3-бөлім

АДАМ ЖӘНЕ ЖАНУАРЛАР ФИЗИОЛОГИЯСЫ

Раздел 3

ФИЗИОЛОГИЯ ЧЕЛОВЕКА И ЖИВОТНЫХ

Section 3

HUMAN AND ANIMAL PHYSIOLOGY

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ZFET (Zebrafish embryo toxicity test) as a model test for determination of heavy metals embryo toxicity

ZFET (Zebrafish embryo toxicity test) is established by OECD as a mandatory step for testing the toxicity of water pollutants and manufacturing sewage waters. As the result of present investigation it was shown the dose-dependent embryolethal but not teratogenic effect of CdSO4. Concentration of 3,5 mM CdSO4 may be considered as minimal 100% lethal concentration for 3 h exposition. Maximum embryos death by 24 h as the result of deep life-incompatible malformations and subsequent embryos coagulation. Survived embryos showed no significant signs of abnormality and developed normally. At the same time Pb(CH3COO)2 in studied concentrations acted as disruptor of normal embryogenesis, however there was no embryo lethal effect observed. Approximately 80% of embryos exposed to 8,125 mM and 812,5 mM Pb(CH3COO)2 showed signs of scoliosis, accompanied by tail tip malformations covering an average 40% of exposed embryos. It is also possible that observed negative effects of lead are the result of increased chorion permeability and alteration of water and salt balance in embryo. Based on the results of experiments it was concluded that cadmium is considered to be highly toxic to zebrafish embryos, dose comparison testify the threefold degree cadmium toxicity to zebrafish embryos comparing with lead.

Key words: Danio rerio, embryos, embryo toxicity, heavy metals.

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ZFET (жолақты даниодағы эмбриотоксиндікті анықтайтын тест) ауыр металдардың эмбриотоксиндігін зерттеу үшін модельді тест

Ағынды суларды және суда ерігіш ксенобиотиктердің улылығын зерттеуде міндетті турде ОБСЕ-ге ZFET усынылады. Бұл тәжірибеде кадмидің аумақтық эфектісі болмаған кезде эмбрионға CdSO4 мөлшерге тәуелді летальді эфектісінің әсерін көрсеткен. Эмбриондардың 3 сағат экспозициянан кейін 100% өлім тудыратын CuSO4 3,5 мМ концентрациясы әсері ең аз концентрация ретінде қарастыруға болады. Эмбриондардың көпшілігінің өмірмен сыйыспайтын терең кемістіктерінің нәтижесінде, және одан кейінгі эмбриондардың коагуляциясы 24 сағаттан байқалды. Аман қалған эмбриондар дамуында бұзылу айқын белгілері болған жоқ және инкубациядан бұрын әдеттегідей дамыған. Pb(CH3COO)2 эмбриондардың даму процесін бұзушы, зерттеліп жаткан концентрацияға әекет ететін зат ретінде,(дамудың бұзылу спектрі перикард ісігіне, кеуде және құйрық бөлімінің қисайуына әкеледі.) бірақ летальді әсер етпейді. 8,125 мМ және 812,5 мМ Pb(CH3COO)2 әсеріне ұшыраған эмбриондардың шамамен 80% сколиоз, (40% эмбриондардың байқалды) құйрығы бұзылу белгілері көрсетілді. Бұл байқалған қолайсыз әсерлер ұрықтың су-тұз балансының және хорион өткізгіштігінің бұзылу нәтижесі болып табылады деп болжанып отыр. Зерттелген активті концентрациясын салыстырғанда қорғасын ацетатына қарағанда кадмий сульфаты Danio rerio эмбриондарға үш есе улы болады.

Түйін сөздер: Danio rerio, эмбрион, эмбрион уыттылығы, ауыр металдар.

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ZFET (тест на токсичность для эмбрионов полосатого данио) как модель для исследования эмбриотоксичности тяжелых металлов

ZFET (исследование токсичности для эмбрионов полосатого данио) установлен ОБСЕ в качестве обязательного этапа при исследовании токсичности водных ксенобиотиков и сточных вод. По результатам исследования было показано дозо-зависимое летальное действие CdSO4 на эмбрионы при отсутствии тератогенного эффекта кадмия. Концентрация 3,5 мМ CdSO4 можно рассматривать как минимальную концентрацию, вызывающую гибель 100% эмбрионов при 3 ч экспозиции. Гибель большинства эмбрионов наблюдалась к 24 ч как результат глубоких уродств, несовместимых с жизнью, и последующей коагуляции эмбрионов. Выжившие эмбрионы не имели явных признаков нарушения развития и развивались нормально до вылупления. В тоже время Pb(CH3COO)2 в исследуемых концентрациях действует как вещество, нарушающее процессы нормального развития эмбрионов, но не оказывает летального действия. У приблизительно 80% эмбрионов экспонированных с 8,125 мМ и 812,5 мМ Pb(CH3COO)2 отмечались признаки сколиоза, сопровождаемые нарушениями формирования хвоста (отмечались у 40% эмбрионов). Предполагается, что наблюдаемые негативные эффекты являются результатом повышения проницаемости хориона и нарушения водно-солевого баланса эмбриона. Сравнение активных концентраций исследуемых металлов позволяет заключить, что сульфат кадмия на три порядка более токсичен для эмбрионов Danio rerio, чем ацетат свинца.

Ключевые слова: Danio rerio, эмбрионы, эмбриотоксичность, тяжелые металлы.

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ZFET (Zebrafish embryo toxicity test) AS A MODEL TEST FOR DETERMINATION OF HEAVY METALS EMBRYO TOXICITY

Introduction

An enormous amount of chemicals produced and sold throughout the world dictates systematic generation and evaluation of ecotoxicological data of chemicals that is critical for risk assessment and the safety of both man and environment. Emphasizing the persistent environmental pollutants, heavy metals are highly toxic to terrestrial and aquatic organisms, but for all that the toxicity of heavy metals is predominantly investigated on model terrestrial organisms whereas their impact on aquatic organisms is less cleared. The fact is that metals are non biodegradable and accumulate in the food chain, make them deleterious to the aquatic organisms and consequently to human being who consume fish as a food source [1]. Determination of heavy metals concentrations in cultured and wild fish species reveals heavy metals concentrations greater than the upper level of intake in food for human consumption according to WHO [2] arguing that heavy metals can be strongly accumulated and biomagnified along the aquatic food chains.

Toxicological studies mainly deal with adult animals or refer to field fish populations, meanwhile the reproductive ability and early life stages of fish, like eggs and larvae are particularly sensitive to contaminants, contributing a gross mortality of polluted fish population. Toxicity tests using early life stages of fish are of great importance in assessing risks to growth, reproduction and survival in polluted environments and are important tools for good environmental monitoring. In the field of environmental and toxicology methodology researches are directed for development and application of sensitive tests providing both advantages of *in vitro* and *in vivo* investigations, cheap and representative, considering ethical recommendations and animal welfare restrictions.

There are three main fish species recommended for investigations on xenobiotics embryotoxicity/teratogenicity as well as adult acute toxicity tests – zebrafish (*Danio rerio*), fathead minnow (*Pimephales promelas*) and medaka (*Oryzias latipes*), between them the zebrafish is considered to be most suitable for studies as the one with most rapid development (for 72 h) make it ideal for high-throughput screening and well studied morphology, physiology and genetics [3]. Multiple results indicate that zebrafish embryos

are useful alternative method for traditional toxicity and teratogenicity testing. Adult zebrafish (*Danio rerio*) are easily cared and maintained, reach sexual maturity after three months, females can spawn hundreds of eggs per spawning and the whole year. The chorion is transparent and the whole development may be observed via microscope until hatching. Morphology, anatomy and individual development are completely investigated, genome is fully sequenced. A remarkable similarity in cellular structure, signaling processes, anatomy and physiology exist among zebrafish and other high-order vertebrates, including human, particularly in early stages of development [4].

ZFET (Zebrafish Embryo Toxicity Test) based on morphological observation of embryos mortality and teratogenicity is established by OECD and ECVAM as mandatory component of chemical testing [5]. Moreover since zebrafish embryos up to 96 h post fertilization are not considered as test animals, because they do not feed externally, the ZFET is not regarded an animal test [6]. Unfortunately, in the CIS the ZFET is not widespread and is in disfavor as the result of menthal indolence and prejudice against a fish as alternative for animal embryo testing. As the cheapest, rapid and informative test for chemicals embryotoxicity the ZFET should precede routine embryotoxicity tests on mammals' models as first-line screening test for developmental abnormalities. Perturbed development can manifest as morphological malformations, behavioral abnormalities or death of the embryos. Besides, even for most well studied xenobiotics their effects on developing aquatic organisms are not cleared as well as effective doses for embryos. Multiple data suggest that embryos are much more sensitive for toxicants that adult fishes, so when establishing and checking maximum permitted concentrations of pollutants one should take into consideration the embryotoxicity/teratogenicity effects. The embryonic zebrafish model offers multiple advantages for metal embryotoxicity screening: power of whole-animal investigation, convenience of cell culture, high rate of individual development, optical transparence make it possible to investigate embryotoxicity throughout embryological period using simple microscopic techniques. Thus in this paper, zebrafish embryos, which represented an attractive model for studying the toxic mechanisms of environmental chemicals were used to examine the individual effects of two heavy metals, cadmium and lead in ZFET (Zebrafish Embryo Toxicity Test).

Materials and methods

Livestock of adult zebrafish (*Danio rerio*) was used for obtaining the embryos. Producers were kept in 50 L aquaria under constant flow through conditions 23±1 ºC and 12 h light/dark cycle, fed by live and dry food once daily. The female is considered to be ready for spawning when protruding its belly. The nest (male:female ratio 2:4) for spawning was transferred into smaller sterile aquaria (15 L), preliminary filled with preheated (up to 26±1 ºC) clean water since the night so that the very early light (intensive natural light is the better) would trigger the spawning. The bottom of spawning aquaria was covered with plastic greed with a mesh size of 1,25 mm to prevent egg predation. Usually the next early morning spawning occurred, adult fish were removed and transferred into the home aquaria, eggs were collected and transferred into sterile vial with clean preheated water (t 26±1 ºC) for further investigations [5].

The glass vessel contained eggs was examined under a stereo microscope with a minimum magnification x4 to identify the fertilized embryos. All experiments were started from early epibolia stage (~ 6 hpf – hours post fertilization). At this stage it is easily to select normally developing embryos from false-developed (this may spontaneously occurs in fishes) and coagulated embryos and avoid false-positive results. All embryos with observable abnormalities were discarded. The exposition was started by addition of the test substances for fertilized eggs. The test concentrations of heavy metals used in current experiments were follows: $3{,}5x10^{-3}$M, $1{,}75x10^{-4}$M, $3{,}5x10^{-5}$M, $3{,}5x10^{-6}$M of $CdSO_4$ exposition for 3 h, then incubation media was replaced; $8{,}125x10^{-3}$M, $81{,}25x10^{-3}$M, $812{,}5x10^{-3}$M of $Pb(CH_3COO)_2$ exposition throughout the 72 h of incubation. All metal solutions were prepared by dilution of stock solutions on bidistilled water.

For exposition each test group (20 eggs per dish, dish triplets for each group) was placed into sterile plastic Petry dishes with preheated pure water medium (t 27±1 ºC), preliminary saturated with oxygen and the test concentrations were added [5]. Methylene blue was added to medium to prevent egg affection by fungi and for better recognizing normal embryo from coagulated ones. Petry dishes with embryos were sealed and placed into incubator APT.lineTM B28 (Binder, Germany) for further development under 27±1 ºC. The medium was replaced daily with new, pure preheated and oxygen-

saturated one, containing correspondent metal concentration if needed.

Eggs were investigated under stereo microscope at 10 hpf (hours post fertilization), 24 hps, 48 hpf and 72 hpf (after hatching) for mortality and malformations. Coagulated eggs were calculated and discarded. The ebryotoxicity was calculated as mortality based on four lethal endpoints attributed to ZFET. An embryo is defined to be dead if one of following endpoint is observed: a) coagulation of the embryo; b) non-detachment of the tail; c) non-formation of somites; d) non-detection of heartbeat (after 48 hpf) [5]. As the rate of embryonic development varies with temperature, water quality and other laboratory conditions one should stage embryos morphologically but not only considering the hours post fertilization (indeed it is quite difficult to catch proper time of fertilization). Taking this into account we used recommended time-points but all embryos were staged as described by Kimmel at al [7]. Teratogenic effects were recorded according to Nagel (table).

Table – Lethal and teratogenic effects observed in zebrafish embryos up to 72 hpf [8]

Category	Physiological/dysmorphogenic effect
Lethal effect	Coagulated egg
	No heart beat (only after 48 hpf)
Teratogenic effect	Malformation of head
	Malformation of eyes
	Malformation of otoliths
	Malformation of tail
	Malformation of tail tip
	Scoliosis
	Deformity of yolk
	Edemas
	Growth retardation

There is divergence in duration of a zebrafish embryotoxicity test – OECD guidelines establish test end at 48 hpf (before hatching), Aquatic Ecology and Toxicology Department of Zoology University of Heidelberg (fist-rate in zebrafish investigations in Europe) – at 72 hpf [5, 8]. We considered tests to be ended at 72 hpf, because until hatching it is quite difficult to recognize curvatures of chorda as embryo is coiled in chorion. Microscopic examination of live embryos was performed using stereo microscope DM 143 (Motic, China) and Motic Images Plus 2.0 software. For further assessment survived embryos after hatching were fixed in 4% formaldehyde. For microscopic examination fixed embryos were washed from fixatives, stained with Mayer hematoxylin, differentiated in acidic 70% ethyl alcohol, dehydrated in series of ethyl alcohol of increasing strength, clearing in xylene, than whole-mount preparations were prepared using Biomount embedding medium. Slides were examined using DM 300 microscope (Leica, Germany) with in-built digital camera, images were captured and processed using Biovision 4.0 software.

Acceptance criteria were following: a) the parent fertility rate should be ≥70%; b) the water temperature should be maintained at 27±1ºC in all test plates all over total duration of the test; c) overall survival rate in the control groups should be ≥ 90% until the end of exposure. All test groups were represented in triplets. The statistical analysis was performed using Microsoft Office Exel statistic software, data were represented as a mean and error of mean.

As regard to ethics within the chorion, fish embryos are not subjected to Directive 86/609/EEC, which regulates the use of animals in scientific experiments and exposure of zebrafish embryos to up to 5 days post fertilization is possible without interfering with present animal welfare legislation.

Results and discussion

The increasing number of chemicals –water pollutants suggests search for new sensitive tools for investigation of xenobiotics toxicity. Despite all efforts to reduce chemical pollution of waters fish populations have not recorded in many regions. Given the importance of fish in aquatic pollution monitoring fish have intensively been implemented in aquatic toxicity testing regulations. Since 2005, fish embryo toxicity testing has been made mandatory for routine sewage surveillance in Germany [9].The point is that gross of MPC (maximum permissible concentrations) for waters used for fishery were established for adults. However some chemicals are more toxic to fish embryos than to adult fish, some are less toxic. In an independent approach to analyze the correlation between ZFET and acute fish data for approximately 90% of tested substances LC_{50} value was within the range documented for conventional acute fish LC_{50} [9]. Thus some European countries are now moving away from acute fish testing toward alternative test methods such as ZFET. The number of zebrafish-related publications averages around 3500 annually [10].

There are numerous advantages for the use of zebrafish as a toxicological model species. The main benefits regard to their size, husbandry and fertility [10]. Zebrafish adults are approximately 2-3 cm long, reducing housing space and husbandry costs, each female may produce up to 300 eggs. Because of the external development and small size of zebrafish embryos they may be tested together using a single cell-culture plate or series of petri dishes to provide several experimental replicates at one time. The chorion of zebrafish is transparent, thus their optical clarity allows for easy developmental staging and assessment of toxicity endpoints without disturbing natural process of embryogenesis and throughout all development period. Small size of embryos and hatched larvae allows the whole-mount staining and assessment. As the embryonic development is considered to be most vulnerable and valuable stage of a fish life cycle it is important to determine individual toxicity of well-spread pollutant with the aim of reconsideration of MPC_{fish}. From a review on approximately 150 toxicological studies using different life-stages of fish, there was concluded that in at least 80% of the cases long-term toxicity could be predicted by results from studies with early life-stages [9]. In our experiments we have investigated lethal and dismorphogenic effects of two well-spread heavy metals – cadmium and lead in the form of water-dissolved salts in zebrafish embryotoxicity test.

Eggs of zebrafish are telolethital characterized by separate and non-dividing yolk. The experiments were started not immediately after spawning rather after 5-6 hpf at the 70%-epiboly stage. The choice of this stage is justified by that fact that at the very early stages of development (cleavage and blastula period) it is difficult to distinguish unfertilized and fertilized eggs. So called false activation by medium and temperature changes is possible for these fishes contributing to false-negative results. The gross mortality of eggs relates to cleavage stages so it was considered worth starting experiments at late epiboly stage. Up to 24 hpf the gastrulation and segmentation periods are finished so that somites, optic vesicle, lens and otic placode are distinguishable. To 48 hpf pigmentation and heartbeat occurs, strong circulation is visible and embryo spontaneously moves in chorion, yolk ball forms great extension, tail detaches from the yolk. Time period from 48 to 72 hpf is recorded as hatching period – for end of this period well pigmented embryo hatches and wide open mouth protruding anterior to eye.

The control of embryos mortality was performed throughout all development period. The proper investigation of malformations was performed after hatching. The embryos investigated in all experimental groups were staged as being at late pec-fin and early protruding mouth stages thus at hatching period (fig. 1, A). Also there is some heterogeneity at the hatching time – individuals within a single development clutch hatch sporadically during the 72 hpf.

Morphogenesis of many of the organ rudiments are now complete and slows down considerably, with some notable exceptions including the gut and its associated organs [7]. Visually rapid development of rudiments of pectoral fins, the jaws and the gills might be recorded. The head-trunk angle increases between 20 and 70 hpf as the consequence of straightening of the embryo. The pectoral fin has a flat flange and held back along the side of the body, and extends posteriorly to cover more than half of the yolk ball. At dorsal view the width of the head at the eyes exceeds the width of the yolk ball. The taper of the yolk ball is more prominent. The ventral cartilage of the mandibular arch (Meckel's cartilage) and ventral element of the hyoid arch are large supportive structures of the lower jaw, beneath oral cavity (fig. 1, D). In later embryos the mouth is wide open and it protrudes anteriorly just beyond the eye. Pigmentation of the retina is so dense that its opacity nearly hides the lens. Melanophores occupy each lateral stripe on the trunk and head (fig. 1). The heart is prominent, beating strongly and full of circulating blood. That flows in all of the aortic arches and through the subclavian loop.

In all experiments concurrent control groups showed no more than 10% for lethal/malformed embryos so that experiments were considered to fulfill acceptance criteria. The cadmium appears to be more toxic than lead in acute exposure, that was in accordance with our results. Thus embryos exposure to cadmium for 3 h led to immediate coagulation of exposed embryos. Three investigated concentrations – $3{,}5x10^{-3}$M, 1, $75x10^{-4}$M $2CdSO_4$ and $3{,}5x10^{-5}$M $CdSO_4$ caused mortality in 100% of exposed embryos (fig. 2, A), so that concentration of $3{,}5x10^{-5}$M $CdSO_4$ may be considered as minimal 100% lethal concentration for 3 h exposition. The only group where survived embryos were observed was the one exposed to $3{,}5x10^{-6}$M $CdSO_4$ – cumulative mortality for 24 hpf was no more than 60%. Embryos death by 24 hpf was the result of deep life-incompatible malformations and subsequent embryos coagulation. Survived embryos showed no significant signs of abnormality and developed normally for the next 48 h (fig 2, B). Hence the cadmium sulfate in investigated concentrations possesses rather embryo

lethal but teratogenic effect by 48 hpf and regarding to concentrations used is considered to be highly embryo toxic to zebrafish embryos.

To investigate lead embryo toxicity we chose $Pb(CH_3COO)_2$ as organic salts of lead are considered to be more toxic than inorganic. The mortality and dysmorphogenic effects resulting from embryos exposure to lead acetate are shown at the figure 3. In all investigated groups the embryo mortality was not exceed 10% and do not significantly differed from the control thus considering that lead in studied concentrations did not show embryo lethal action. Rather, as shown at the figure 3, A the $Pb(CH_3COO)_2$ caused malformations in an average 90% of exposed embryos. Types of malformations and their contribution to overall abnormalities are shown on figure 3, B-D. The maximum of observed abnormalities related to malformations of spinal cord and related structures – tail and tail tip (fig.1 A – C). Notochord curvatures in various planes lead as well as may be the result of defects in structure of muscular tissue and influence the making of excretory system.

When larvae transfer to active feeding those curvatures might be the reason for increased larvae mortality. The significant malformations may be considered those that cover approximately and more than 50% of exposed embryos.

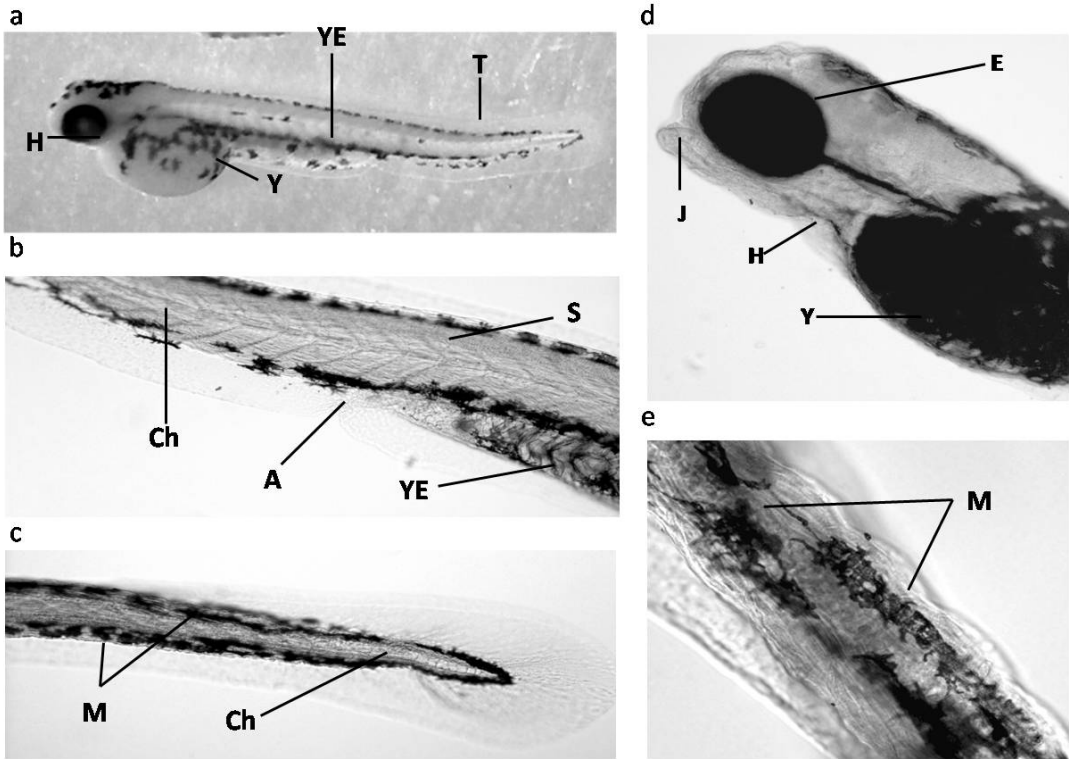

Figure 1 – Zebrafish (*Danio rerio*) embryos exposed to $8{,}125*10^{-3}$M lead acetate. A – pec-fin stage, x 35, stereomicroscope image; B – D protruding mouth stage, x 100, whole-mount; E – pec-fin stage, x 400, whole-mount. Note the pathological trunk (B), tail (A) and tail tip (C) curvatures. Abbreviations: A – anal gut opening, Ch – notochord, E – eye, J – jaws, H – heart, M – melanocytes, S – somites, T – tail, Y – yolk, YE – yolk extension

Approximately 80% of embryos exposed to $8{,}125x10^{-3}$M and $812{,}5x10^{-3}$M $Pb(CH_3COO)_2$ showed signs of scoliosis. Last are well seen in late stages of development, after hatching when embryo is free from chorion and notochord curvatures may be well distinguished. Notochord curvatures most probably are the result of both muscles paralysis and abnormalities of bone formation due to toxic action of lead acetate. Scoliosis usually accompanied by tail tip malformations (fig. 1, C) covering an average 40% of exposed embryos in all experimental groups, deficient segmentation and decreasing and malformation of muscle segments. Other prevalent embryos abnormalities referred to hydropic cavi-

ties. Mostly they are observed in pericardium and abdominal cavity, occasionally – in the brain. In our experiments pericardial edemas were observed in groups exposed to $812{,}5x10^{-3}$M and $81{,}25x10^{-3}$M $Pb(CH_3COO)_2$ in the ratio of 10% and 60% of exposed embryos correspondingly. In those embryos heart is in a form of thin tube and pericardium is swelled as the result of pathological fluid accumulation. Edemas arise in embryos when water balance is disturbed significantly. In our experiments there was observed accumulation of methylene blue that was added to medium to prevent fungi infection in pigment cells that testify to increasing chorion permeability. This phenomenon never occurs during development of control or cadmium treated embryos. Increased chorion permeability may lead to disturbance in water and salt balance in embryo and thus contributes to observed edema. Partly this depends on the ability of a compound to pass through the chorion. Chorion acts as a barrier for chemicals whose molecular weight is too high to enter embryo via the pores within the chorion, but their toxicity can occur after hatch. It is assumed that heavy metals are known to be blocked by the chorion via binding, most likely via complexation by anionic charged groups, possibly thiol-groups, which are abundantly present in the chorion. [11]. Nevertheless this study and other references indicate that heavy metals are highly toxic to embryos, means that whether cadmium and lead are able to pass through chorion causing direct toxicity or disrupt its permeability. Ansari S. had studied influence of three heavy metals (Zn, Ni and Cr) in ZFET and considered that very low concentrations of all the three metals are effective to kill the 10%, 50% and 90% of the total population accordingly [1]. It was also found that the embryos of zebrafish were less sensitive to heavy metals as compared to larvae, due to the presence of chorion. Abnormal locomotory activity, morphological abnormalities was also observed.

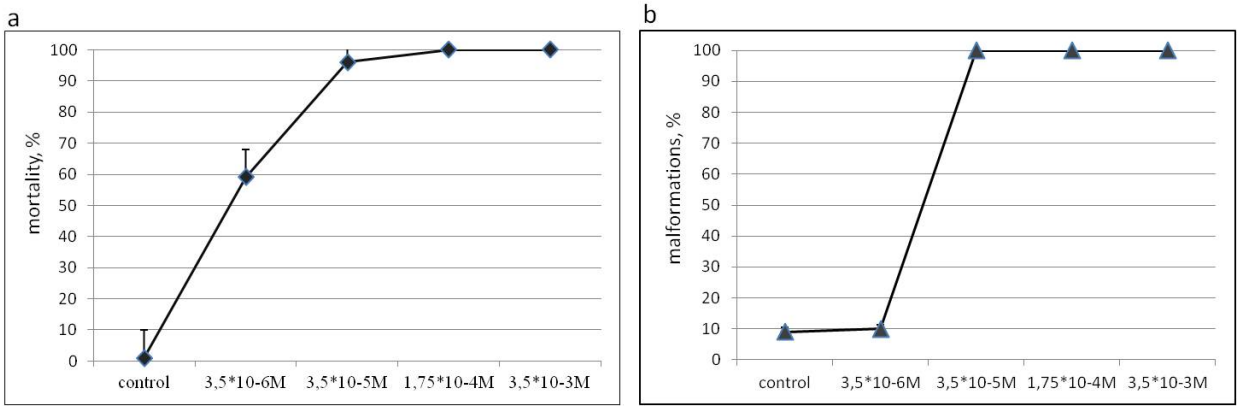

Figure 2 – Overview of the lethal and dismorphogenic effects of cadmium sulfate in zebrafish (*Danio rerio*) embryos after 48 hpf. The standard deviation for each parameter is the result of three independent runs.

Mechanisms of developmental toxicity have been partially explained for only a few toxicants, and there is no chemical for which it is fully explained [10]. Indeed it is very little known about mechanisms of embryo lethal and dismorphogenic effects of cadmium and lead in fish. Concerning cadmium and lead their chemical coordination and oxidation-reduction properties have given them an additional benefit so that they can escape control mechanisms such as homeostasis, transport, compartmentalization and binding to required cell constituents. These metals bind with protein sites which are not made for them by displacing original metals from their natural binding sites causing malfunctioning of cells and ultimately toxicity [12]. Metals get to fish organism different ways: directly from water by gills and skin or by alimentary tract (with food). The largest quantities of cadmium and copper are accumulated in metabolically active tissues (e.g., liver, kidney, alimentary tract, spleen), where they are bound to metallothioneins, one molecule of which can sequester 6–7 cadmium molecules [13, 14]. There is less known about metallothioneins induction in embryos and larvae. Cd multiple effects on cells also express in the affection of essential cellular processes such as cell division, proliferation, differentiation and apoptosis. Cd triggers cell apoptosis, both in vitro and in vivo in several models. Commonly, Cd can induce apoptosis via a caspase-dependent pathway or a

caspase-independent pathway based on the different Cd exposure conditions. Caspase 3 induction in response to cadmium was recorded in the following fish species: cinnamon clownfish (*Amphiprion melanopus*), rock bream (*Oplegnathus fasciatus*), European sea bass (*Dicentrarchus labrax*), zebrafish (*Danio rerio*), large yellow croaker (*Pseudosciaena crocea*), Atlantic salmon (*Salmo salar*), Nile tilapia (*Oreochromis niloticus*) and Medaka (*Oryzias latipes*) [15]. There was shown dose response acute toxicity, abnormal somite patterning and ectopic apoptosis induction when exposing zebrafish embryos to cadmium, and acute toxicity after lead exposure [10] as well as both lethal and sub-lethal effects such as 24 hpf death and 72 hpf delayed hatching [16]. Discussing the mechanisms of cadmium embryo toxicity, there was evidence that CdSO4 caused significant oxidative stress, including decreases in the reduced glutathione (GSH) level, inhibition of superoxide dismutase (SOD) activity, as well as increases in malondialdehyde (MDA) content in zebrafish embryos [16].

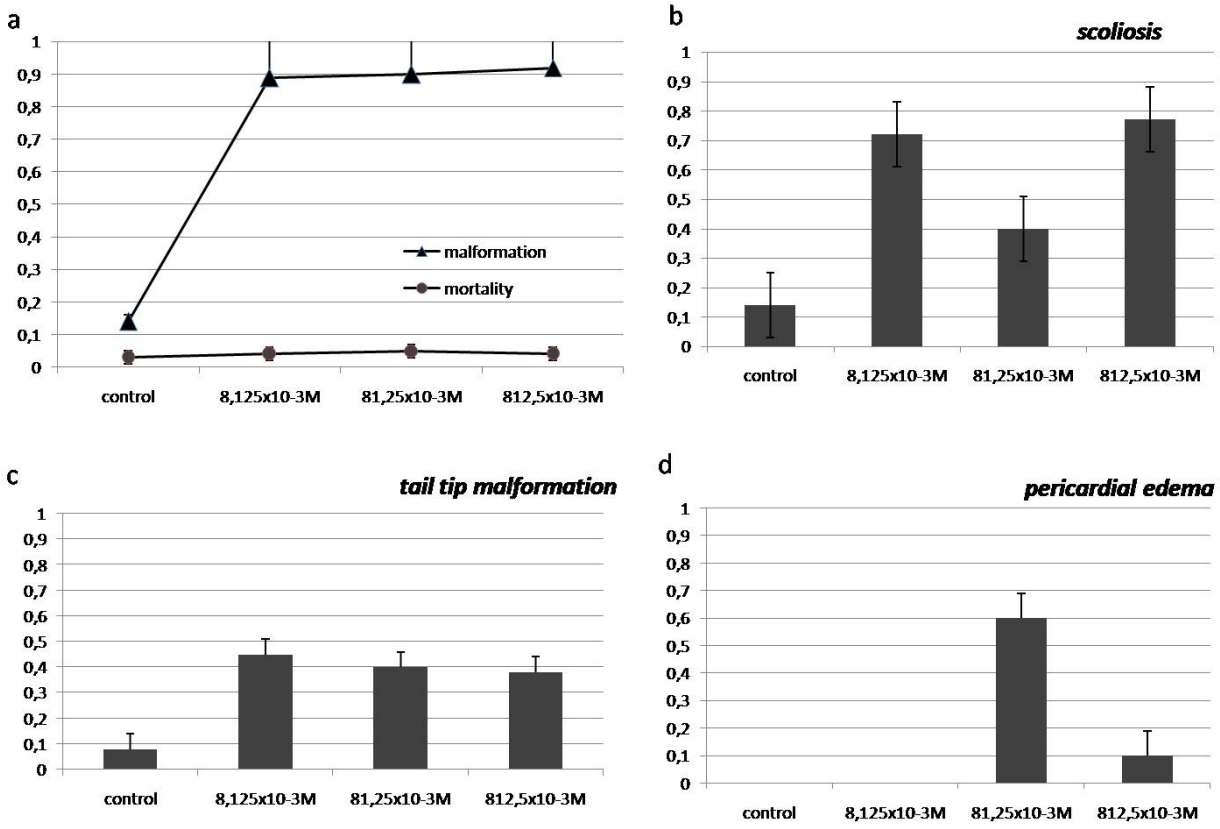

Figure 3 – Summary of the lethal and dismorphogenic effects (A) and individual morphologic malformations (B-D) in zebrafish (*Danio rerio*) embryos after 72 h exposure to lead acetate. Y-coordinate refers to decimal value. The standard deviation for each parameter is the result of three independent runs

In current study lead acetate in opposite do not cause any lethal but rather physiological effects related to water/salt balance and muscles contraction, as well as abnormal formation of notochord. There was no dose-dependency in any of observed malformations, so it is preliminary to consider about teratogenic effects of the lead, but toxic effects leading to malformations should be take into consideration. Despite enveloping almost all exposed embryos these malformations were light thus didn't cause embryo coagulation, nevertheless they may cause remote lethality after hatching when proceed to active feeding. It is also possible that observed negative effects of lead are the result of increased chorion permeability and alteration of water and salt balance in embryo. The ionic mechanism of lead toxicity occurs mainly due to the ability of lead metal ions to replace other bivalent cations like Ca2+, Mg2+, Fe2+ and monovalent cations like Na+, which ultimately disturbs the biological

metabolism of the cell [12]. The ionic mechanism of lead toxicity causes significant changes in various biological processes such as cell adhesion, intra- and inter-cellular signaling, protein folding, maturation, apoptosis, ionic transportation, enzyme regulation, and release of neurotransmitters. Lead can substitute calcium even in picomolar concentration affecting protein kinase C, which regulates neural excitation [17]. Thus cadmium and lead bioaccumulation and toxicity was recorded in many cyprinid fishes and other laboratory and commonly used fish. The correlation analysis indicated that the toxic effect of each heavy metal on a given cyprinid fish species was similar and that there is little effect of habitat and size at maturity on heavy metal toxicity. As a result, reliable toxicity data can be obtained on the basis of acute toxicity in early life stages [17].

Based on the results of experiments it was concluded that cadmium is considered to be highly toxic to zebrafish embryos, dose comparison testify the hundredfold cadmium toxicity to zebrafish embryos comparing with lead. Lead acetate has no lethal but gross but slight malformation effects which probably are due to altered chorion permeability. In conclusion, this study indicates that the presence of heavy metals in aquatic ecosystems can significantly interfere with the distribution of species, mainly due to their high toxicity to fish embryo and larvae. Therefore, early life-stage of zebrafish provides an ideal model for studying the adverse effects of heavy metals. Maximum permitted concentration for waters used in fishery was take into account when calculating minimal heavy metal doses for current experiments and thus it is clear that fish embryos are much more susceptible to metal's MPC than adult fishes and additional experiments to reestablish hygienic regulations worth providing for protection and conservation of native fish populations in Kazakhstan.

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