic crustacean, *Cypridopsis hartwigi*, was less sensitive with lethal concentrations of 4 mg/litre for a 24-h exposure, 2mg/litre for a 48-h exposure, and 0.12mg/litre for a 96-h exposure. For TBT acetate, the LC_{100} was 0.15mg/litre for a 72-h exposure of Daphnia and 0.15mg/litre for a 96-h exposure of Cypridopsis. The LC_0 was 0.075mg/litre for both species (Dobson and Cabridenc, 1990). As per EPA., 2003 criterion, to protect freshwater aquatic life from chronic toxic effects the limit of TBT in freshwater is 0.072µg/L. As a part of determining this limit, the invertebrate species, *Gammarus pseudolimnaeus* exposed to TBT concentrations and find that 96-hr LC_{50}s is 3.7µg/L.

In the current experiments *G.fossarum* after exposure to 2.5µl/l TBT (1.075mg Sn/l), showed effects with increasing locomotion (a sign of avoidance behaviour) and 100% mortality is observed visually after 20 hr. At TBT concentration of 1µl/l (0.43mg Sn/l) movement activity reduced and after 24 hr exposure 50% mortality observed visually. Test results leads to the conclusion that TBT concentration of 1µl/l was 24-hr LC_{50} in *G.fossarum*. While comparing the results of current experiments with TBT on *G.fossarum* and results of previous experiments with TBT on different species indicates that *G.fossarum* was more sensitive than other *Gammarus* species and copepods.

V. CONCLUSION

Findings like, increased stress ventilation along with decreased locomotion in 0.5 µl/l TBT, decreased locomotion with 50% mortality in 1µl/l TBT and avoidance behaviour with 100% mortality in 2.5µl/l TBT, shows that *G.fossarum* behaviour can be used for monitoring the both acute and chronic toxicity down to a very low concentrations per litre levels even on other freshwater invertebrate species. The results of the experiments conducted shows that the micro litres of TBT in water will increase the mortality of *Gammarids* species and their response to these type of toxicants (environmental problems) indicates the importance of *G.fossarum* to use as an ideal organism for studies on freshwater with toxic substances (Schmidlin et al., 2015).

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