Early Safety Assessment of Human Oculotoxic Drugs Using the Zebrafish Visualmotor Response.

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Abstract

Introduction: Many prescribed drugs can adversely affect the eye by causing damage to the function of visual pathways or toxicity to the retina. Zebrafish have the potential to efficiently predict drugs with adverse ocular effects at pre-clinical stages of development. In this study, we explore the potential of using a semi-automated visual behaviour assay to predict drug-induced ocular toxicity in wild-type zebrafish larvae. **Methods**: 3 dpf larvae were treated with six known oculotoxic drugs and five control drugs in embryo medium containing 0.1% DMSO. After 48 hours, larvae were assessed using the visualmotor response (VMR), an assay which quantifies locomotor responses to light changes; the optokinetic response (OKR), a behavioural assay that quantifies saccadic eye responses to rotating stimuli; and the touch response, a locomotor response to tactile stimuli. **Results**: 9 of 10 negative control drugs had no effect on zebrafish visual behaviour. 5 of the 6 known oculotoxic drugs (digoxin, gentamicin, ibuprofen, minoxidil and quinine) showed adverse effects on zebrafish visual behaviour assessed by OKR or the more automated VMR. No gross morphological changes were observed in treated larvae. The general locomotor activity of treated larvae, tested using the touch response assay, showed no differences with respect to controls. Overall the VMR assay had a sensitivity of 83%, a specificity of 100% and a positive predictive value of 100%. **Discussion**: This study confirms the suitability of the VMR assay as an efficient and predictive pre-clinical approach to evaluate adverse ocular effects of drugs on visual function *in vivo*.

Keywords: visual behaviour assays; drug treatment; ocular toxicity; visual function; visualmotor response; zebrafish.

[1]Abbreviations.

[1] *dpf, days post fertilization; ERG, electroretinogram; hpf, hours post fertilization OKR; optokinetic Response; OMR, optomotor Response; VMR, visualmotor response.*

1. Introduction:

Adverse drug reactions can negatively impact upon patient welfare and can curtail further development of promising drugs. Indeed, of drugs entering clinical development, drug-induced organ toxicity is the leading factor associated with failure to reach the market (Kola & Landis, 2004). The toxic effects of drugs on vision are significant, with ~6.8% of drugs removed from clinical trials because of visual toxicity (Richards, et al., 2008). Notably, adverse effects associated with drug-induced ocular toxicity are difficult to manage once they occur, and even though cardiovascular and gastrointestinal toxicity are of higher incidence, ocular toxicity has the highest negative influence on drug development (Redfern, et al., 2008; Verdugo-Gazdik, Simic, Opsahl, & Tengowski, 2006). Thus, there is a need for efficient and predictive pre-clinical assays of ocular toxicity that can eliminate drugs that induce visual toxicity at earlier stages in development.

Many prominent FDA/EMA approved drugs adversely affect vision by functional or morphological damage to tissues in the visual pathway (Fraunfelder & Fraunfelder, 2004; Santaella & Fraunfelder, 2007). Here, we test the toxicity of cisplatin, digoxin, gentamicin, ibuprofen, minoxidil and quinine, on zebrafish visual behaviour. Cisplatin cross-links DNA and is a chemotherapeutic prescribed for solid tumours (Plummer, et al., 2011). However, cisplatin can also produce irreversible occulotoxic effects including optic neuritis and retinal ischemia (Caraceni, Martini, Spatti, Thomas, & Onofrj, 1997; Kwan, Sahu, & Palexes, 2006). Optic neuritis is caused by immune-mediated destruction of the myelin sheath surrounding the optic nerve, leading to disrupted signal transmission between the eye and brain (de Seze, 2012). Cisplatin can also cause papilledema; a swelling of the optic disc caused by increased intracranial pressure (Schmid, Kornek, Scheithauer, & Binder, 2006). Cisplatin accumulation in the macular area initiates damage to the optic nerve and results in irreversible blindness (Al-Tweigeri, Magliocco, & DeCoteau, 1999). Although not a first-choice drug, digoxin is used to treat several cardiac dysfunctions by inhibiting the sodium/potassium ATPase pump (Lawrenson, Kelly, Lawrenson, & Birch, 2002). Digoxin is also known to inhibit this exchanger in photoreceptor cells and administration of digoxin is associated with altered colour perception and blurred vision (Lawrenson, et al., 2002). The aminoglycoside antibiotic, gentamicin, is linked with conjunctivitis, in which accumulated drug irritates the conjunctival epithelial cells causing ocular burning (Thomas, Galiani, & Brod, 2001). Retinal detachment and lamellar liposomal inclusions have been identified in animals receiving gentamicin treatment because of abnormal deposition of lysosomal cells in the retinal pigment epithelium (RPE) (D'Amico, et al., 1985). The anti-inflammatory agent ibuprofen, a non-selective cyclooxygenase inhibitor, causes optic neuritis and visual disturbances, perhaps by altering retinal blood flow (Gamulescu, Schalke, Schuierer, & Gabel, 2006; Haefliger, Meyer, Flammer, & Luscher, 1994). Minoxidil, a prostaglandin I2 inhibitor, can cause bilateral optic neuritis. A report suggests this is mediated by induction of peroxides in the retinal vasculature (Gombos, 1983). Finally, the anti-malarial agent quinine, can cause blurred vision, optic neuritis and night blindness by directly damaging photoreceptor and "ON" type ganglion cells (Dyson, Proudfoot, Prescott, & Heyworth, 1985).

Zebrafish (*Danio rerio*) are freshwater fish belonging to the family Cyprinidae. These vertebrate model organisms are routinely used for research in genetics and developmental biology (Fadool & Dowling, 2008). Zebrafish also possess many attributes advantageous for pharmacological and toxicological research. These include; uncomplicated husbandry, small size, simplicity of generating offspring, high fecundity, external fertilisation and rapid development (Taylor, Grant, Temperley, & Patton, 2010). The established efficacy of several small molecules in zebrafish embryos provides *proof-of-principle* for efficient, medium-scale pharmacological screens (Fadool & Dowling, 2008; Taylor, et al., 2010). As, *in vitro* and *in vivo* results often poorly correlate, zebrafish offer the advantage of a complex physiological environment that is absent in *in vitro* systems (Barros, Alderton, Reynolds, Roach, & Berghmans, 2008). Many of the major organs of zebrafish are formed within 24 hours (Phillips, et al., 2011). In relation to vision, the larval and adult zebrafish eye shows similar morphology to the human eye (Goldsmith & Harris, 2003). Development of the zebrafish eye commences as early as 11 hours post fertilization (hpf) and by 3 days post-fertilisation (dpf) the morphology of the eye has the main characteristics of an adult eye (Goldsmith & Harris, 2003). The large eye size compared to the rest of the body reflects the importance of vision in zebrafish. Nascent visual behaviour responses are first seen in 3 dpf larvae and these mature significantly by 5 dpf (Easter & Nicola, 1996; Yin, et al., 2012). Behavioural tests have long been used to assess vision and to screen for visual deficits (Brockerhoff, et al., 1995). An optomotor response assay (OMR) examines the position of zebrafish placed in elongated chambers to moving black and white stripes presented underneath the transparent chambers (Neuhauss, et al., 1999; Richards, et al., 2008). The optokinetic response (OKR) assay is commonly used assay to examine larval eye movements to a rotating black-and-white striped drum (Brockerhoff, 2006; Fleisch & Neuhauss, 2006). Recently, semi-automated systems for quantifying the locomotor movements of zebrafish larvae in response to light stimuli have been developed. The visualmotor response (VMR) assay allows for the simultaneous monitoring of individual larvae in wells of a 96-well plate in response to lights being turned ON or OFF (Emran, Rihel, & Dowling, 2008; Yin, et al., 2012).

This study supports previous studies reporting that zebrafish are an appropriate model to assess drug-induced visual toxicity (Richards, et al., 2008). Here, we compare the OKR and VMR activity of 5 dpf wildtype larvae treated with drugs known to cause visual toxicity in humans. Five of the six tested drugs, resulted in altered visual locomotor behaviour in zebrafish, without effects on a tactile locomotor response. In addition, we demonstrate the utility of the semi-automated VMR assay to more efficiently identify drugs inducing adverse visual effects.

2. Methods

2.1. Zebrafish Husbandry and Maintenance

Wild-type stocks of the Tuebingen (Tu) and AB strains of zebrafish were maintained on a 14 hour light/10 hour dark cycle at 28°C according to standard procedures (Westerfield, 2000). Male and female adults were placed in breeding tanks following their afternoon feed or 1-2 hours before the end of the light period. Embryos were obtained by natural spawning and developmental stages were determined by morphology and development time (Kimmel, Ballard, Kimmel, Ullmann, & Schilling, 1995). Embryos were raised in embryo medium (0.137 M NaCl, 5.4 mM KCl, 5.5 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 1.3 mM $CaCl_2$, 1.0 mM $MgSO_4$ and 4.2 mM $NaHCO_3$) containing methylene blue on a 14:10 light: dark cycle at 28°C. Media was changed daily. All experiments were carried out under ethical approval granted by the UCD Animal Research Ethics Committee.

2.2 Drug Preparation & Treatment:

Drugs were obtained from the following suppliers: digoxin (Tocris Biosciences 20830-75-5) minoxidil, (Tocris Biosciences 38304-91-5), gentamicin (Gibco Life Technologies 15750-045), ibuprofen (Cayman Chemicals 15687-27-1), cisplatin (Sigma Aldrich 15663-27-1), quinine (Sigma Aldrich 6119-70-6), kanamycin (Sigma Aldrich 246-933-9), streptomycin (Sigma Aldrich 223-286-0), chloramphenicol (Sigma Aldrich 200-708-1), penicillin-G (Sigma Aldrich 113-98-4) and ampicillin (Sigma Aldrich 200-708-1).). Drugs solutions were prepared using 0.1% v/v DMSO/Embryo media as solvent. Stock solutions of 10 mM were stored at –20 degrees Celsius. On the day of treatment, unless stated otherwise, 10 µM dilutions of the drugs were prepared. 0.1% v/v DMSO/ Embryo media was used as the vehicle control. Larvae were treated with drugs in a volume of 400 µl, from 3 to 5 dpf, in 48-well plates with 5 larvae per well. The fish were removed from drug in a fume hood before being assayed.

2.3 Optokinetic Response Assay:

Single larvae were immersed in a Petri dish containing 9% methylcellulose (Sigma Aldrich #9004-65-3) to retard swimming behaviour and placed inside a drum with black and white stripes comprising 18^o per stripe and a contrast of 99% (Brockerhoff, 2006). The drum was rotated at 18 rpm for 30 seconds clockwise and then 30 seconds anti-clockwise. The number of eye saccades produced in response to the rotation of the drum was manually recorded and the average number of saccades per minute quantified.

2.4 Visualmotor Response Assay:

The VMR assay quantifies the locomotor behaviour of zebrafish larvae to light changes using an infrared tracking system (Emran, et al., 2008). Individual larvae are placed in wells of a 96 well clear polystyrene plate (Whatman #7701-1651 square, flat bottom wells of 650 µl volume) and immersed in 600 µl of embryo medium. Unless stated otherwise, 12 larvae were used per treatment group. The plate was then placed in a Zebrabox recording chamber

(Viewpoint Life Sciences, France) and the light driving parameters were set to 1 hour and 40 minutes. Within this time period, the light is set to ON for the first 30 minutes to allow a period of settling. Following this, there are four periods where the light changes from ON to OFF and vice versa in twenty-minute intervals. The detection sensitivity is set to 10, the activity burst threshold set to 25 and the activity freeze threshold set to 3. The activity burst threshold corresponds to a given surface change by more than 25 pixels from one image to the next and activity above this threshold is regarded as burst activity. The activity freezing threshold corresponds to a given surface changing by less than 3 pixels from one image to the next and is regarded as freezing as having no activity. Locomotor activity between the two thresholds values is regarded as normal activity. The activity of individual larvae is measured in milliseconds per second (ms/s). The analysis of the complex data produced by the VMR assay was achieved by customised MS Excel macro sheets. The "*overall activity*" of larvae within a treatment group for the 1 hour 40 minute assay is plotted as an activity trace. The average ON and OFF peak traces are calculated from the average responses of larvae from 100 seconds before to 300 seconds after the lights go ON and OFF. There are two such changes within the 1 hour and 40 minute runtime of the assay and these are averaged. Finally, the "average overall, pre OFF, OFF peak, pre ON and ON peak" activities are graphed. The "average overall activity" is the mean activity of the larvae during the entire assay. "Pre OFF and ON activity" are the average activities of larvae 100 seconds prior to the respective light changes. "OFF and ON peak" activities are the average maximum activity of larvae within a treatment group during the first 5 seconds after the light change.

2.5. Touch response assay:

Zebrafish larvae in the test drug were placed individually in wells of a 96 well plate. A pipette tip or 22 G gauge needle was used to touch the caudal fin of the larvae. The locomotor response of larvae to this tactile stimulus was manually recorded using a dissecting microscope and categorized into normal, reduced or absent response.

2.6. Statistical and Mathematical Analysis.

Statistical analyses of the OKR and VMR assays was evaluated using a one way ANOVA and a post-hoc Dunnett's multiple comparison test, comparing drug-treated larvae to vehicle controls. The positive predictive value $^{(\text{true positives}}/_{\text{all positives})}$, sensitivity $^{(\text{visualtox. positives}}/_{\text{all oculotoxic compounds})}$ and specificity $^{(\text{total visualtox. positives}}/_{\text{all true positives})}$ of the VMR assay was calculated from the indicated formulae.

3. RESULTS

In light of the need to develop improved assays that can predict drug-induced visual toxicity in humans, we evaluated the potential of visual behaviour assays in zebrafish. Previously, gentamicin, digoxin, cisplatin, minoxidil, quinine and ibuprofen, drugs known to be toxic to the human eye, were investigated for oculotoxic effects in zebrafish larvae treated from 3-8 dpf using the OMR assay (Richards, et al., 2008). Here, we evaluated the capability of the zebrafish OKR and VMR assays to predict visual toxicity.

3.1 The zebrafish OKR is highly predictive of drugs that induce visual toxicity in humans:

For the OKR assay, wild-type larvae were treated with 10 µM drug from 3 to 5 dpf. At day 5, all larvae were washed with embryo medium, placed individually within the rotating drum and the number of saccadic responses to the moving black and white stripes quantified. All known oculotoxic compounds showed a significantly ($p \leq 0.0001$) reduced number of OKR saccades, except cisplatin (**Fig. 1**). Indeed, all known oculotoxic drugs tested, except for cisplatin, reduced the number of saccades by ~1.5 to 2 fold.

3.2 Known oculotoxic drugs do not alter gross morphology or general locomotor behavior:

To determine if the known oculotoxic drugs had general toxic effects on zebrafish larvae, we assessed the gross morphology of treated zebrafish and their locomotor response to a tactile stimulus (**Fig. 2**). For the touch response, a 22 G gauge needle was used to touch the caudal fin of treated larvae and the resulting swimming response was classified into normal, reduced or absent (**Fig. 2**). Compared to 0.1% DMSO-treated control larvae, all larvae treated with the known oculotoxic drugs displayed a normal touch response. In agreement, the gross morphology of treated larvae was also indistinguishable from vehicle-treated controls, with normal length and width, prominent pigmented eyes and inflated swim bladders. In summary, the known human oculotoxic compounds appear to induce specific toxic effects on zebrafish visual behavior, and did not alter gross morphology or general locomotor behavior.

3.3 Known oculotoxic drugs reduce the overall VMR profile:

Recently, we and others have utilised the zebrafish VMR assay as a more automated and more high-throughput approach to quantify visual behaviour in zebrafish larvae (Emran, et al., 2008; Yin Jun 2012). Thus, we sought to determine the suitability of the VMR assay to efficiently predict human drugs that induce visual toxicity. For the VMR, treated larvae are placed in individual wells of a 96-well plate and the locomotor activity of larvae in response to changes from lights ON to OFF, and vice versa, every 20 minute are automatically recorded by an infrared camera. In the overall activity traces, averaged for $n > 36$ larvae (**Fig. 3**), vehicle-treated larvae are observed to have a basal level of activity which dramatically alters upon a light change. In agreement with the OKR analyses, larvae treated with cisplatin exhibited overall VMR profiles indistinguishable from control larvae. However, gentamicin-,

digoxin-, minoxidil-, quinine- and ibuprofen-treated larvae all dramatically suppressed the waves of increased or decreased activity in response to lights OFF or ON, respectively.

3.4 Known oculotoxic drugs reduce the VMR ON and OFF peaks

Other important features of the VMR are the peak ON and OFF responses that occur immediately after light changes (**Fig. 4**). These peaks are significant readouts of visual behaviour as they are absent in eyeless or phototransduction mutant zebrafish (Emran, et al., 2008). The peak traces are calculated from the average responses of larvae at 100 seconds before, and up to 300 seconds after, the lights go ON or OFF. In the ON and OFF peak activity traces, averaged from 2 responses for n > 36 larvae (**Fig. 3**), vehicle-treated control larvae exhibited significant peak increases in activity following light changes. Notably, 5 of the 6 known oculotoxic drugs significantly impaired the ON or OFF peak VMR responses (**Fig. 4**).

3.5 Summary of VMR changes in larvae treated with oculotoxic drugs.

Additional measures of the VMR including *overall, pre-ON, pre-OFF, ON peak* and *OFF peak* activities were quantified (**Fig. 5**). The overall activity is the average activity of larvae over the entire 1 hour 40 minute assay. The pre-OFF and -ON activities are the average activities of larvae 100 seconds prior to the respective light changes. OFF and ON peak activities are calculated from the average maximum activity of larvae during the first 5 seconds after the light change. Except for cisplatin, all of the oculotoxic drugs significantly reduced the overall, pre-ON and pre-OFF activities compared to the 0.1% DMSO control. The OFF peak is significantly reduced by gentamicin, digoxin, quinine and minoxidil treatment and the ON peak is significantly reduced with gentamycin digoxin, quinine and ibuprofen. Thus, quinine, minoxidil, gentamicin and digoxin affected both the ON and OFF peak, whereas interestingly minoxidil only affected the OFF peak (**Fig. 5**).

Overall, VMR analyses of the 6 known oculotoxic compounds and the 5 control compounds resulted in 5 true positives, potentially 1 false negative, 1 false positives and 9 true negatives. Thus, the VMR assay for drugs causing visual toxicity has a sensitivity of 83%, a specificity of 90%, and a positive predictive value of 83%. In summary, we conclude that the VMR is an appropriate alternative screen to identify and predict drugs that cause visual toxicity *in vivo*.

4. Discussion

4.1 –The Need for Predictive Assays of Drug-Induced Visual Toxicity.

Only 10% of lead drugs enter the market after clinical trials and adverse side effects contribute significantly to attrition rates, and the high costs incurred with drug development failure (Cuatrecasas, 2006). It is not uncommon for these effects to be discovered only after a drug has been marketed. Adverse effects of drugs on vision are often unrecognized, overlooked or misdiagnosed. These effects can occur with drugs prescribed for indications outside of the eye, but also with the increasing number of drugs targeted for delivery to or action in the eye, (Edelhauser, et al., 2010). Although they occur only rarely, ocular side effects can be severe, so it is essential that both patients and physicians are informed. There are also ethical considerations as many assays of visual toxicity utilise higher order vertebrates. Thus, there is a need to develop assays in organisms, with less ethical concerns, that more efficiently detect adverse visual effects associated with existing drugs and drugs in development.

4.2 -Current Assays for Visual Toxicity

In vitro assays *e.g.* corneal epithelial cells and *ex vivo* assays *e.g.* the enucleated rabbit eye or bovine cornea, offer some advantages including ease of use, reduced expense and reproducibility. However, the complex physiological environment is absent in these systems. Non-invasive, *in vivo* assays have also been developed including: pachymetry, a measurement of corneal thickness; tonometry, a measurement of intra-ocular pressure; electroretinography, a physiological measurement of retinal function; and in vivo confocal microscopy, an advanced laser scan of the cornea (Pauly, et al., 2007). However, these tests often only evaluate a single parameter related to visual morphology of function, they can be technically challenging and labour intensive, and they utilise significant numbers of higher vertebrate animals.

4.3 -Assays for Visual Toxicity in Zebrafish Larvae.

Richards *et al* (2008) previously reported that 14 out of 19 known human oculotoxic compounds exerted visual toxicity effects in zebrafish larvae using the OMR assay (Car, 2006). Richards *et al* used drug concentrations ranging between 10 µM to 30 µM and treated from 3 to 8 dpf (Richards, et al., 2008). From that list, 6 compounds; gentamicin, digoxin, quinine, ibuprofen, minoxidil and cisplatin, were tested here, investigating the potential of the VMR as an improved assay to identify/predict oculotoxic drugs. Here, all oculotoxic drugs were tested at 10 µM concentration from 3-5 dpf, and all but cisplatin showed adverse effects on the zebrafish VMR: ibuprofen and minoxidil significantly reduced the ON or OFF VMR peak, whereas, gentamicin, digoxin and quinine significantly reduced the ON and OFF VMR peak. In summary, the VMR assay is a semi-automated screen that can detect drugs exhibiting ocular toxicity.

In zebrafish, the OKR and OMR assays have previously been applied to investigate the ocular toxicity of drugs (Richards, et al., 2008). These assays involve manually placing small numbers of sample groups inside a channel or a rotating drum (Brockerhoff, 2006). Although, there are newer systems to automate aspects of the assays and analysis (Mueller, Schnaedelbach, Russig, & Neuhauss, 2011), they are not currently suitable for medium- to large-scale drug screens. Disadvantages of these assays for drug screens include: restriction to screening one test compound per assay, large volumes of media (and drug) required, and group behaviour which can affect the locomotor activy of individual fish (Brockerhoff, 2006; Mueller, et al., 2011; Richards, et al., 2008). Here, we demonstrate that the VMR assay offers a more efficient, alternative approach to assess visual toxicity in zebrafish. The VMR assay allows for the quantification of 96 larval responses at a time and takes less than 2 hours to run. The VMR assay is based on rapid bursts of activity that wild-type larvae produce immediately after light changes. The presence and intensity of this peak response is indicative of visual function. For instance, Emran et al (2008) report that eyeless *rx3*$^{-/-}$ mutants don`t exhibit a VMR response to light changes and that *no optokinetic response c (nrc)* mutants, which have abnormal photoreceptors, have a diminished VMR (Emran, et al., 2008). Here, we report that adverse effects of oculotoxic drugs at 10 uM on visual function can be identified using the zebrafish VMR assay In the case of quinine, a larger fold reduction (~4 fold) in visual response was achieved with the VMR then the OKR (~1.5 fold). Secondly, within 1 hour 40 mins ~12 drugs were tested in 96 larvae using the VMR, whereas the same analysis would take ~5 hours by the OKR. In summary, the VMR is an alternative approach to identify and predict ocular visual toxicity of drugs at a pre-clinical stage.

Figure legends:

Figure 1. The effect of oculotoxic compounds on 5 dpf larval OKR after 48 hours of treatment. The graph depicts the number of saccades per minute observed in fish treated with known oculotoxic compounds or 0.1% DMSO control. All tested oculotoxic compounds, except cisplatin, significantly (***, $p<0.0001$) reduce by 1.5 -2 fold the number of eye saccades per minute. *n=30 larvae per group, experiment repeated 3 times*.

Figure 2. (a) The touch response of oculotoxic drug-treated larvae. All treated larvae have a normal swimming response to tactile stimuli, comparable to DMSO treated control larvae showing that non-visual motor responses are unaffected. *n ≥ 36 larvae, experiment repeated 3 times* (b) Gross morphology of drug-treated larvae. Dorsal (1-7) and lateral (8-14) images of 5 dpf fish after treatment with known oculotoxic drugs or 0.1% DMSO for 48 hours. No significant differences in gross morphology were discerned relative to the 0.1% DMSO control. *n ≥ 12 larvae, experiment repeated 3 times*.

Figure 3. The effect of oculotoxic drugs on overall zebrafish VMR activity. Graph tracing the overall locomotor activity of drug-treated (red trace) and DMSO treated controls (blue trace) during the 1 hour 40 minute test period. Yellow and black bars represent periods of lights ON and OFF, respectively. All oculotoxic drugs, except cisplatin, reduce the increases or decreases in VMR activity upon light changes, compared to controls. *n ≥ 36 larvae, experiment repeated 5 times.*

Figure 4. The effect of oculotoxic drugs on the average ON and OFF peak VMR responses. Digoxin, gentamicin, and ibuprofen significantly reduce the ON peak response. Minoxidil significantly reduces the OFF peak response. Quinine significantly reduces the ON and OFF peak VMR responses. *n = 36 larvae per drug, experiment repeated 5 times.*

Figure 5. Summary of effect of oculotoxic drugs on VMR parameters. The graph depicts the average overall, ON peak, OFF peak, pre-OFF and pre-ON activities of control and oculotoxic compounds. Quinine and minoxidil significantly affect the OFF peak. All compounds, except minoxidil & cisplatin, significantly (*; $p<0.05$) reduce the ON peak. *n ≥ 36 larvae per drug, experiment repeated more than 3 times.*

Supplementary Figure 1. The effect of control antibiotic drugs on overall zebrafish VMR activity and ON and OFF peak activity. Graph tracing the overall locomotor activity of drug-treated (red trace) and DMSO treated controls (blue trace) during the 1 hour 40 minute test period. Yellow and black bars represent periods of lights ON and OFF, respectively. None of the control antibiotics adversely affect the VMR activity *n ≥ 36 larvae, experiment repeated 5 times.*

Supplementary Figure 2. The effect of control pharmacological modulators of neurotransmission on the overall zebrafish VMR activity and ON and OFF peak activity. Graph tracing the overall locomotor activity of drug-treated (blue trace) and DMSO treated controls (red trace) during the 1 hour 40 minute test period. Yellow and black bars represent

periods of lights ON and OFF, respectively. None of the control antibiotics adversely affect the VMR activity *n ≥ 36 larvae, experiment repeated 5 times.*

References

Al-Tweigeri, T., Magliocco, A. M., & DeCoteau, J. F. (1999). Cortical blindness as a manifestation of hypomagnesemia secondary to cisplatin therapy: case report and review of literature. *Gynecol Oncol, 72*, 120-122.

Barros, T. P., Alderton, W. K., Reynolds, H. M., Roach, A. G., & Berghmans, S. (2008). Zebrafish: an emerging technology for in vivo pharmacological assessment to identify potential safety liabilities in early drug discovery. *Br J Pharmacol, 154*, 1400-1413.

Brockerhoff, S. E. (2006). Measuring the optokinetic response of zebrafish larvae. *Nat Protoc, 1*, 2448-2451.

Brockerhoff, S. E., Hurley, J. B., Janssen-Bienhold, U., Neuhauss, S. C., Driever, W., & Dowling, J. E. (1995). A behavioral screen for isolating zebrafish mutants with visual system defects. *Proc Natl Acad Sci U S A, 92*, 10545-10549.

Car, B. (2006). Enabling Technologies in Reducing Drug Attrition Due to Safety Failures. *Int. Drug Disc., 1*, 53-56.

Caraceni, A., Martini, C., Spatti, G., Thomas, A., & Onofrj, M. (1997). Recovering optic neuritis during systemic cisplatin and carboplatin chemotherapy. *Acta Neurol Scand, 96*, 260-261.

Cuatrecasas, P. (2006). Drug discovery in jeopardy. *J Clin Invest, 116*, 2837-2842.

D'Amico, D. J., Caspers-Velu, L., Libert, J., Shanks, E., Schrooyen, M., Hanninen, L. A., & Kenyon, K. R. (1985). Comparative toxicity of intravitreal aminoglycoside antibiotics. *Am J Ophthalmol, 100*, 264-275.

de Seze, J. (2012). Atypical forms of optic neuritis. *Rev Neurol (Paris), 168*, 697-701.

Dyson, E. H., Proudfoot, A. T., Prescott, L. F., & Heyworth, R. (1985). Death and blindness due to overdose of quinine. *Br Med J (Clin Res Ed), 291*, 31-33.

Easter, S. S., Jr., & Nicola, G. N. (1996). The development of vision in the zebrafish (Danio rerio). *Dev Biol, 180*, 646-663.

Edelhauser, H. F., Rowe-Rendleman, C. L., Robinson, M. R., Dawson, D. G., Chader, G. J., Grossniklaus, H. E., Rittenhouse, K. D., Wilson, C. G., Weber, D. A., Kuppermann, B. D., Csaky, K. G., Olsen, T. W., Kompella, U. B., Holers, V. M., Hageman, G. S., Gilger, B. C., Campochiaro, P. A., Whitcup, S. M., & Wong, W. T. (2010). Ophthalmic drug delivery systems for the treatment of retinal diseases: basic research to clinical applications. *Invest Ophthalmol Vis Sci, 51*, 5403-5420.

Emran, F., Rihel, J., & Dowling, J. E. (2008). A behavioral assay to measure responsiveness of zebrafish to changes in light intensities. *J Vis Exp*.

Fadool, J. M., & Dowling, J. E. (2008). Zebrafish: a model system for the study of eye genetics. *Prog Retin Eye Res, 27*, 89-110.

Fleisch, V. C., & Neuhauss, S. C. (2006). Visual behavior in zebrafish. *Zebrafish, 3*, 191-201.

Fraunfelder, F. W., & Fraunfelder, F. T. (2004). Adverse ocular drug reactions recently identified by the National Registry of Drug-Induced Ocular Side Effects. *Ophthalmology, 111*, 1275-1279.

Gamulescu, M. A., Schalke, B., Schuierer, G., & Gabel, V. P. (2006). Optic neuritis with visual field defect--possible Ibuprofen-related toxicity. *Ann Pharmacother, 40*, 571-573.

Goldsmith, P., & Harris, W. A. (2003). The zebrafish as a tool for understanding the biology of visual disorders. *Semin Cell Dev Biol, 14*, 11-18.

Gombos, G. M. (1983). Bilateral optic neuritis following minoxidil administration. *Ann Ophthalmol, 15*, 259-261.

Haefliger, I. O., Meyer, P., Flammer, J., & Luscher, T. F. (1994). The vascular endothelium as a regulator of the ocular circulation: a new concept in ophthalmology? *Surv Ophthalmol, 39*, 123-132.

Kimmel, C. B., Ballard, W. W., Kimmel, S. R., Ullmann, B., & Schilling, T. F. (1995). Stages of embryonic development of the zebrafish. *Dev Dyn, 203*, 253-310.

Kola, I., & Landis, J. (2004). Can the pharmaceutical industry reduce attrition rates? *Nat Rev Drug Discov, 3*, 711-715.

Kwan, A. S., Sahu, A., & Palexes, G. (2006). Retinal ischemia with neovascularization in cisplatin related retinal toxicity. *Am J Ophthalmol, 141*, 196-197.

Lawrenson, J. G., Kelly, C., Lawrenson, A. L., & Birch, J. (2002). Acquired colour vision deficiency in patients receiving digoxin maintenance therapy. *Br J Ophthalmol, 86*, 1259-1261.

Mueller, K. P., Schnaedelbach, O. D., Russig, H. D., & Neuhauss, S. C. (2011). VisioTracker, an innovative automated approach to oculomotor analysis. *J Vis Exp*.

Neuhauss, S. C., Biehlmaier, O., Seeliger, M. W., Das, T., Kohler, K., Harris, W. A., & Baier, H. (1999). Genetic disorders of vision revealed by a behavioral screen of 400 essential loci in zebrafish. *J Neurosci, 19*, 8603-8615.

Pauly, A., Brignole-Baudouin, F., Labbe, A., Liang, H., Warnet, J. M., & Baudouin, C. (2007). New tools for the evaluation of toxic ocular surface changes in the rat. *Invest Ophthalmol Vis Sci, 48*, 5473-5483.

Phillips, J. B., Blanco-Sanchez, B., Lentz, J. J., Tallafuss, A., Khanobdee, K., Sampath, S., Jacobs, Z. G., Han, P. F., Mishra, M., Titus, T. A., Williams, D. S., Keats, B. J., Washbourne, P., & Westerfield, M. (2011). Harmonin (Ush1c) is required in zebrafish Muller glial cells for photoreceptor synaptic development and function. *Dis Model Mech, 4*, 786-800.

Plummer, R., Wilson, R. H., Calvert, H., Boddy, A. V., Griffin, M., Sludden, J., Tilby, M. J., Eatock, M., Pearson, D. G., Ottley, C. J., Matsumura, Y., Kataoka, K., & Nishiya, T. (2011). A Phase I clinical study of cisplatin-incorporated polymeric micelles (NC-6004) in patients with solid tumours. *Br J Cancer, 104*, 593-598.

Redfern, W. S., Waldron, G., Winter, M. J., Butler, P., Holbrook, M., Wallis, R., & Valentin, J. P. (2008). Zebrafish assays as early safety pharmacology screens: paradigm shift or red herring? *J Pharmacol Toxicol Methods, 58*, 110-117.

Richards, F. M., Alderton, W. K., Kimber, G. M., Liu, Z., Strang, I., Redfern, W. S., Valentin, J. P., Winter, M. J., & Hutchinson, T. H. (2008). Validation of the use of zebrafish larvae in visual safety assessment. *J Pharmacol Toxicol Methods, 58*, 50-58.

Santaella, R. M., & Fraunfelder, F. W. (2007). Ocular adverse effects associated with systemic medications : recognition and management. *Drugs, 67*, 75-93.

Schmid, K. E., Kornek, G. V., Scheithauer, W., & Binder, S. (2006). Update on ocular complications of systemic cancer chemotherapy. *Surv Ophthalmol, 51*, 19-40.

Taylor, K. L., Grant, N. J., Temperley, N. D., & Patton, E. E. (2010). Small molecule screening in zebrafish: an in vivo approach to identifying new chemical tools and drug leads. *Cell Commun Signal, 8*, 11.

Thomas, T., Galiani, D., & Brod, R. D. (2001). Gentamicin and other antibiotic toxicitiy. *Ophthalmol Clin North Am, 14*, 611-624.

Verdugo-Gazdik, M. E., Simic, D., Opsahl, A. C., & Tengowski, M. W. (2006). Investigating cytoskeletal alterations as a potential marker of retinal and lens drug-related toxicity. *Assay Drug Dev Technol, 4*, 695-707.

Westerfield, M. (2000). A guide for the laboratory use of zebrafish (Danio rerio). *University of Oregon Press., 4th ed*.

Yin, J., Shine, L., Raycroft, F., Deeti, S., Reynolds, A., Ackerman, K. M., Glaviano, A., O'Farrell, S., O'Leary, O., Kilty, C., Kennedy, C., McLoughlin, S., Rice, M., Russell, E., Higgins, D. G., Hyde, D. R., & Kennedy, B. N. (2012). Inhibition of the Pim1 oncogene results in diminished visual function. *PLoS One, 7*, e52177.

Yin Jun , L. S., Francis Raycroft , Sudhakar Deeti, Alison Reynolds, Kristin M. Ackerman, Antonino Glaviano, Sean O'Farrell , Olivia O'Leary, Sarah McLoughlin, Megan Rice, Eileen Russell,

Desmond G. Higgins, David R.Hyde & Breandan N. Kennedy* (2012). INHIBITION OF THE PIMl ONCOGENE RESULTS IN DIMINISHED VISUAL FUNCTION. *Plosone*.

Figure 1.

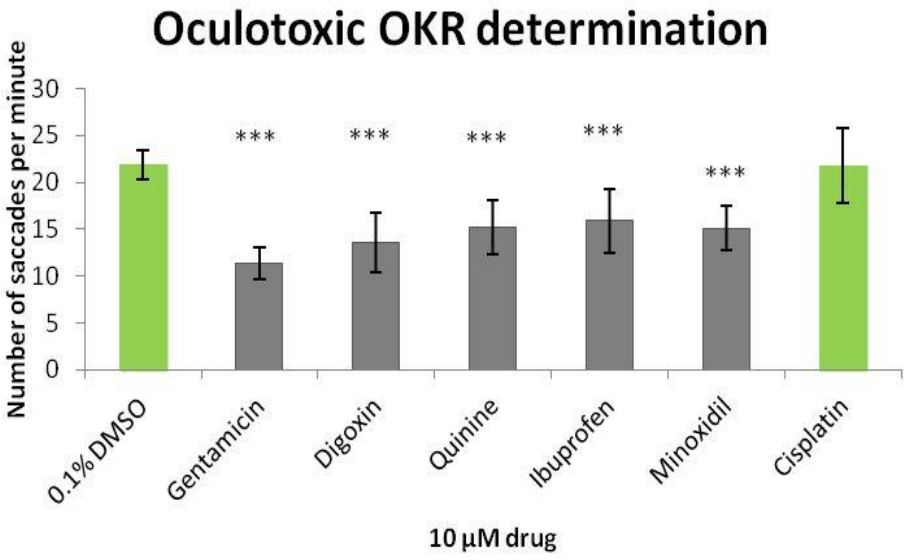


Figure 2. Non-visual behavioural responses & gross morphology of oculotoxic drug-treated larvae

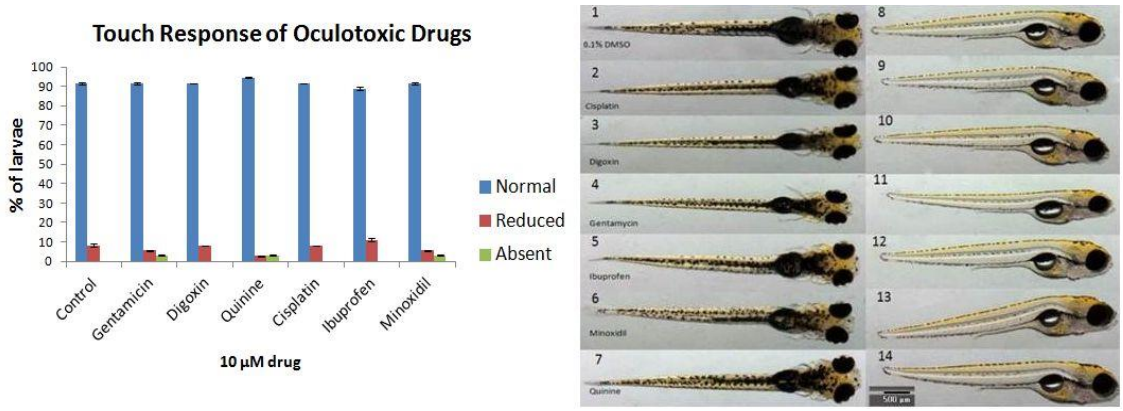


Figure 3. Overall VMR activity traces

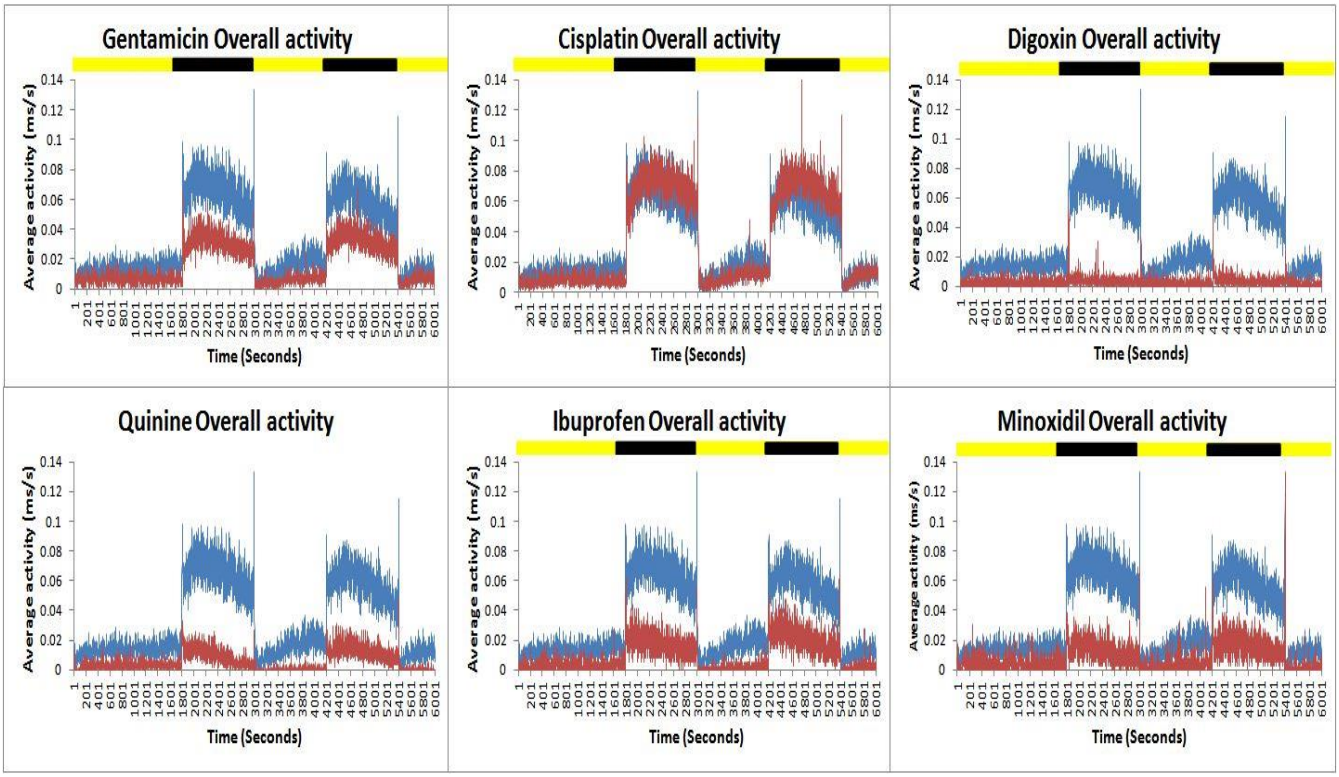


Figure 4. ON/OFF Peak VMR TRACES

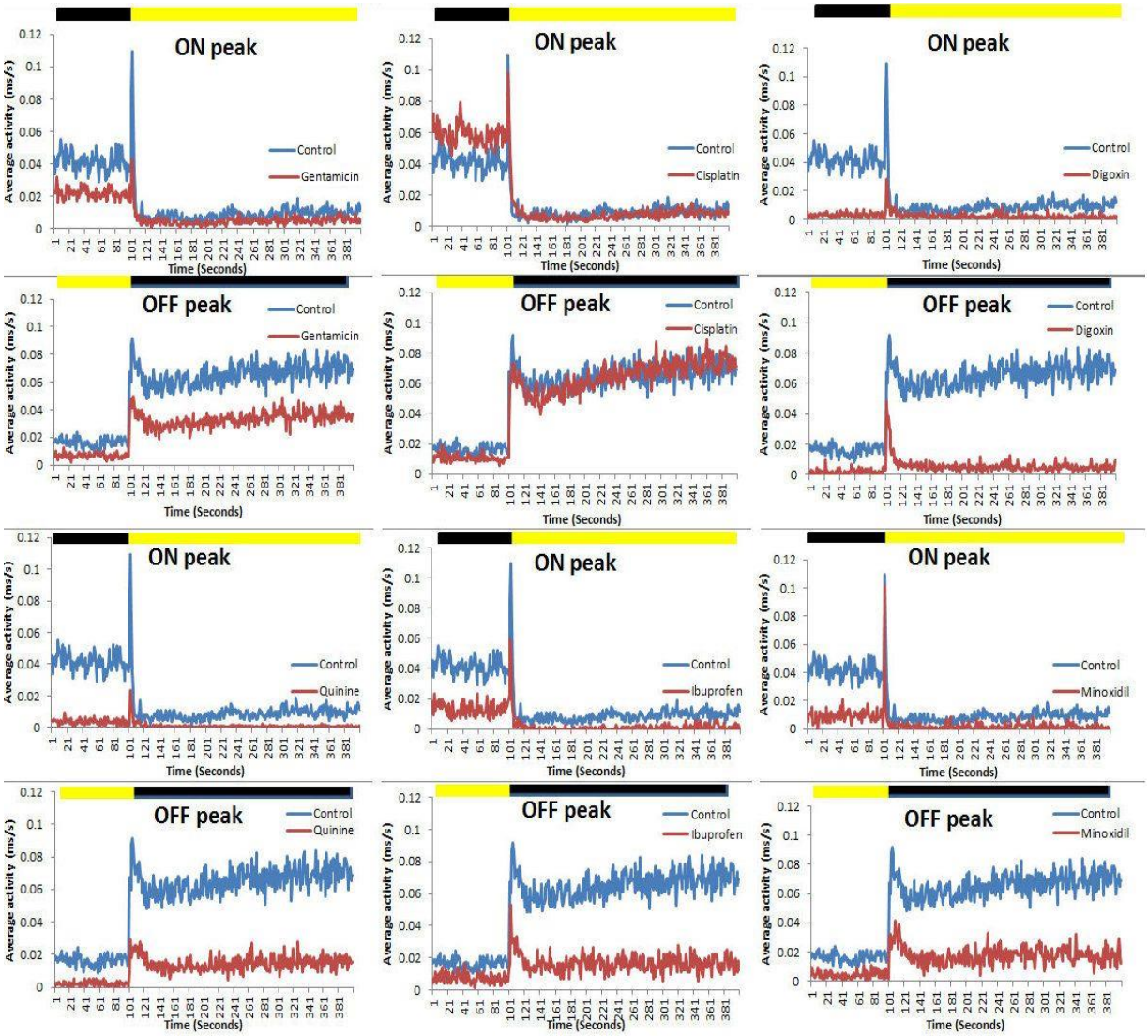


Figure 5. Summary of VMR

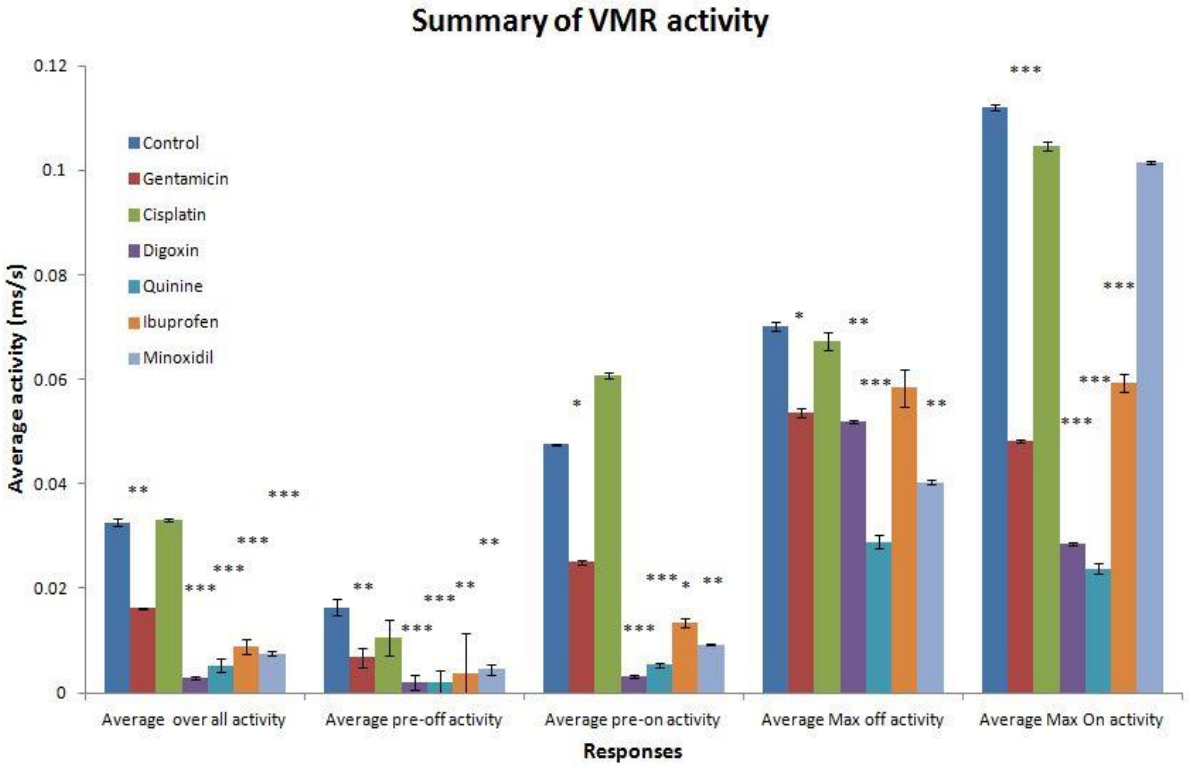
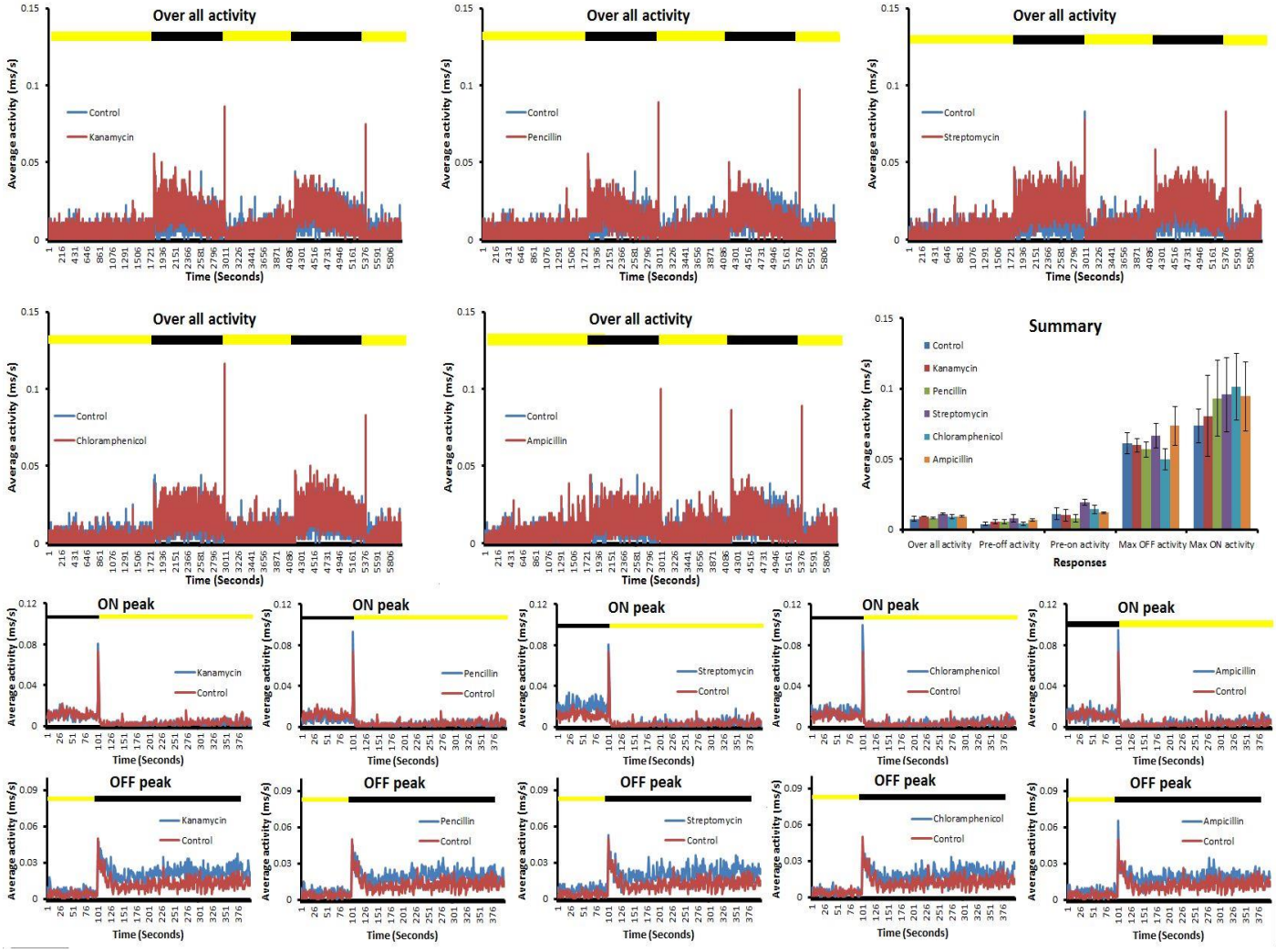


Table 1: Summary of the pharmacological, behavioural and morphological effects on known oculotoxic compounds in zebrafish larvae. *NT; not tested.*

Compound	OMR *(Richards et al. 2008)*	**OKR assay**	**Effects on VMR at 10 µM**		**Touch Response**	**MTC (µM)**	**Morphological defects**
			ON peak	OFF peak			
Cisplatin	**Reduced**	No affect	No affect	No affect	No affect	>150	None
Digoxin	**Reduced**	**Reduced**	**Reduced**	**Reduced**	No affect	>200	None
Ibuprofen	**Reduced**	**Reduced**	**Reduced**	No affect	No affect	>250	None
Gentamicin	**Reduced**	**Reduced**	**Reduced**	**Reduced**	No affect	>350	None
Minoxidil	**Reduced**	**Reduced**	No affect	**Reduced**	No affect	>150	None
Quinine	**Reduced**	**Reduced**	**Reduced**	**Reduced**	No affect	>250	None
Kanamycin	NT	NT	No affect	No affect	NT	NT	None
Penicillin	NT	NT	No affect	No affect	NT	NT	None
Streptomycin	NT	NT	No affect	No affect	NT	NT	None
Chloramphenicol	NT	NT	No affect	No affect	NT	NT	None
Ampicillin	NT	NT	No affect	No affect	NT	NT	None
Histamine	NT	NT	No affect	No affect	NT	NT	None
Atropine	NT	NT	No affect	No affect	NT	NT	None
Cimetidine	NT	NT	**Reduced**	No affect	NT	NT	None
Clonidine	NT	NT	No affect	No affect	NT	NT	None
Lap4	NT	NT	No affect	No affect	NT	NT	None

Supplementary Figure 1



Supplementary Figure 2