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Zebrafish (*Danio rerio*): A Pioneer Model in Medical Chemical Defense Researches

Zebra balığı (*Danio rerio*): Tıbbi Kimyasal Savunma Araştırmalarında Öncü Model

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ABSTRACT

Preparing the highest level of preparedness plans and treatment procedures against the adverse effects of chemical warfare agents (CWA) is the most important component of chemical defense. Currently, the zebrafish (*Danio rerio*) model, which exhibits superior qualities compared to rodents for evaluating CWA effects, is the most important subject of this study. An overview of the zebrafish model and existing research using this model in CWA studies is presented. The anatomical and physiological features of the zebrafish have made it a popular model organism. Literature studies have shown that the zebrafish model is a versatile model for observing the effects of CWA.

Key Words

Chemical warfare agent, zebrafish model, medical chemical defense.

ÖZ

Kimyasal savaş ajanlarının (KSA), olumsuz etkilerine karşı en üst düzeyde hazırlık planlarının ve tedavi prosedürlerinin hazırlanması kimyasal savunmanın en önemli bileşenidir. Günümüzde KSA etkilerini değerlendirmek için kemirgenlere göre daha üstün nitelikler sergileyen zebra balığı (*Danio rerio*) modeli bu çalışmanın ana konusudur. KSA çalışmalarında, zebra balığı modeli ve bu modeli kullanan mevcut araştırmalar ile ilgili genel bir değerlendirme yapılmıştır. Zebra balığının anatomik ve fizyolojik özellikleri onu popüler bir model organizma haline gelmiştir. Zebra balığı modelinin KSA'nın etkilerini gözlemlemek için çok yönlü ve mükemmel bir model olduğu yapılan literatür çalışmaları ile ortaya konulmuştur.

Anahtar Kelimeler

Kimyasal savaş ajanı, zebra balığı modeli, tıbbi kimyasal savunma.

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INTRODUCTION

In the last two decades, globalization, chemical proliferation, and easy access have increased the potential use of chemical warfare agents. In addition to globalization and chemical proliferation, terrorist attacks and threats by non-state actors also necessitate the search for new detection and treatment options for these agents [1].

According to the Chemical Weapons Convention, all chemicals, their precursors, and toxins are defined as chemical warfare agents (CWAs) when used for purposes other than those specified in the convention [2]. In addition, they cause economic and political problems. They also cause death, injury, and adverse conditions in humans, animals, and plants [3]. CWAs are classified according to their mode of action as blister agents, nerve agents, pulmonary (choking) agents, blood poisoning agents, riot control agents, and toxic chemicals of biological origin (Figure 1). Blister agents enter the body through the respiratory tract and skin. They affect the eyes, skin, respiratory, and the hematopoietic system. Sulfur mustard (HD), nitrogen mustard (NH), and Lewisite (L), are included in this group. The characteristic cutaneous manifestations of SM, the most common CWA agent, play an essential role in the differential diagnosis. SM is a significant threat due to its easy synthesis [4-6]. Furthermore, the mechanisms underlying exposure to these agents are still poorly understood. Lung irritants are toxic chemical agents that affect the respiratory tract, causing pulmonary edema and death if left untreated. This group includes phosgene (CG), diphosgene (DP), chlorine (CL), and chloropicrin (PS). Organophosphorus (OP) compounds constitute the structure of nerve agents considered in the class of CWAs. OP compounds are dual use. These compounds could be used as both pesticides and CWA's. Possible OP pesticide exposure poses a risk for all groups, especially during pregnancy and childhood. Sarin (GB), Tabun (GA), Cyclo-Sarin (GF), Soman (GD), third generation nerve agents, VX and fourth generation nerve agents, Novichok, are included in this group. OP compounds and chemical agents exert their effects by inhibiting the enzyme acetylcholinesterase (AChE) [4,5,7]. Due to this inhibition, muscarinic and nicotinic symptoms and many toxic effects are observed [7]. Blood poisoning agents exert their effects by inhibiting the enzyme Cytochrome C oxidase. Oxygen utilization by the cells is blocked, causing suffocation in the body. Depending on the concentration, death occurs within minutes. Hydrogen cyanide (AC) and cyanogen chloride (CK) are in this group [4,5].

Riot control agents are chemical agents with low toxicity and short duration of action which are designed to temporarily incapacitate people by creating fear and panic. 2-chloroacetophenone (CN), 2-chlorobenzalmalonitrite (CS), and Dibenz (b,f)-1,4-oxazepine (CR) belong to this group. Biological organisms produce toxins. Ricin and saxitoxin belong to this group. Ricin toxin exerts its effect by disrupting cellular protein synthesis. It is widely used, especially for assassination purposes. Saxitoxin disrupts nerve conduction and causes paralysis. [4,5,8].

More detailed and measurable research approaches are needed to reduce the risks associated with CWAs, which pose a significant threat to human health, and to improve the range of medical defense procedures. The

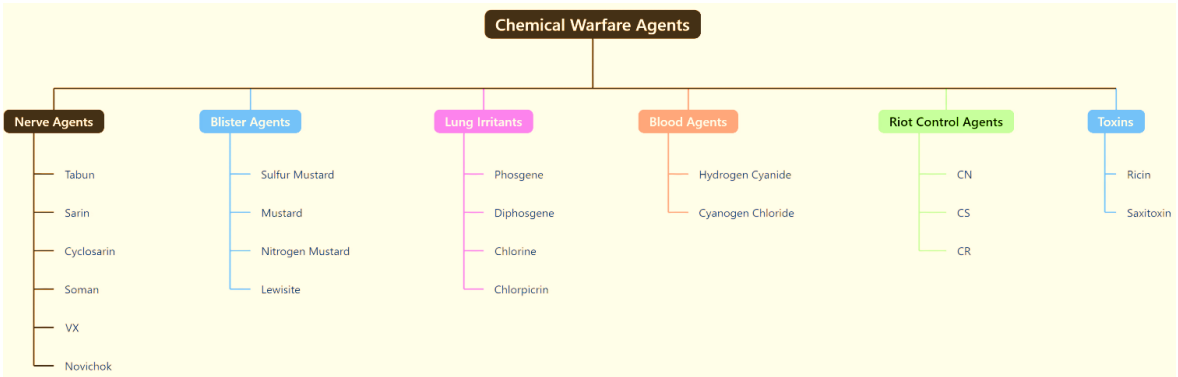

Figure 1. Classification of chemical warfare agents.

zebrafish model, which provides a superior alternative to rodent models, has emerged in studies conducted in the last two decades. Zebrafish is a preferred model for researches in various biochemical disciplines. Zebrafish embryos and larvae have the potential to provide valuable new information about chemical poisoning, drug development studies, genetic studies, diagnosis, and treatment strategies. They are currently used extensively as a high-throughput screening model [9,10]. This review will provide an overview of the zebrafish model and existing research using this model in the assessment of CWA exposure.

Zebrafish as a Developmental Model

Zebrafish is a freshwater fish native to South Asia. Studies on zebrafish began in the 1960s. The use of this fish as a model was pioneered by George Streisinger and colleagues at the University of Oregon [11]. Zebrafish has been used as a model in studies for the last 40 years and the number of studies in this field has been increasing rapidly. (Figure 1.) When the keyword "zebrafish model" is searched in the Pubmed search engine by filtering the abstract and the whole article, it is seen that 24,766 studies were conducted between 1982 and 2024. As a vertebrate model and due to its biological development, it provides superiority over rodent models as a popular and influential model organism for toxicology research [11]. Then, a more customized search was performed in the Pubmed search engine. The words "zebrafish model, chemical warfare agent" were typed into the search engine and abstract, and all articles were filtered. It was observed that 39 studies were conducted between 1991 and 2024 and that these studies increased over the years (Figure 3).

There are many reasons for using zebrafish as model organisms [1,11-13]. The genetic structure of zebrafish is 70% similar to human genes. This genetic similarity supports zebrafish as a reliable model for identifying human diseases and understanding their conditions. Their rapid development, reproductive processes, external development of embryos, and transparency allow real-time observations of developmental processes (Figure 4). The ease of genetic manipulation allows genetic modifications. The low maintenance cost is an advantage over rodents and other vertebrate models. It is preferred for large-scale studies due to its small size and simple housing requirements. Zebrafish is suitable for the high -throughput screening model in toxicology and therapeutic agent studies. At the same time, zebrafish exhibit a range of behaviors that can be observed and measured. Behavioral analyses provide essential information in studies assessing chemical exposure's neurotoxic effects.

Zebrafish is anatomically and physiologically similar to humans and is a vertebrate animal. Having organs and tissues such as the brain, sensory organs, heart, liver, pancreas, kidney, intestine, bone, muscle, etc. (Figure 5) facilitates the study of vertebrate biological processes [15]. These properties allow the study of organ-specific toxicities and systemic effects of chemical agents. Embryos develop all organs within 48 hours after fertilization. These characteristics mentioned above increase the popularity of zebrafish, and studies in this field are increasing rapidly.

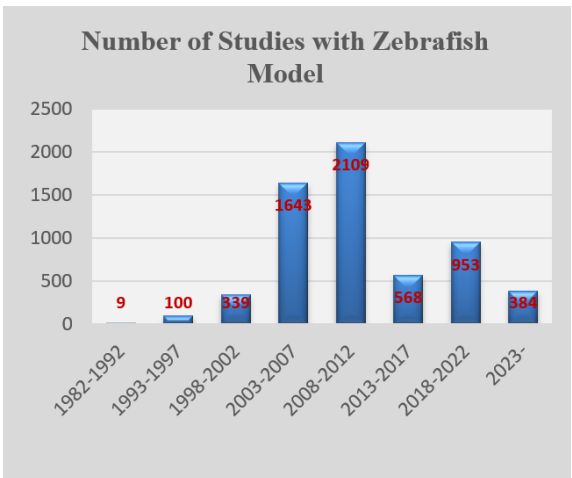

Figure 2. Number of Pubmed publications with zebrafish model between 1982-2025.

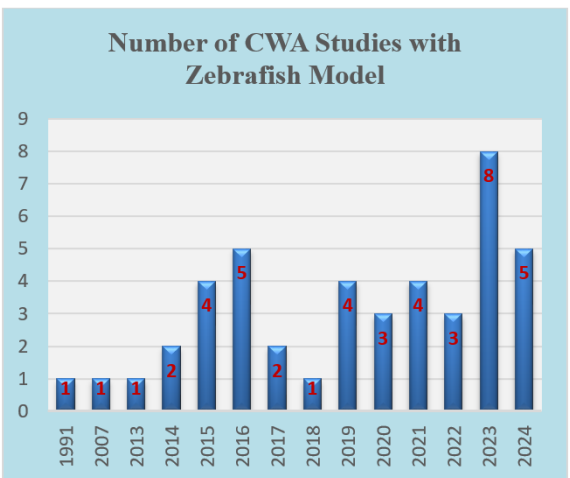

Figure 3. Number of Pubmed publications with CWA and zebrafish model between 1991-2024.

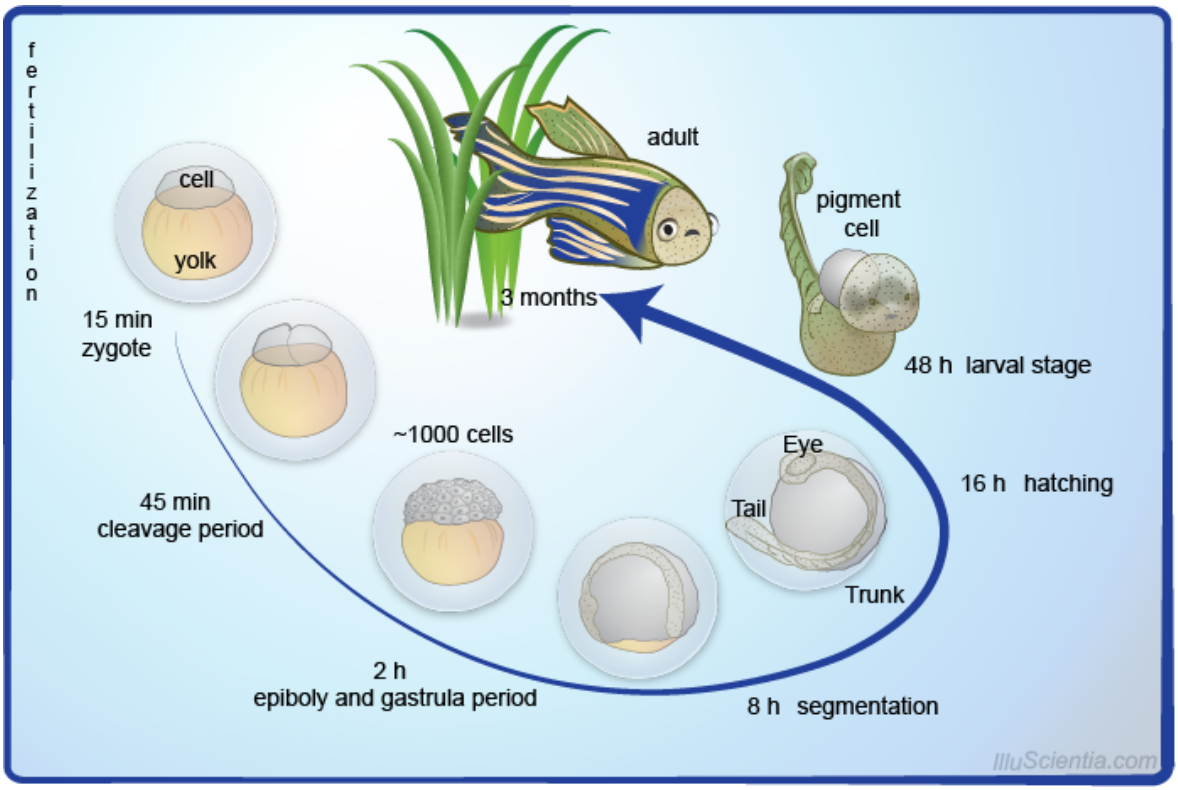

Figure 4. Life cycle of a zebra fish from fertilization to an adult fish (Image used without editing) [14].

There is a wide variety of zebrafish strains worldwide, but the most frequently used strains in research are AB, Ekkwill, Wild Indian Karyotype (WIK), Casper, Nadia, and Tubingen (TU). [16]. Each of these strains has different genetic characteristics or phenotypes, and different offenses are preferred depending on the specificity of the research. The AB strain is a laboratory-grown strain that is widely used in developmental and toxicological research. WIK and Tubingen strains are also frequently used in conjunction with AB [17,18].

Zebrafish as a Model of Toxicology

Zebrafish is a model organism that plays a vital role in chemical risk assessment. This model is the vertebrate model widely used and recommended by institutions such as Environmental Protection Agency, (EPA) Organization for Economic Cooperation and Development (OECD), and International Organization for Standardization (ISO) [20]. These institutions recognize the zebrafish model as a reliable and efficient model for chemical studies. This is important in supporting the validity and reliability of zebrafish studies in scientific research.

The "Zebrafish Embryo Acute Toxicity Test" (ZFET) was published by the OECD in 2013 to assess the toxicity of chemicals with the zebrafish embryo model. This test is used to determine the concentrations of chemicals that cause acute toxicity in fish in aquatic environments. For the test, zebrafish embryos are used and exposed to a toxic chemical for 96 hours. Using the data obtained, the lethal concentration ($_{LC50}$) of the chemicals on the embryo is calculated. In addition, the toxic effects of the chemicals on aquatic organisms are evaluated by monitoring behavioral changes in embryos exposed to the toxic chemical at 24, 48, 72 and 96 hours [21]. Standard protocols have been prepared under this directive. To assess the validity of the ZFET, the OECD conducted trials on 20 different chemicals, and tests were carried out in 11 different laboratories [22]. The study results revealed that ZFET is a valid method for toxicity assays. Zebrafish are a popular model for studying neuronal development, all aspects of developmental so neuroscience can be studied in zebrafish. The ability of zebrafish to respond to chemicals in water has made it a powerful model for toxicological studies. The advantages mentioned above of zebrafish have made it a preferred model for chemical toxicity research [11]. However, it is not preferred for research on the respiratory and reproductive systems due to anatomical differences [16].

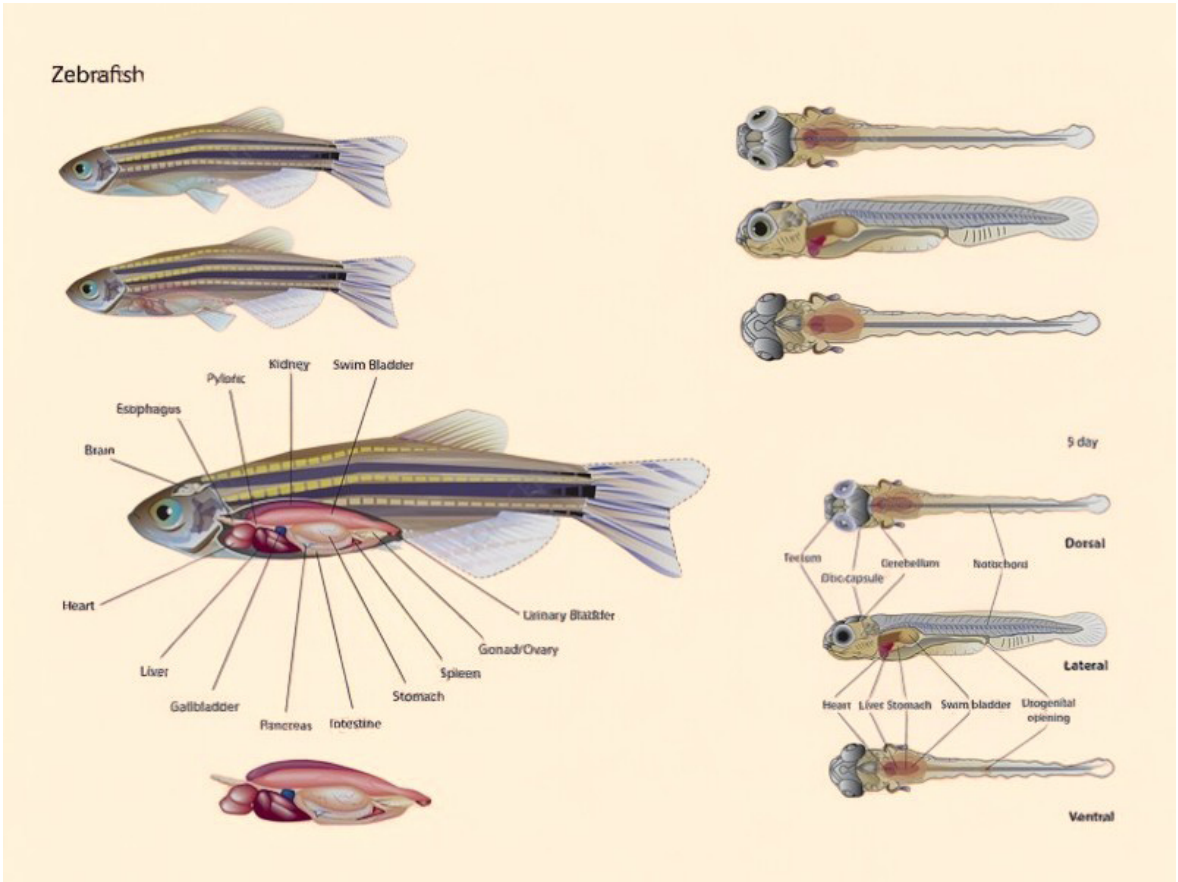

Figure 4. Life cycle of a zebra fish from fertilization to an adult fish (Image used without editing) [14].

RESULTS and DISCUSSION

Zebrafish is seen as an essential model for the study of OP compounds and the effects of CWAs. Although there are many different mechanisms in the pathophysiology of OP and nerve agents, standard treatment only addresses specific mechanisms. The World Health Organization (WHO) has stated the need to study newer and more effective antidotes instead of conventional treatments [23]. Zebrafish has emerged as an animal model with a rising momentum in the research of CWAs involving human toxicology.

A literature review by Koenig et al. examined the literature using zebrafish models for OP and nerve agent exposure. Research on AChE inhibition due to exposure to OP compounds and nerve agents has only been conducted on rodent models. Thanks to genetic manipulation and high screening capabilities, these kinds of research have been done to observe subacute effects and effects on neuronal development and behavior. However, more research must be done on oxime reactivators, anticholinergics and anticonvulsants pharmacologics. The study emphasizes that zebrafish model is a highly efficient system for evaluating AChE reactivators and antidotal therapies against OP and nerve agent exposure [7].

In the study with the zebrafish model, the effects of OP exposure were used to screen for new therapeutics. It was reported to be a suitable model for screening new oxime antidotes against OP-inhibited AChE. The concentrations determined for chlorpyrifos oxon (CPO) and dichlorvos (DDVP) indicated that CPO plays an important role in AChE inhibition. Aldoxime antidotes also showed similar properties in AChE reactivations. At the end of the study, zebrafish were shown to be a good model for the investigation of antidotes against OP exposure [24].

Another study by Koenig et al. evaluated therapeutics in a larval zebrafish model for exposure to OP-containing pesticides and nerve agents. It concluded that zebrafish larvae are a good screening model [25]. This is directly related to neurological development and emphasizes

the critical role of AChE in developmental processes. AChE is an enzyme that hydrolyzes acetylcholine at cholinergic and neuromuscular synapses to choline and acetate and thus plays a vital role in regulating nerve conduction. The substantial toxicity of OP and nerve agent exposure is manifested by AChE inhibition. In another phase of the study, zebrafish larvae were exposed to OP pesticides including (chlorpyrifos (CP), paraoxon-ethyl (PO), diisopropylfluorophosphate (DFP), chlorpyrifos oxon (CPO), and nerve agents (GB, GD, and GF). Six-day-old (pdf) zebrafish larvae were exposed to different concentrations of GB, GD, GF, PO, CPO, DFP, and CP, and LC_{50} concentrations were determined at 30, 60, 90, and 120 minutes. When these LC_{50} concentrations were applied to zebrafish larvae, it was found that nerve agents were more toxic than OP pesticide compounds, CP was the least toxic, and CPO was the most toxic [25].

In another phase of the study, using different exposure times and LC_{50} concentrations, the ability of three oxime compounds (2-PAM, MMB-4, and MINA) to reactivate AChE inhibited by nerve agents and OP pesticides was evaluated. Pesticides with OP compounds were more sensitive to oxime treatment at different concentrations for 20 min than nerve agents. Oxime treatments significantly reactivated AChE inhibited by CPO, and 2-PAM was the most effective oxime. All three oximes were found to be significantly effective against GB exposure. However, no oxime was effective in GD exposure [25]. It could be said that the results of this study are compatible with rodent models, and it is a valuable and valid model when animal welfare is also considered.

Jin et al. attempted to develop new antidotes against the toxic effects of OP in an in vivo zebrafish model. Atropine and pralidoxime (2-PAM) are against the toxic effects of OP and nerve agent, but the efficacy of these treatments is limited. Therefore, a zebrafish screening model was developed for newly synthesized antidotes to reduce the toxic effects of OPs. A pilot study including of 1200 drugs currently in use identified 16 compounds that suppressed OP toxicity in a zebrafish model [12]. The mechanisms of some compounds are still unclear. The Zebrafish model holds promise for identifying these mechanisms and finding new treatments against OP toxicity.

The zebrafish model was used with OP pesticide CPO and paraoxon (POX) and ethyl 4-nitrophenyl methyl phosphonate (NEMP) and isopropyl 4-nitrophenyl) and VX nerve agent. A new method called vivo zebrafish OP-antidote test (ZOAT) was developed to test the efficacy of antidotes against isopropyl 4-nitrophenyl methyl phosphonate (NIMP) exposure. ZOAT is the general name of the study in which in vivo AChE activity, reactivation capacity of antidotes, VMR (Visual Motor Response) test, EFPMR (Electric Field Pulse Motor Response) test and central and peripheral nervous system functions were evaluated separately. Before the test, larvae were starved for 17 hours and living conditions specific to the study were prepared. For each test, groups of 9-12 larvae were formed and 7-day-old larvae were exposed to OP compounds for one hour. Antidote toxicity tests were performed to determine the safe concentrations of antidotes. Then OP toxicity test, ACHE activity tests, motor response tests, BBB (blood-brain barrier) crossing test and data analysis were performed. Pyridinium oximes (2-PAM, OBX, and HI-6') were applied against OP pesticide and nerve agent exposure, and the AChE reactivation capacity of the antidote, its different protective efficacy on the central nervous system and peripheral nervous system, and its ability to cross the blood-brain barrier were evaluated. All OP compounds inhibited AChE activity to certain levels, while AChE activity was 100% in the control group. The activity of oximes differed depending on the type and concentration of OP compounds used. Also, the reactivity of AChE may vary depending on the type of OP compounds and oximes used. It was stated that the ZOAT method provides a suitable model for the identification of more effective treatments against OP exposure. This method may contribute to developing new antidotes that can be used in humans. It has been emphasized that it is important to determine the in vivo systemic toxicity of the compound to be included as an antidote in such studies before the study to determine the concentration of unobserved effect [26].

In a study by Krystle et al., the developmental effects of POX on zebrafish embryos were examined. It was found that AChE activity increased in healthy zebrafish embryos according to developmental stages, while AChE was inhibited by POX exposure, but this inhibition was reversible when transferred to clean water after 48 hours. It was also observed that POX at concentrations of 31.2-500 nM caused AChE inhibition in zebrafish embryos, but this effect did not negatively affect secondary neuron development. After 26 hours of exposure, tail contractions were observed even in minimal AChE ac-

tivity [27]. This also shows the sensitivity of zebrafish embryos are to POX exposure. In previous studies, spontaneous movements and behavioral changes in embryos and larvae were considered consistent with AChE inhibition. However, in this study, it was stated that such effects may be independent of AChE inhibition.

Most studies on CWAs using the zebrafish model are related to OP compounds and nerve agents. Research with other CWAs is somewhat less. There are studies to fill these gaps, but more numbers should be included. One of the studies conducted for this purpose concerns organoarsenic chemicals. These chemicals started to be used as CWAs in the early 20th century due to their high toxicity.

Organoarsenic CWAs include diphenylchlorarsine (Clark I), 10-chloro-5,10- dihydrophenarsazinine (Adamsite), and phenyldichloroarsine (PDCA), triphenylarsine (TPA), The acute toxicity thresholds of CWA degradation products diphenylarsinic acid (Clark I[ox]), phenarsazinic acid (Adamsite[ox]), phenylarsonic acid (PDCA[ox]), and triphenylarsine oxide (TPA[ox]) were determined using zebrafish embryo model. The study also analyzed the mRNA expression of five genes encoding antioxidant enzymes in zebrafish embryos. Clark I, Adamsite, and PDCA showed lethal effects at deficient concentrations in zebrafish embryos. In contrast, TPA and its degradation products did not show acute toxicity but only affected the transcription of antioxidant genes [28].

A limited number of studies on SM, which is a blister agent group, have been conducted using the zebrafish model. In a study by Wang et al. on the toxic effects of SM exposure in zebrafish larvae, new information was presented on chondrogenesis disorders, how the c-Fos/AP-1 inhibitor T-5224 attenuates them, and their treatment. SM has been reported to reduce the survival rate and hatching rate of zebrafish larvae, cause pericardial edema and inhibit the growth rate of the head. It was found that SM caused adverse effects on cartilage development in zebrafish larvae, altered mRNA expression of cartilage-related genes such as fosap and col2a1a, increased c-fos protein expression, and decreased COL2A1 expression. It has also been reported that SM-induced cartilage development disorders are associated with c-Fos/AP-1 pathways and T-5224 ameliorates SM-induced disorders by inhibiting c-Fos/AP-1. It was also emphasized that the negative effects of SM on lung bronchial cartilage structure should be studied [29].

Another study using zebrafish embryos evaluated the photomotor response (PMR) test following short and long-term exposure to neurotoxic chemicals and chemical weapons precursors. The PMR was assessed as the response of a zebrafish embryo to light stimuli 24 hours after fertilization. This response was used to assess the presence of toxicity. Zebrafish embryos were exposed to chemicals that inhibit aerobic respiration (cyanide, etc.), AChE inhibitors, and various chemical weapons precursors for short (1 hour) and long (16 hours+) periods. The effects of the chemicals on the PMR were analyzed; 10 of the 20 were influential on the PMR, and eight were effective in both short- and long-term tests. Chemical weapon precursors reduced all PMR phases based on their general toxicity properties, while aerobic respiration inhibitors and OPs showed different effects in different phases [30]. In this study, unlike the others, cyanide, one of the blood poisoning agents, was used. Cyanide is a CWA that shows its effect by disrupting the electron transport chain and blocking oxygen use. As a result of cyanide exposure, PMR effects of cyanide exposure were in the form of decreased or complete disappearance of embryos' responses to light stimuli. Depending on the cyanide dose and exposure duration, PMR changes were observed after both short-term and long-term exposure [30]. In the zebrafish model, cyanide's neurotoxic potential and efficacy were once again demonstrated in agreement with other rodent models. These effects of cyanide on zebrafish embryos provide the basis for more detailed toxicological studies.

Since cyanide is a chemical agent that causes death within minutes if left untreated, affects the cardiovascular, central nervous system and causes loss of metabolic function, antidote treatment should be initiated as soon as possible. A study was conducted using a zebrafish and rabbit model to discover new biomarkers and antidotes against cyanide exposure. By screening 3120 small molecules, four new antidotes were discovered that inhibit cyanide toxicity, with riboflavin (vitamin B2) being the most effective. Changes in bile acid and purine metabolism were observed in zebrafish as a result of cyanide exposure, particularly an increase in inosine levels. Based on this effect, inosine was proposed as a potential biomarker of cyanide exposure. It suggests that it may be a valuable biomarker for monitoring cyanide toxicity. The metabolic effects of cyanide observed in

zebrafish agreed with the rabbit model. Similar metabolic changes were observed in humans treated with nitroprusside. Riboflavin has shown its protective effect as the most effective antidote to regulate these metabolic disturbances. The fact that it can be taken orally makes it even preferable [31]. Testing antidote studies related to cyanide exposure on the zebrafish model may be a valuable tool to examine the effects of cyanide toxicity at the molecular and cellular levels and test potential antidotes. However, it may need to be validated with different model systems.

The role of nitric oxide (NO), which plays a vital role in biological systems and respiratory control, was examined in adult and larval zebrafish models. NO is a compound that provides signal transduction between cells regulating acute hypoxic ventilation in mammals. However, its role in respiratory control during fish development is unclear. It was shown that NO plays an inhibitory role in respiration in adult zebrafish, whereas it plays an excitatory role in larvae. This suggests that NO plays a changing role in respiratory control throughout zebrafish development [32]. To observe the effects of NO, adult zebrafish were exposed to sodium nitroprusside (SNP) contained iron and cyanide groups. When used for research purposes, SNP causes the release of NO in the body. SNP releases nitric oxide through biochemical processes. Because of this property, SNP is used to increase nitric oxide levels in experimental settings.

After NO administration, there was a significant decrease in respirations in adult zebrafish. This reduction is particularly pronounced under hypoxic conditions. Nitric oxide in neuroepithelial cells is considered an oxygen chemoreceptor in fish, suggesting it may play a central role in respiratory control. In contrast to adult zebrafish, NO plays a respiratory-enhancing role in larval zebrafish. This suggests that NO shows a different effect during the developmental stages of larvae. Inhibition of nitric oxide synthesis in larvae reduced the respiratory response, especially under hypoxic conditions. This suggests that NO plays a vital role in adapting larvae to hypoxic conditions [32]. These findings reveal that NO functions as a central modulator of respiratory control in larval zebrafish and that this function is distinct compared to adult zebrafish. The research revealed that nitric oxide plays an essential role in zebrafish respiratory control and that this role varies according to developmental stages.

Lung irritants, a major threat to military and civilian populations, are highly toxic gases that cause significant damage to the respiratory tract and pulmonary edema. During World War I, studies on the chlorine gas zebra model, which changed the course of the war and caused the death and exposure of hundreds of thousands of soldiers, have not been conducted in this context. However, studies have been conducted using chlorine solutions. Kent et al. investigated the toxicity of chlorine solutions used for disinfection of zebrafish embryos. It was observed that chlorine toxicity in zebrafish embryos varied depending on embryo development, with six hpf post-fertilization embryos being more resistant to the toxic effects of chlorine than 24 hpf post-fertilization embryos. This suggests that embryos are less sensitive to the toxic effects of chlorine at early developmental stages. The toxicity rate depends on the development of the embryos, but also on the properties of the chlorine solution. A 40% mortality rate was observed in 6 hpf embryos, and a 60% mortality rate was observed in 24 hpf embryos exposed to 100 ppm concentration of unbuffered chlorine solution for 10 minutes [33]. The pH level is an essential factor for the study, the chlorine water mixture raises the pH level. In the study, buffered chlorine solutions with pH 7 were more toxic than unbuffered pH 8-9. Exposure to chlorine solutions at concentrations of 50-100 ppm for 5 minutes and exposure to 100 ppm for 5 minutes both resulted in low rates of mortality and malformations. This suggests that short-term exposure does not cause significant toxicity to the embryo. Exposure to 150 ppm and above caused significant malformations and high mortality rates in embryos. This indicated that the duration and concentrations of chlorine exposure had significant effects on zebrafish embryos [33]. These findings emphasize that the pH level of chlorine has a vital role in toxicity. This study is essential in examining the effects of chlorine concentration, exposure time, and pH levels on embryo health. It is predicted that the zebrafish model can be used in toxicity studies of chlorine gas, which has an essential place among chemical warfare agents, evaluation of development, behavioral tests, and histopathological studies and can shed light on future studies.

Studies with the zebrafish model have made great progress in the last four decades. It becomes more popular as it offers high throughput scanning. Due to its anatomical, physiological, and genetic characteristics, economical production and maintenance, and ability to reproduce in large numbers quickly, zebrafish are prefer-

red over other rodents. Toxicity studies with chemical warfare agents and the zebrafish model are essential today. Based on the information above, the Zebrafish model is an excellent and versatile model for observing the effects of CWAs

However, this model has some disadvantages. Anatomical and physiological differences of zebrafish limit the study of some agents. The living conditions of zebrafish and the way they are exposed to chemical agents may cause some differences. Although there is 70% genetic similarity, genetic differences limit studies.

This rapid increase in the use of the zebrafish model brings along some ethical problems. As with other animal models, the "3R Principle" (Replacement, Reduction, Refinement) should be followed in the zebrafish model and animal welfare should be prioritized.

References

1. R.T. Peterson, C.A. MacRae, Changing the scale and efficiency of chemical warfare countermeasure discovery using the zebrafish, Drug Discov Today Dis Models, 10 (2013) e37–e42.
2. V. Pitschmann, Overall view of chemical and biochemical weapons, Toxins, 6 (2014) 1761–1784.
3. L. Szinicz, History of chemical and biological warfare agents, Toxicology, 214 (2005) 167–181.
4. S. Sezigen Chapter 1: Introduction and History: S. Sezigen, L. Kenar (Editors:), Practical guide for the medical management of chemical war casualties, organization for the prohibition of chemical weapons (OPCW) (Turkish Translation), University of Health Sciences, Ankara, Turkey, 2016.
5. R. Black, Problems in toxicology, basic aspects of chemical warfare toxicology, development of chemical warfare agents, historical use and properties (Vol. 1, 1st ed., S. Sezigen, Turkish Translation.), EMA Tıp Kitapevi, Ankara, Turkey, 2021.
6. S. Sezigen, K. Ivelik, M. Ortatatli, M. Almacioglu, M. Demirkasimoglu, R.K. Eyison, Z.I. Kunak, L. Kenar, Victims of chemical terrorism, a family of four who were exposed to sulfur mustard, Toxicol Lett., 303 (2019) 9–15.
7. J.A. Koenig, T.L. Dao, R.K. Kan, T. Shih, Zebrafish as a model for acetylcholinesterase-inhibiting organophosphorus agent exposure and oxime reactivation, Ann N Y Acad Sci., 1374 (2016) 68–77.
8. 8. M. Schwenk, Chemical warfare agents Classes and targets, Toxicol Lett., 293 (2018) 253–263.
9. A.J. Hill, H. Teraoka, W. Heideman, R.E. Peterson, Zebrafish as a model vertebrate for investigating chemical toxicity, Toxicological Sciences, 86 (2005) 6–19.
10. S. Burgess, New techniques for using zebrafish as a model for development, Methods, 39 (2006) 181–182.
11. J.R. Meyers, Zebrafish: Development of a vertebrate model organism, Curr Protoc Essent Lab Tech., 16 (2018) e19.
12. S. Jin, KS. Sarkar, Y.N. Jin, Y. Liu, D. Kokel, T.J. Van Ham, L.D. Roberts, R.E. Gerszten, CA. MacRae, RT. Peterson, An in vivo zebrafish screen identifies organophosphate antidotes with diverse mechanisms of action, J Biomol Screen., 18 (2013) 108–115.
13. N.A. Ducharme, LE. Peterson, E. Benfenati, D. Reif, CW. McCollum, JÅ. Gustafsson, M. Bondesson, Meta-analysis of toxicity and teratogenicity of 133 chemicals from zebrafish developmental zoxicity studies, Reprod Toxicol., 41 (2013) 98–108.
14. IlluScientia, Life cycle of a zebra fish from fertilization to an adult-zebrafish_cycle.png (2013), https://upload.wikimedia.org/wikipedia/commons/f/f9/Zebrafish_Cycle.png (accessed October 10, 2024)
15. C.A. MacRae, RT. Peterson, Zebrafish-based small molecule discovery, Chem Biol., 10 (2003) 901–908.
16. T. Teame, Z. Zhang, C. Ran, H. Zhang, Y. Yang, Q. Ding, M. Xie, C. Gao, Y. Ye, M. Duan, Z. Zhou, The use of zebrafish (Danio rerio) as biomedical models, Anim Front., 9 (2019) 68.
17. G. Audira, P. Siregar, S.A. Strungaru, J.C. Huang, C. Der Hsiao, Which zebrafish strains are more suitable to perform behavioral studies? A comprehensive comparison by phenomic approach, Biology (Basel), 9 (2020) 1–22.
18. P.F. Silva, C. Garcia-de-Leaniz, F.A.M. Freire, V.A.M. Silveira, A.C. Luchiari, Different housing conditions for zebrafish: What are the effects? behavioural processes, 209 (2023) 104886.
19. J. Brock, Research illustration, Francis crick institute science photo library, Zebrafish anatomy illustration (2024) https://www.sciencephoto.com/contributor/fxb/ (accessed October 03, 2024).
20. S. Singh, K. Gautam, SS. Mir, S. Anbumani, Genotoxicity and cytotoxicity assessment of "forever chemicals" in zebrafish (Danio rerio), Mutat Res Genet Toxicol Environ Mutagen, 897 (2024) 503788.
21. J. Buschmann, The OECD guidelines for the testing of chemicals and pesticides, Methods Mol Biol., 947 (2013) 37–56.
22. F. Busquet, R. Strecker, J.M. Rawlings, S.E. Belanger, T. Braunbeck, GJ. Carr, P. Cenijn, P. Fochtman, A. Gourmelon, N. Hübler, A. Kleensang, M. Knöbel, C. Kussatz, J. Legler, A. Lillicrap, F. Martínez-Jerónimo, C. Polleichtner, H. Rzodeczko, E. Salinas, K.E. Schneider, et al., OECD validation study to assess intra- and inter-laboratory reproducibility of the zebrafish embryo toxicity test for acute aquatic toxicity testing, Regulatory Toxicology and Pharmacology, 69 (2014) 496–511.
23. M. Faria, N. Garcia-Reyero, F. Padrós, P.J. Babin, D. Sebastián, J. Cachot, E. Prats, M. Arick, E. Rial, A. Knoll-Gellida, G. Mathieu, F. Le Bihanic, B.L. Escalon, A. Zorzano, A.M.V.M. Soares, D. Ralduá, Zebrafish models for human acute organophosphorus poisoning, Scientific Reports, 5 (2015) 1–16.
24. H.R. Schmidt, Z. Radić, P. Taylor, E.A. Fradinger, Quaternary and tertiary aldoxime antidotes for organophosphate exposure in a zebrafish model system, Toxicol Appl Pharmacol., 284 (2015) 197–203.
25. J.A. Koenig, C.A. Chen, T.M. Shih, Development of a larval zebrafish model for acute organophosphorus nerve agent and pesticide exposure and therapeutic evaluation, Toxics, 8 (2020) 1–11.

26. L.E. Dubrana, A. Knoll-Gellida, L.M. Bourcier, T. Mercé, S. Pedemay, F. Nachon, A.G. Calas, R. Baati, M. Soares, P.J. Babin, An antidote screening system for organophosphorus poisoning using zebrafish larvae, ACS Chem Neurosci., 12 (2021) 2865–2877.
27. K.L. Yozzo, S.P. McGee, D.C. Volz, Adverse outcome pathways during zebrafish embryogenesis: A case study with paraoxon, Aquat Toxicol, 126 (2013) 346–354.
28. W. Wilczynski, T. Brzeziński, P. Maszczyk, A. Ludew, M.J. Czub, D. Dziedzic, J. Nawala, S. Popiel, J. Beldowski, H. Sanderson, M. Radlinska, Acute toxicity of organoarsenic chemical warfare agents to Danio rerio embryos, Ecotoxicol Environ Saf., 262 (2023) 115116.
29. H. Wang, C. Ma, C. Liu, L. Sun, Y. Wang, J. Xue, B. Zhao, W. Dong, The c-Fos/AP-1 inhibitor inhibits sulfur mustard-induced chondrogenesis impairment in zebrafish larvae, Chemosphere, 359 (2024).
30. C.M. Carbaugh, M.W. Widder, C.S. Phillips, D.A. Jackson, V.T. DiVito, W.H. van der Schalie, K.P. Glover, Assessment of zebrafish embryo photomotor response sensitivity and phase-specific patterns following acute- and long-duration exposure to neurotoxic chemicals and chemical weapon precursors, Journal of Applied Toxicology, 40 (2020) 1272.
31. A.K. Nath, L.D. Roberts, Y. Liu, S.B. Mahon, S. Kim, J.H. Ryu, A. Werdich, J.L. Januzzi, G.R. Boss, G.A. Rockwood, C.A. MacRae, M. Brenner, R.E. Gerszten, R.T. Peterson, Chemical and metabolomic screens identify novel biomarkers and antidotes for cyanide exposure, FASEB J., 27 (2013) 1928–1938.
32. C.S. Porteus, J. Pollack, V. Tzaneva, R.W.M. Kwong, Y. Kumai, S.J. Abdallah, G. Zaccone, E.R. Lauriano, W.K. Milsom, S.F. Perry, A role for nitric oxide in the control of breathing in zebrafish (Danio rerio), J Exp Biol., 218 (2015) 3746–3753.
33. M.L. Kent, C. Buchner, C. Barton, R.L. Tanguay, Toxicity of chlorine to zebrafish embryos, Dis Aquat Organ, 107 (2014) 235–240.