Evaluating the biological impact of polyhydroxyalkanoates (PHAs) on developmental and exploratory profile of zebrafish larvae

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SUPPLEMENTARY METHODS

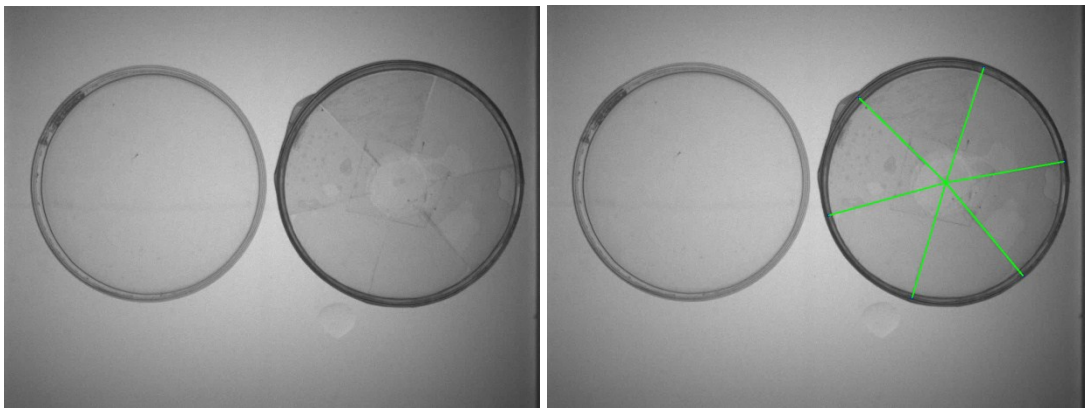
1. Self-designed video-tracking Software introduction

The video recording system and software are self-made. We use the camera to record the behavior of the zebrafish. The camera is fixed on a tripod. The camera is over the test apparatus of zebrafishs. Each record video is 15 minutes. The frame rate is 30 f/s.

The video resolution is 1024×768.

The software based on the Visual studio 2010 and Opencv 2.4.6 was made. The software system is divided into three modules: region partition module, tracking module and parameter calculation module.

Region partition module is responsible for automatic detection of the six regional boundaries. Our method use the Hoff circle detection algorithm to detect the test apparatus, then we use the Hoff line detection algorithm to detect the regional boundaries. If the image quality is poor, the software will prompt the operator to click each of the six regional boundary points. The regional boundaries are detected by this way. Region partition result is shown in **Fig S1**.



(a)Original image **(b)Region partition image**

Fig S1 Region partition result

Tracking module is responsible for automatic detection of the behavior of the zebrafish. We use the average background modeling algorithm to obtain the background. First of all, background image accumulation is made. Users choose the number of image accumulation according to the image quality. Let us suppose that the accumulation background image is M frames, then, calculating mean value of each pixel of the M frames as its background model. When the current frame is detected, the difference pixel value d(x,y) can be obtained by using the current frame pixel value I(x,y) minus the pixel value U(x,y) in the same position of background model. The software compares the U(x,y) with the threshold value TH. TH is chosen by the adaptive algorithm. If TH<d(x,y), the value is 1, else the value is 0. At last, the output image can be obtained. Foreground segmentation result is shown in **Fig S2**. Further, our method limit the zebrafish maximum movement distance in order to reduce the error detection. Tracking result is shown in **Fig S3**.

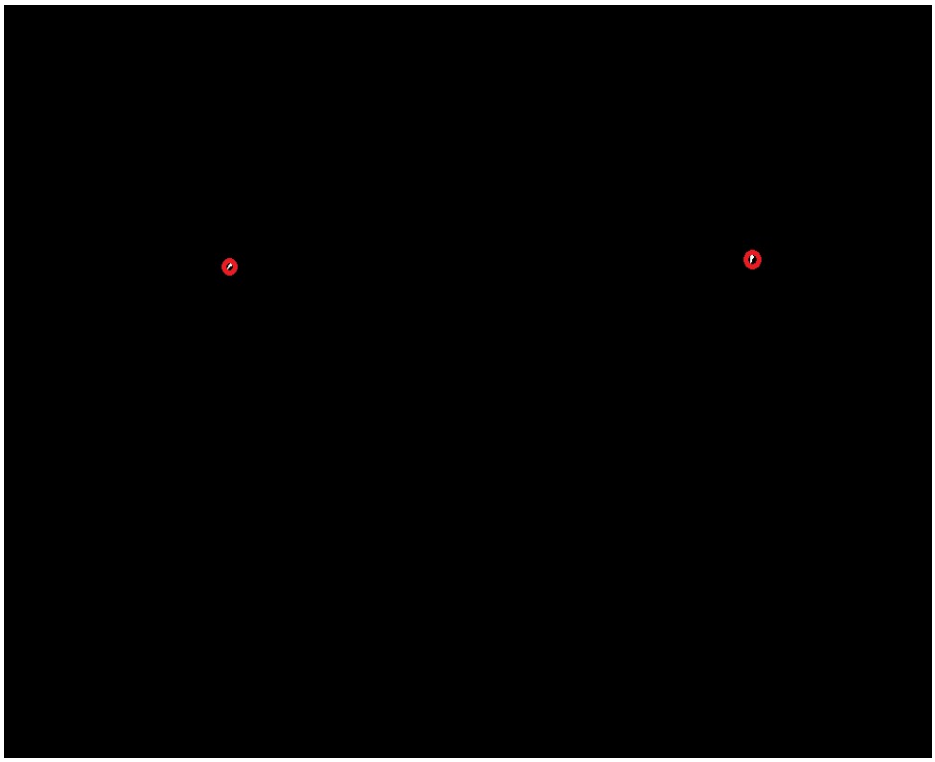


Fig S2 Foreground segmentation result

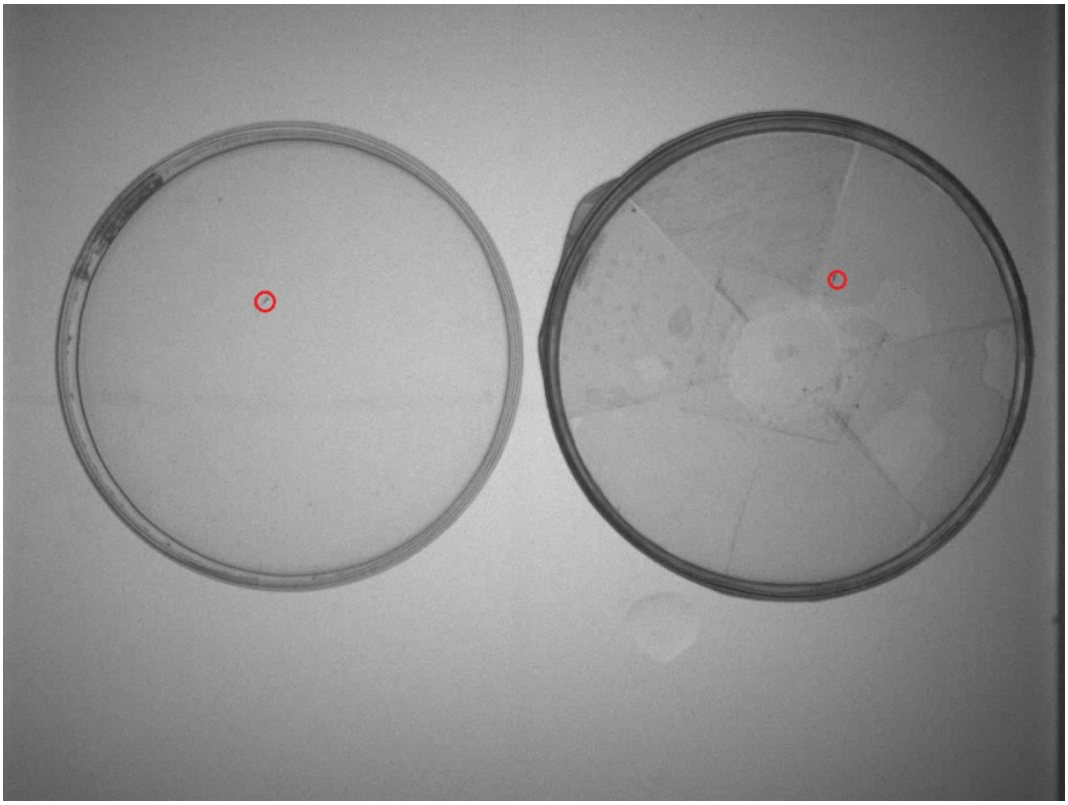


Fig S3 Tracking result

Parameter calculation module is responsible for automatic experimental parameters calculation. In tracking module, the zebrafish position of each frame is obtain, the position sequence is sorted by time. By calculating, movement, speed and color region can be obtained. Many other parameters can be obtained from parameter calculation module.

2. **Open field test hardware introduction**.

The experimental setup is composed by light source (alternate white and infrared lights arrays in a platform as showed in **Fig. S4**), infrared camera (30 frames/s) located above and standard and color enriched open field apparatus as a pair in the middle of camera vision. As in **Fig.S5B**, the color enriched open field test apparatus is a glass Petri dish (9 cm in diameter) are delineated into inner transparent area (a concentric circle of dish 2 cm in diameter) as a starting location and outer zones which is equally divided into six sector region. Each region has been colored yellow,

red, green, orange, blue, and black by color photographic filters to make sure the photo-permeability of visible and infrared light from the light source. The standard open field apparatus (left) is identical to the color enriched open field apparatus except that the bottoms and walls of each six radial area is transparent.

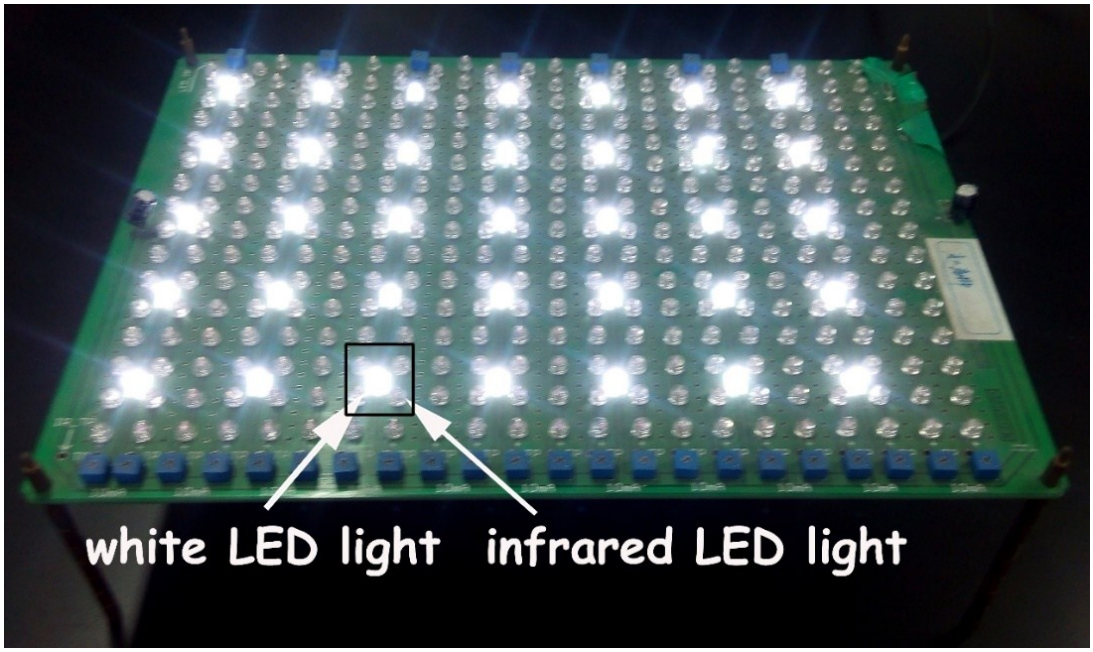


Fig. S4. White and infrared distribution and formation in the light source platform. Lager white arrow mark the White LED light; smaller white arrow indicate the infrared LED light. As the black rectangle indicate, one light unit is composed of one White LED in the middle, and four infrared LED surrounded. In this light source, there are 35 light units equally placed in the circuit board enabling the homogeneous and uniformed light output.

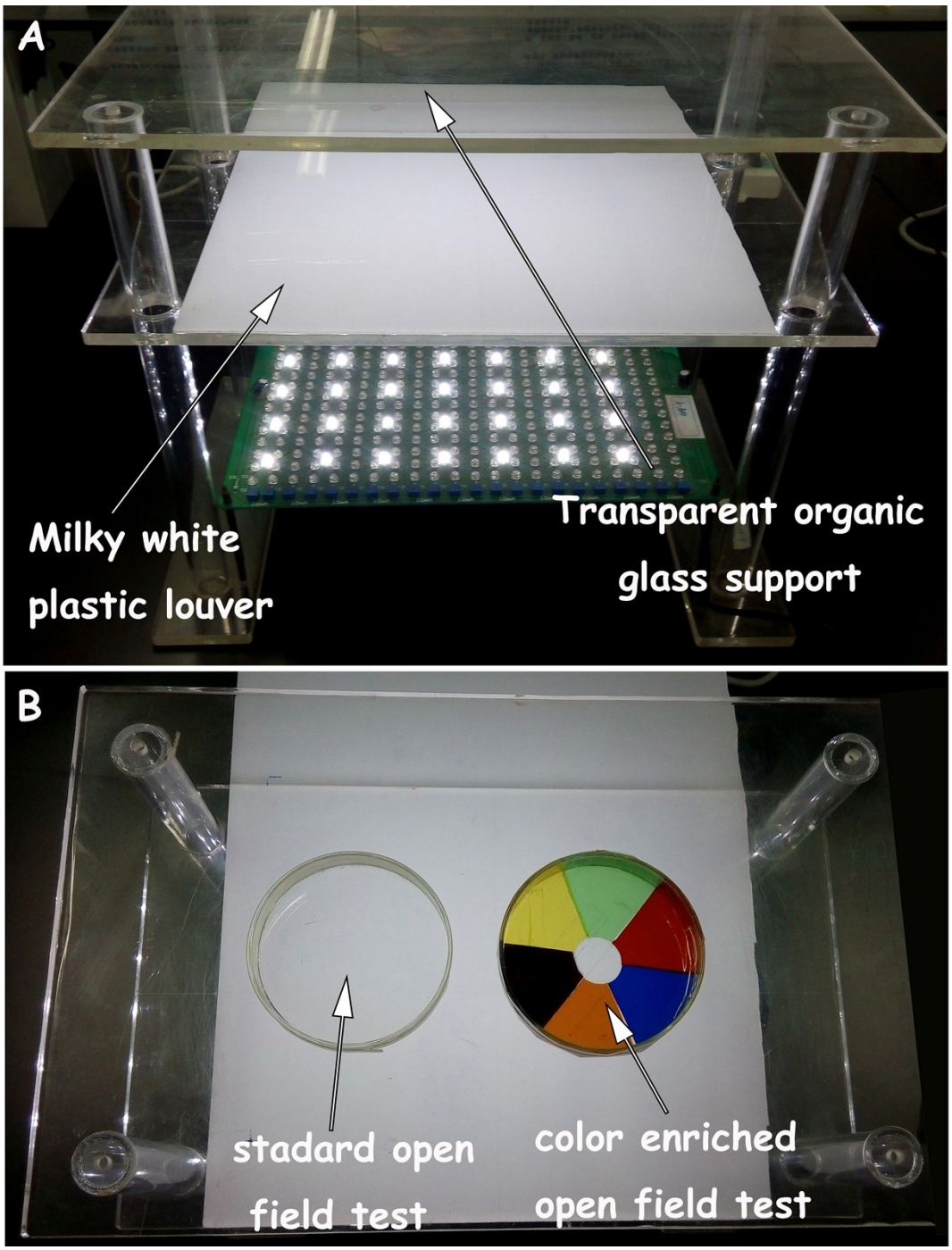


Fig. S5. behavioral experimental platform formation. (A) Light pathway in this experiment. The holder is made up of transparent organic glass enabling the white and infrared light pass though. To make the white and infrared light intensities equal in the viewing area, a milky white plastic louver was placed in the middle. (B) Visual effect of equal intensities of the behavioral experiment area. For color enriched open field test, light intensity is balanced in every color segment. No obvious too light or too dark region was found.

SUPPLEMENTARY FIGURE

1. **Transmittance spectra of color filters used in color enriched open field test**

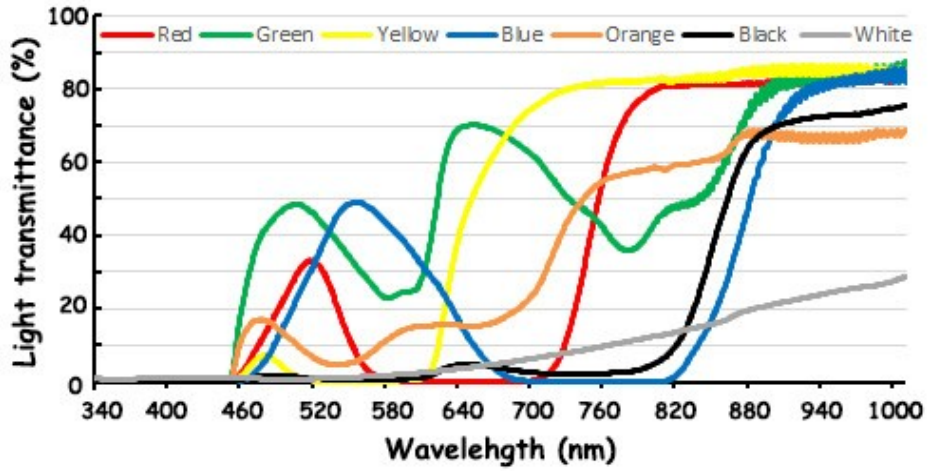


Fig.S6. Transmittance spectra of color filters. Transmittance peak value for orange: 475.5nm (16.969%); yellow: 478nm (7.214%); green: 501nm (48.607%); red: 515.5nm (33.131%); 551nm (49.048%); black: 640.5nm (4.661%); white: 657nm (4.428%).

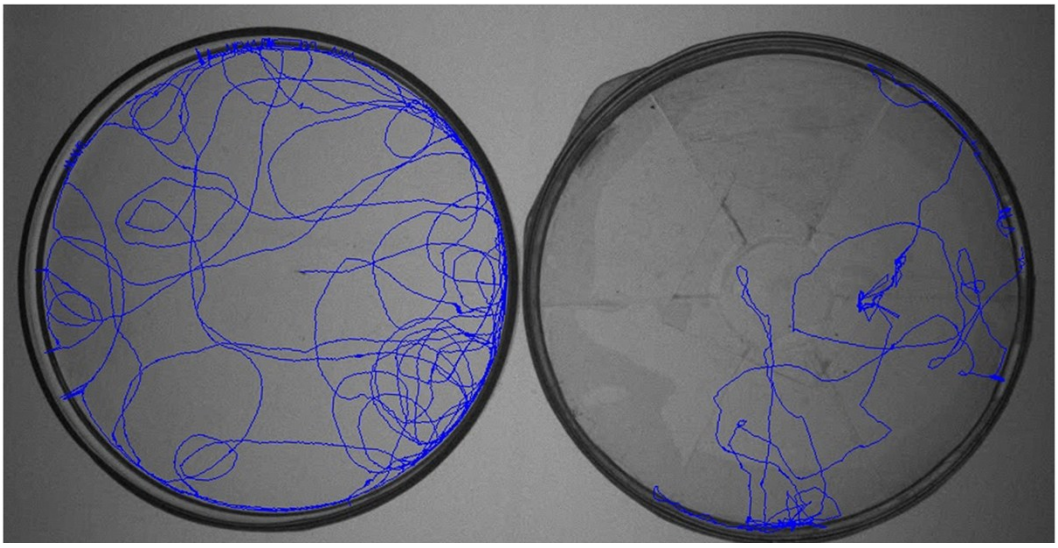


Fig.S7. Schematic figure of the Infrared light intensity distribution captured by infrared camera.

2. **Effects of PHAs microinjection on embryonic mortality.**

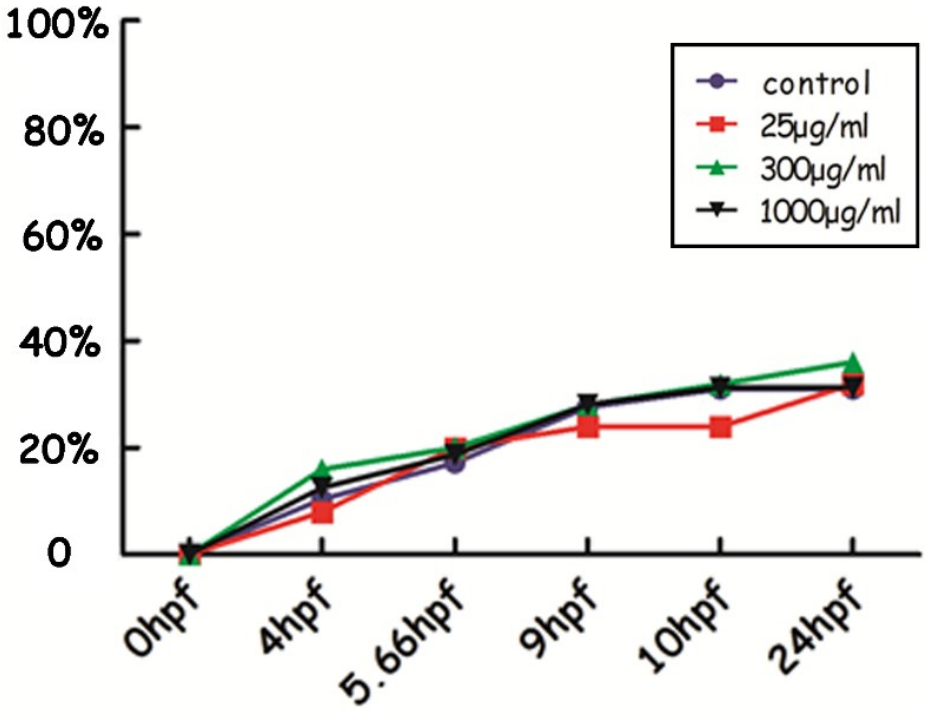


Fig.S8. Effects of PHAs microinjection on embryonic mortality.

SUPPLEMENTARY TABLE

1. **Significance for TDM, time spent and Number of entries per zone in each PHAs concentration injected group.**

Significance / Parameters / Concentration	Percentage TDM per zone (Fig.7Ba)	Percentage of time spent per zone (Fig.7Bb)	Number of entries per zone (Fig.7Bc)
0 µg/mL	F(6,161)=2.2344 ,p=0.0324	F(6,161)=2.2364 ,p=0.0423	F(6,161)=1.6596 ,p=0.1342
0.25 µg/mL	F(6,161)=2.2330 ,p=0.0426	F(6,161)=2.2503 ,p=0.0411	F(6,161)=1.8177 ,p=0.0987
2.5 µg/mL	F(6,161)=2.5109 ,p=0.0238	F(6,161)=2.3113 ,p=0.0362	F(6,161)=1.9672 ,p=0.0733
25 µg/mL	F(6,161)=2.3261, p=0.0351	F(6,161)=2.1927, p=0.0463	F(6,161)=2.1641, p=0.0491
75 µg/mL	F(6,161)=2.5952 ,p=0.0199	F(6,161)=2.0056 ,p=0.0613	F(6,161)=2.2718 ,p=0.0393
300 µg/mL	F(6,161)=3.3033 ,p=0.0043	F(6,161)=2.6746 ,p=0.0168	F(6,161)=2.8237 ,p=0.0122
1000 µg/mL	F(6,161)=3.1665 ,p=0.0058	F(6,161)=3.2253 ,p=0.0051	F(6,161)=3.3971 ,p=0.0035
1500 µg/mL	F(6,161)=4.1255 ,p=0.0007	F(6,161)=3.0609 ,p=0.0073	F(6,161)=3.8823 ,p=0.0012

Table S1. Significance of one way ANOVA for TDM (Fig.7Ba), time spent (Fig.7Bb) and Number of entries (Fig.7Bc) per zone in each PHAs concentration injected group in color enriched open field test.