Universe Scientific Publishing

ORIGINAL ARTICLE

Toxiological effects of p-xylene on the juvenile flounder(Paraichthys OLIVACEUS)

Jin Wu

National Marine hazard Migration Service, State Ocean administration Beijing 100194, China

Abstract: Acute and sub-chronic toxicity tests usingParaichthys OLIVACEUSWere conduit-ted, in order to evaluate the toxicological effects of one of the most typical hazardous and marvelous sessions, p-xylene. the cultural effects studied in the present study include the acute lethal effect, growth inhibition, as well as biomarkers which could indicate the genotoxicity, neurotoxicity and of p-xylene. the re-sults showold that the 96 h LC_{50}Of p-xylene on JuvenileP. OLIVACEUSWas 45.7 mg · L^{-1}. Exposure to p-xylene with concentration no less than 2.3 mg · L^{-1}Causeed significant growth inhibition of the Covenant. MDA content and DNA

Damage in fish liver was significant increased and the activity of AChE in fish brain was significant inhibited after 4.6 and 9.2 mg · L^{-1}P-xylene exposure for 28 days. Exposure to 9.2 mg · L^{-1}P-xylene for 28 days used sig-significant reduce of total employee counts and lysozyme activity. these results indicate that the long-term effect of p-xylene lead to oxidative stress and DNA damage. the results-oriented in this study provided data for marine ecotoxiologicalassessment of p-xylene and could contribute to a bet-ter understanding of the toxiologicalmechanism of p-xylene in fish.

Keywords: P-xylene;Paraichthys olivaceu; Lethal effect; chronic toxicity

P-xylene is an important industrial raw material and widely used in pesticides.,Plastics and fiber synthesis.2015Annual global production of p-xylene3 696Ten thousand tons, of which China's annual output reached882Million tons, accounting for the annual production of global p-xylene23%.Studies on mammals show that p-xylene is neurotoxic,Genetic poison

Currently, p-xylene has been listed by the International Maritime Organization as a dangerous chemical with high leakage risk.[4].2007P-xylene Leakage Occurred on a foreign ship at hengjida Xin Chemical Wharf, Zhuhai Port, China.400ASCENDING p-xylene pours into the sea[5].Currently, there are few studies on the toxicity of p-xylene to aquatic organisms..Van jawai and Zhou Qixing[6]The acute lethal effect of xylene on zebrafish was studied. However, the sensitivity of sea fish and freshwater fish to xylene may be different..Therefore, it is urgent to understand the toxic effects of hazardous chemicals such as para-xylene on marine life, especially economic species, so as to provide evidence for risk assessment of marine hazardous chemicals leakage..

In recent years, China's aquaculture industry has developed rapidly.2012China's sea Culture Area2 180 927Hectares, breeding production1 643.81Million tons of marine fish100Ten thousand tons[7].PARALICHTHYS OLIVACEUS(Paraichthys OLIVACEUS)It is a valuable cold and warm marine fish with high economic value.10In the past few years, it has gradually become an important breeding fish species in the Yellow Sea and Bohai Sea of China.[8].Xylene including o-xylene,M-xylene and p-xylene3.In this study, the most widely used paraxylene was used to

study the lethal effect and Growth Inhibition Effect of paraxylene on juvenile Paralichthys olivaceus through acute and sub-chronic exposure tests, determination of lipid peroxidation in the liver of juvenile Paralichthys olivaceus under p-xylene Stress,DNADamage Effect,AChE activity in Brain Tissue(AChE),Analysis of the Genetic Toxicity of p-xylene to Japanese flounder (Paralichthys olivaceus),In order to provide a scientific basis for the marine eco-toxicological evaluation of p-xylene, the neurotoxicity and immune toxicity of p-xylene were studied..

1. Materials and Methods (Materials and Methods)

1.1 Experimental fish

Juvenile Japanese flounder (Paralichthys olivaceus) from the Yellow Sea Water of Yantai Haiyang City Breeding Factory.Specifications for laboratory fish2.One is after hatching DAverage body length of juvenile4 ~ 6 cm, The average weight is(4.2 ±0.3) gUsed in acute toxicity tests.Another specification is after incubation6A month of juvenile average body length6 ~ 8 cmAverage weight(6.7 ± 0.6) gWas for growth experimental and Chronic Toxicity Test.Experimental fish Of10 dFoster conditions:Dissolved Oxygen7.0 ~ 7.5 mg in L^{-1}Salinity33~ 35Temperature(18 ± 1)PH 8.1.Foster during feeding Granville brand fish feed every day feeding of for experimental with fish weight3%.

1.2 Acute Toxicity Test

Acute toxicity test referenceOECD (Organization. E-Conomic cooperation, development)Chemicals test guidelines for[9]Of is set5A exposure concentration and1A blank control every group is set3A parallel.Exposure concentration respectivelyNatural 20,40,80, And320 mg in L^{-1}.This experiment using semi-static test methods every24Hour for a replacement95%The test liquid test during continuous inflatable and as far as possible to keep the sink in seal state.P-xylene exposure experimental conditions and foster during keep consistent.In exposure96 hDuring the processing group random6Times take water samples determination water in p-xylene concentration6Times average said p-xylene of actual concentration.Water in p-xylene content determination reference top empty-Capillary column gas chromatography method

(GB/T-5750.8-2006)[10]TheAgilent 6890NStyle Gas Chromatography Capillary chromatographic columnFFAP 25 m × 0. 32mm;Carrier gas for high purity nitrogen;Gas for pure hydrogen;Into-like temperature150;Column temperature50Detector temperature160;Line Flow Rate

CM s^{-1};Shunt than10:1;Into-like of800MuL.Fish death of judgment standard for glass rod touch tail no any-Should be.Test data using probability unit analytic method to find96 hThe semi-lethal concentration(LC_{50})And its95%Confidence Interval.

1.3 28 dChronic exposure experimental

1.3.1 28 dChronic exposure test design

According to the acute toxicity of experimental results select96 h-lC_{50}Of 1/20,1/40And1/80As3A sub-chronic toxicity test exposure concentration2.3,4.6And9.2 mg in L^{-1}, Each set3.The experimental conditions are consistent with the acute toxicity test..Before the test starts 28 dAfter that, each group8.Body weight measured by tail test fish for Calculation Changes in weight gain rate and specific growth rate of fish[11].The formulas for weight gain rate and specific growth rate are as follows:Weight gain rate(%) = (W2-W1) * 100/W1., Specific growth rate(%) = 100 x [(LN)W2-lnW1)/T].

Among themW2.AndW1.The weight at the end and at the beginning,TRepresents the time of exposure.DAfter the start of the experiment0,7.,14.,21.And28 dTime sampling for the determination of subchronic toxicity.Randomly selected from each sink during sampling8.Juvenile Japanese flounder (Paralichthys olivaceus)2 mLBlood was taken from the tail vein by syringe and blood cell count was immediately performed.[12].Remaining blood samples in4.Xia, Jing2 000 r. Min^{-1}Centrifugal Separation, collecting serum-80Cryopreservation for the determination of lysozyme activity.After blood extraction, the fish was quickly dissected and the liver and brain tissues were extracted and frozen in liquid nitrogen.-80For Lipid Peroxidation,DNAInjury and ache(AChE)Activity Determination.

1.3.2 Determination of Physiological and Biochemical Indexes

Method for Determination of secondary oxidation product propylene two

0.125 mol · L^{-1}, Na_2.EDTA 0.05 mol · L^{-1})In the ice bath, after homogenization4.,1 200gCentrifugal30 minThe supernatant was collected for the determination of experimental indicators..Take200μLSupernatant, add100μL 20% TCA (Contains1 mmol · $L^{-1}FeSO_4$.)And200μL0.67%Thiobarbituric acid reagent, in90Water Bath10 min, 000 r. Min^{-1}Centrifugal5 min, Take200μLSupernatant in530 nmRead AbsorbanceOd530, UnitNmol · mg^{-1}.Protein Content Determination to bovine serum albumin for standardBrad-FordOf methods[14]Determination.

DNAAlkali helicase of determination methods referenceChingOf methods[15]

Using Fluorescence Spectrophotometer quantitative organization in double-stranded and Single ChainDNA Content,DNAOf integrityFValue said calculation formulaF =(XAudna-XSsDNA)/(XDsDNA-XSsDNA)-InXFor

Is iodide study Acetyl choline wasAChEDecomposition for acetic acid and study choline andDTNBGenerate a of yellow complex in412 nmThe color.Take100MuLOf phosphate buffer in join50 Wall micro-bacteria freeze-dried powder for substratePH = 6.4Of potassium phosphate buffer with substrate suspension take200MuLThe suspension andNatural 20MuLTo be measured liquid serum in96Hole ELISA plate in mixing in570 nmThe measurement AbsorbanceA128Under water bath30 minTermination reaction determination AbsorbanceA2.And the activity of Lysozyme(U in mL^{-1})Measurement style(A1-A2)/A2.

1.4 Data Analysis

All experimental results were3A parallel group of average±Standard Deviation(Means ± S. D)Said.With data softwareSPSS 17.0The single factors Variance Analysis(One-way ANOVA)AndDuncanTest,$P<0.05$Representative difference significantly.

2. Experimental results (REsults)

2.1 Death and growth

Concentration of p-xylene in water of different concentration groups

Degrees respectively(18.25 ±0. 5),(34.52 ±0. 5),(72.63 ±0. 8),(147.24 ± 0.6)And(303.77 ±0. 5) mg · L^{-1}.P-xylene against juvenile PARALICHTHYS OLIVACEUS 96 h-lC_{50}Yes45.7 (33.516 ~ 59.741) Mg-l^{-1}.Regression EquationY=-4.328 + 2.607X.During the experiment, there was no death in the control group.20 mg · L^{-1}The mortality rate was25%,320 mg ·L^{-1}The mortality rate of juvenile Japanese flounder in the exposure group was100%.

Such as chart1.For young Japanese flounder (Paralichthys olivaceus)28 dAfter exposure, gain weight Inhibition of rates and specific growth rates.2.3,4.6And9.2 mg · L^{-1}Exposure group of weight gain rate and specific growth rate and the control group compared by significant suppression($P<0.05$).Which9.2 mg in L^{-1}Of exposure group in brown Japanese flounder (Paralichthys olivaceus juvenile of growth inhibition to achieve highest weight gain rate and specific growth rate respectively for the control group21.27%And23.07%.

2.2 P-xylene on brown Japanese flounder (Paralichthys olivaceus juvenile liver of lipid peroxidation andDNADamage Effect

After different concentration and time of p-xylene exposure after experimental fish liver organizationMDAContent Change as shown in Figure1Shown in:In7/dWhen,2.3 mg in L^{-1}Concentration group inMDAThere was no significant difference in content compared with the control group.(PLess than 0.05).From14 dAt the beginning, each concentration groupMDACompared with the control group, the content showed significant difference.(PLess than 0.05)For each concentration groupMDAThe content showed a trend of first increasing and then decreasing..2.3 mg · L^{-1}And4.2 mg · L^{-1}In the liver of juvenile Japanese flounder (Paralichthys olivaceus)MDAContent in14 dReached the highest value, and the content175.11%And175.85%. 9.2 mg·L^{-1}Observed in the exposure group,MDAContent in21 dTime Reached the highest value, its content accounted for about the control group197.71%.

2.3 Effects of p-xylene on brain tissue of juvenile PARALICHTHYS

OLIVACEUSAcheEnergetic

Impact

Exposure to p-xyleneAcheThere is a significant dose of activity-Effect relation(Figure3):With the increase of exposure time and concentration,2.3,4.6And9.2 mg · L^{-1}Of the exposure groupAcheActivity decreased significantly compared with control group(PLess than 0.05)And in28 dReached the minimum value in the control group.78. 11%,65. 11% 57.40%.

2.4 P-xylene on brown Japanese flounder (Paralichthys olivaceus juvenile of total blood cell quantity and lysozyme activity of influence

P-xylene on brown Japanese flounder (Paralichthys olivaceus juvenile of total blood cells quantity influence significantly(P<0.05)7/dWhen the concentration group total blood cells quantity was significantly higher than that of the control group(P<0.05)14 dAn arcane9.2 mg in L^{-1}Exposure group of blood cells quantity significantly reduce and reached the lowest value blood cells quantity is control group 68.49%.14 dAnd21 dAn arcane2.3And4.6 mg in L^{-1}Concentration group of blood cells quantity still significantly higher than that of the control group and9.2 mg in L^{-1}Concentration group of blood cells quantity lower than control level(Figure4).P-xylene can significantly reduce brown Japanese flounder (Paralichthys olivaceus juvenile serum lysozyme activity(P<0.05)With the exposure time increase(14 ~ 28 d)The exposure group(2.34.2And 9.2 mg in L^{-1})Lysozyme activity was significantly decreased, and the control group in Test During and no significant change.2.3,4.6And9.2 mg in L^{-1}Exposure group respectively in21 dAnd28 dAt to minimum respectively for the control group60.71%,73.91And81.19% (Figure5).

3. Discussion (Discussion)

3.1 P-xylene on brown Japanese flounder (Paralichthys olivaceus juvenile survival and growth of influence has been study show that xylene on Article bass(Morone sax-Atilis),Black stay fish(Pimephales promelas),Mesh (Oryzias latipes)(Poe-cilia reticulata),Rainbow trout(Oncorhynchus mykiss)And Crucian Carp(Carassius auratus)OfLC_{50}Respectively2.0MuL in L^{-1}(96 h),8 870MuG In L^{-1}(96 h),8 800MuG In L^{-1}(96 h),2 600MuG In L^{-1} In ·-1 [18-21].

(96 h)And18 000MuG L (24 h)This experimental study get p-xylene on brown Japanese flounder (Paralichthys olivaceus juvenile96 h-lC_{50}Value45.7 mg in ·L^{-1}.The show that6A fish on p-xylene of sensitivity in turn for hua tiao bass>Rainbow trout>Mesh (Oryzias latipes)>Black stay fish>Crucian Carp>Brown Japanese flounder (Paralichthys olivaceus.According to chemical material of fish toxicity classification standard(National Environmental Protection Bureau Water and Wastewater monitoring analysis methods Editorial Board,2002)Xylene on brown Japanese flounder (Paralichthys olivaceus juvenile of toxicity belongs to medium toxicity and Vanya authority and weeks start

[6]The zebrafish of study results consistent.At present on p-xylene on fish growth suppression of study also no reported this study show that, 2.3,4.6And9.2 mg in L^{-1}This3A exposure group can significantly suppression Japanese flounder (Paralichthys olivaceus juvenile weight of increase and reduce its weight gain rate and specific growth rate, This show that exposure in p-xylene(> 2.3 mg in L^{-1}) 28 dThe brown Japanese flounder (Paralichthys olivaceus juvenile has remarkable inhibition on the growth of.

3.2 P-xylene on brown Japanese flounder (Paralichthys olivaceus juvenile of liver toxicity

Xylene in lactation animal liver in metabolism main by a methyl of oxidation the by cells PigmentCYP450 2E1Will pollution molecular transformation into methyl benzyl alcohol further by biological transformation for methyl malonic acid[22].In this a metabolism process in organization in oxidation free radical increase can be induced by biological in of lipid peroxidation,DNAChain broken Crack and Oxidation Injury So find out these biological markers of activity change can reflect Oxidation stress of there often was as an environment pollution stress of biological markers.

MDA(MDA)Content is lipid peroxidation reaction of decomposition product,MDAOf main is due to without

antioxidant enzyme transformation of superoxide anion and other free radical on cell membrane more unsaturated acid the attack produce.LOOHSuch as have oxide caused by its content can reflect lipid peroxidation of Level[24].JajteSuch.[25]Study found that p-xylene of lipid peroxidation role is its induced mice liver toxicity of Main Way.This experimental study show that,2.3 mg in L^{-1}Concentrations in the liverMDAContent in7 dThere was no significant difference between control group and,14.

After the concentration groupMDAThe content increased significantly;In9.2 mg·L^{-1}Observed in the processing group,MDAContent in21 dReach the highest value.Studies have shown that p-xylene can significantly affect

Fish LiverMDAContent in the liverMDAThe production is mainly due to the insufficient regeneration of glutathione.LoohThe Intramolecular Cyclization of peroxides,Steps like cracking[26].Exposure to p-xylene in this study7 dLipid Peroxidation did not occur at any time, indicating that the antioxidant system in the body can

Yu's Reactive Oxygen Species(ROS)So oxygen free radicals do not affect Liver Cells

Causing significant damage, however, in the high concentration group21 dTimeMDAContent

Significant increase in lipid peroxidation in the liver of young Japanese flounder (Paralichthys olivaceus) exposed to high concentrations for a long time resulted in significant toxicity of oxygen free radicals to the liver cells of young Japanese flounder (Paralichthys olivaceus)..

Studies have shown that xylene exposure can causeDNAInjury.Lu Dan Yu[27]Xylene can induce nucleated cells in peripheral blood of pregnant females.DNAInjury.Ni[28] , The study found that peripheral blood cells of workers exposed to benzene, toluene and xylene could causeDNAInjury.However, the genetic toxicity of p-xylene to aquatic organisms, especially fish, is unknown.

The study also showed that short-term exposure to p-xylene did not causeDNADamage, which is consistent with the results of this experiment..Therefore, the authors believe that the exposure of p-xylene to Brown To leadDNAChain fracture;NiazSuch.[31]The study show that p-xylene belongs to high fat-soluble organic solvent can make the cell membrane production DNACracking enzyme Final CauseDNAChain fracture.By this experimental get of p-xylene lead to the lipid peroxidation injury results the authors speculate that p-xylene caused by the oxidation injury may is caused by Brown Japanese flounder (Paralichthys olivaceus juvenileDNADamage of Main Way.

3.3 P-xylene on brown Japanese flounder (Paralichthys olivaceus juvenile of brain toxicity

AChEIs and neural activities closely related of important material is characterization pollutants neural toxicity of important biological markers[32].San- Swimming and feeding ability has obvious of negative correlation.Le Bris Low fish of feeding activities and then influence fish of growth.This paper results show that p-xylene(2.3 ~ 9.2 mg in L^{-1})Role Under can significantly induced by Brown Japanese flounder (Paralichthys olivaceus juvenile brain tissue inAChEActivity andAChEEnzyme activity decreased and exposure concentration and exposure time and has obvious of negative correlation.So author think p-xylene of neural toxicity may Blood Cells quantity was significantly decreased trend and group was significantly lower than that in the control group.AccordingToxtreeDatabase of chemical material of classification shows that p-xylene belongs to non-polarity anesthesia chemical material its toxic mechanism main because its can non-selective by cell membrane on cells structure caused by injury play its toxicity role[43].A few study also show that long-term exposure in p-xylene conditions under will increase lung tissue cells apoptosis lymphatic cells and renal tubular proximal cells of cell death quantity[44-46]; Snow and[2]The study show that with the exposure dose of increased p-xylene exposure group of cells apoptosis rate significantly increased.So author think high concentration of p-xylene may be caused by the blood cells apoptosis and lead to blood cells quantity reduce and then influence brown Japanese flounder (Paralichthys olivaceus juvenile of blood function reduce its immunity.Lysozyme in fish non-specific immune in role important role is fish immune defense level of another important index[47].This experimental study show that p-xylene exposure of Japanese flounder (Paralichthys olivaceus serum in lysozyme vitality has significant suppression role.General think fish in low concentration of exogenous pollutants stimulation when can cause immune system compensatory stress to Regulation their own state however in high concentration long time exposure under

3.4 P-xylene on brown Japanese flounder (Paralichthys olivaceus juvenile of immune toxicity

Blood indicators are widely used to evaluate fish health and environmental adaptability, and also as toxicological indicators to reflect the impact of pollutants on fish immune levels.[39].Fish blood cells

References

1. Zhang Ting,Fukatsu hara,Snow White,Wait..Study on the neurotoxicity of formaldehyde and xylene in Mice[J].Journal of Environment and Health, 2012, 29 (2): 155-160
2. Zhang T, Yuan f s, Bai X, *et al*. The study on the joint Neurotoxicity of formaldehyde and xylene in mice [J].Journal of Environment and Health, 2012, 29 (2): 155-160 (In Chinese)
3. Snow White,Fukatsu hara,Zhang Ting,Wait..Formaldehyde and xylene combined exposure to smallGenetic Toxicity of rat bone marrow cells[J].Journal of Environment and Health, 2012, 29 (1): 51-54
4. Bai X, Yuan f s, Zhang T, *et al*. genetic Toxicity of form-aldehyde and xylene to mouse bone marrow cells [J]. journal of Environment and Health, 2012, 29 (1): 51-54 (In Chinese)
5. Huang shuhui,Ma jianteng,Janina,Wait..Microcystins-LRToxic Effects of hexylene on Zebrafish Embryos[J].Journal of Environment and Health, 2013, 30 (4): 308-311
6. Huang s h, Ma j t, Zhang N, *et al*. Toxic Effects of microcycles-LR and xylene on Zebrafish Embryos [J].Journal. Environment, Health 2013 30 (4): 308-311 (. Chinese)
7. International Tanker Owners pollution Federation (itopf). techical information Paper (TIP 17): Response. marine chemical incidents [R/OL]. (2014-05-19) [2017-11-15]. http:/www. itopf. COM/knowledge-resources/docu-ments-guides/document/tip-17-response-to-marinechemi-cal-incidents/
8. Chemical Safety Association. P-xylene leaks at Zhuhai Port no casualties [R/OL]. [2017-11-15]. http://www.chemicalsafety.org.cn/detail.php? Oneid = 8089
9. Van BadiaWeeks Qixing.Water TolueneEthylbenzene and xylene on zebrafish of Toxicity Effect[J].Ecological Toxicology report2009 4 (1): 136-141
10. Fan Y WZhou q x. Toxic effects. toluene ethyl-benzene, xylene. waters. zebrafishBrachydanioRerio[J]. Asian Journal. ecotoxicology 2009 4 (1): 136-141 (. Chinese)
11. King dongshiHigh brocade yu.China sea water aquaculture of development and current situation[J].InCountry fisheries2015 (4): 39-42
12. Wang d s, Gao J y. the Development and Present Situation-tion of Marine aquiculture in China [J]. chinese Journal of fishers, 2015 (4): 39-42 (In Chinese)
13. Na n, Guo h r, Zhang s c, *et al*.In VitroAndIn vivoA-cute toxicity of fenpyroximate to flounderParalichthysOLIVACEUSAnd its Gill Cell Line FG [J]. Aquatic Toxicology,2009, 92: 76-85
14. OECD Guideline for the testing of chemicals. Test No 203: fish, acute toxicity test [s]. Paris: OECD, 1992
15. Ministry of Health of the People's Republic of China. GB/T57508-2006Standard Test Methods for drinking water-organic compounds[S].Beijing:China Standard Publishing House, 2006
16. Ng t y t, Chowdhury m j, wood c m. can the biological ligand model predict Cu toxicity across a range of PHS in softwater-accelerated rainbow trout [J]. environment Science & Technology, 2010, 44 (16): 6263-6268
17. Ashida M. Purification and Characterization of pre-phenol-
18. Wills e d. eval. Lipid peroxidation. lipids, Biological Membranes [M]/Snell K mullock B. bio-Chemical Toxicology: a practical Approach. washington: IRL Press, 1987: 127-152
19. Bradford M. A rapid, sensitive method. quantitation. microgram quantities. protein utilizing. principle. protein-dye binding [J]. Analytical Bio-Chemistry 1976 72 (1-2): 248-254
20. Ching e w k Siu w h l Lam p k s *et al*. DNA ad-duct formation, DNA strand breaks. green-lipped mussels (Perna viridis) Exposed. benzo [a] pyrene: Dose-, time-dependent relationships [J]. Marine pollu-tion Bulletin 2001 42 (7): 603-610/