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Bazı Azo Boyalarının QSAR Yöntemi ve Daphnia Magna ile Akut Toksisite Testi ile İncelenmesi

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Öne Çıkanlar:
- Teorik
- Deneysel
- Ekotoksikoloji

Anahtar Kelimeler:
- Aromatik Aminler
- Azo Boyaları
- QSAR
- Teorik Kimya
- Daphnia Magna
- Ekotoksikoloji

ÖZET:

Azo boyalar, tekstil boyalarının en önemli sınıfını temsil eder. Azo boyaların biyotransformasyonu aromatik aminleri serbest bırakabilir. Bazı aromatik aminlerin genotoksik ve/veya kanserojen özelliklere sahip olduğu iyi bilinmektedir. Bu çalışmada, tekstil sektöründe yaygın olarak kullanılan aromatik aminlerden oluşan azo boyalarının çevre üzerindeki etkilerinin akut su toksisitesi testi ile araştırılması amaçlanmıştır. Daphnia Magna kullanılarak yapılan akut su toksisite testi deneysel ve teorik olarak incelenmiştir. Teorik çalışmalarda son zamanlarda ilgi gören ve ülkeler arası yönetmeliklerde de kullanılan OECD QSAR Toolbox programı tercih edilmiştir. Yapılan çalışmalar sonucunda deneysel ve teorik sonuçların paralel sonuçlar verdiği görülmüştür. Bu çalışma, Daphnia Magna üzerinde yapılan akut toksisite testlerinde maliyeti, süreyi ve hayvan ölümlerini azaltmak amacıyla teorik çalışmaların deneysel çalışmalara ikame olabileceğini göstermiştir. Ayrıca, bu çalışma sonuçlarına göre Basic Yellow 28, Dispers Blue 291 ve Dispers Brown 27-1 azo boyalarının ekotoksikolojik olarak toksik olduğu deneysel ve teorik yöntemler ile bulunmuştur.

Investigation of Some Azo Dyes by QSAR Method and Acute Toxicity Test with *Daphnia Magna*

Highlights:
- Theoretical
- Experimental
- Ecotoxicology

Keywords:
- Aromatic amines
- Azo Dyes
- QSAR
- Theoretical Chemistry
- Daphnia Magna
- Ecotoxicology

ABSTRACT:

Azo dyes represent the most important class of textile dyes. Biotransformation of azo dyes can release aromatic amines. It is well known that some aromatic amines have genotoxic and/or carcinogenic properties. In this study, it was aimed to investigate the effects of azo dyes, which are composed of aromatic amines, and are widely used in the textile industry, on the environment using acute water toxicity test. The acute water toxicity test using Daphnia Magna was investigated experimentally and theoretically. The OECD QSAR Toolbox program, which has recently attracted attention in theoretical studies and is also used in international regulations, has been preferred. As a result of the studies, it was seen that the experimental and theoretical results gave parallel results. This study showed that theoretical studies can be substituted for experimental studies in order to reduce cost, time, and animal mortality in acute toxicity tests on Daphnia Magna. In addition, according to the results of this study, it was found by experimental and theoretical methods that Basic Yellow 28, Disperse Blue 291, and Disperse Brown 27-1 azo dye are ecotoxicologically toxic.

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Ethics Committee Approval: In the animal experiment in this article, Daphnia Magna species was used and Ethics Committee Approval certificate is not required as this species is not included in the definition of experimental animal.

INTRODUCTION

In general, dyes are a fairly common practice used to change the color properties of different substrates. First, substances with coloring properties were obtained from natural sources such as animals or vegetables. Today, natural dyes have been replaced by synthetic dyes and the use of synthetic dyes has increased considerably. Hundreds of new colored compounds are released every year and take their place in the market (Ventura-Camargo et al., 2013). The majority of synthetic dyes are dye groups known as azo dyes. In a study, it was seen that approximately half of the dyes available in the market contain dyes belonging to the azo dye group (Majcen-Le et al., 1997). These dyes are used in the textile industry to color fibers with various raw materials. Azo dyes are easily synthesized, and have excellent fixing and holding properties. It can also offer a wide variety of colors compared to natural dyes (O'Neill et al., 1999).

Substances defined as azo dyes are synthetic dyes known as amines or phenol diazotized amines containing an azo group (N=N-) in their structure. Almost all of the dyes used in textiles are azo dyes (Carliell et al., 1995). Aromatic amines are used as raw materials or intermediates in the synthesis of azo dyes. Recent studies have observed that aromatic amines, especially aromatic amines carried into consumer products, pose a risk to human health due to their toxicological, ecotoxicological, mutagenic, and/or carcinogenic properties. Aromatic amines are generally defined as chemical compounds that have one or more aromatic rings in their molecular structure bearing one or more amino substituents (Brüschweiler et al., 2017).

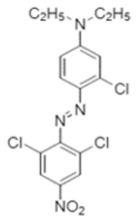
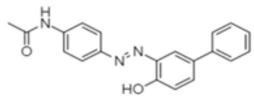
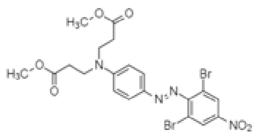
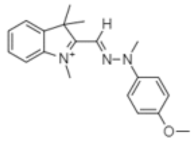
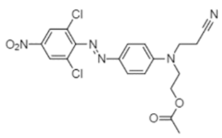
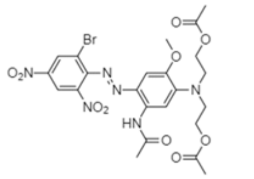
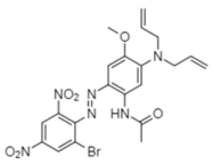
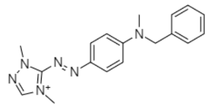
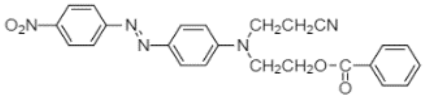
While increasing industrialization causes environmental pollution, the discharge of toxic wastes from various industries also affects water resources, soil fertility, aquatic organisms, and ecosystem integrity. Textile dyeing industries, one of these industries, release a large amount of wastewater to the environment after dyeing. Textile processing industries mostly use azo dyes. It is affected by toxicity in aquatic organisms (fish, algae, bacteria, etc.) as well as in animals. Among the dyes, especially the chronic effects of azo dyes have been studied over the years (Correia et al., 1994). A large number of azo dyes are used in the dyeing of textile products. Studies have observed that approximately 10% of the dyes in the dyeing process are not bound to the fibers and therefore released into the environment (Hildenbrand et al., 1999).

Polluting dye residues include azo dyes, which are discharged in large quantities directly into water bodies, and this characterizes an important pathway of environmental contamination (Pearce et al., 2003). According to some studies, approximately 10-15% of the dyes used by industries are lost during the dyeing process and thus released into the environment (Nam et al.,2000; Jarosz-wilkolazka et al., 2002). In another study, it was predicted that these values could be up to 50% higher (O'Neill et al., 1999). However, the exact data on the amount of dye released into the environment is not known yet (Ekici et al., 2001). The use of azo dyes in textile colors is of great interest, as environmental concerns may arise due to the high volume of water involved in the dyeing process. When a dye is used in this process, some of it is not subject to adhesion to the fibers remaining in the water bath. As a result, large volumes of wastewater containing dyes and related excipients are produced and can be released into the environment (Umbuzeiro et al., 2004; Umbuzeiro et al., 2005).

On the one hand, azo dyes meet the needs of people, on the other hand, they cause ecological and toxicological changes in hydric sources, soil, and atmosphere. The presence of dyes in water causes problems in the body and can have a negative impact on public health (Achwal, 1997). Azo dyes do not degrade readily under natural conditions and are not typically removed from wastewater by conventional wastewater treatment systems (Puvaneswari et al., 2006).

Aquatic environments are extremely important to the world's population because they are used as resources for water, agricultural activities, and animal production, and are also associated with recreational activities. Rivers, lakes, and oceans are the end targets of many pollutants from industrial, agricultural, and domestic activities (Ohe et al.,2004). With toxicity tests on fish and other aquatic organisms, it can be determined at what concentration a substance is harmful to organisms, and at which concentrations it has a visible effect. Using the results obtained from these tests, it is possible to determine the maximum concentrations for a water creature, evaluate the chemical measurements in the water source and make decisions accordingly and predict limitations. In these tests, while all other conditions are kept constant, trials are carried out by changing only the level of the factor and the concentration of the toxic substance (Bulut et al., 2013).

Table 1. Chemical structures of the studied azo dyes

Azo Dye	Structure	Azo Dye	Structure
Disperse Brown 27-1		Disperse Yellow 27	
Disperse Brown 19		Basic Yellow 28	
Disperse Orange 30		Disperse Blue 79	
Disperse Blue 291		Basic Red 46	
Orange 73-1			

Acute aquatic toxicity in Daphnia Magna is one of these tests. A recent study showed that Daphnia is highly sensitive to aniline and that aniline has a species sensitivity distribution. These data show that anilines can act in a different mode of action in Daphnia Magna. However, it is not yet known whether water fleas are the only sensitive species to aromatic amines (Ramos et al., 2002). Given the limited experimental data available on the toxicity of dyes and pigments, the development of prediction models appears to be of time interest (Jillella et al., 2021)

In this study, azo dyes commonly used in the textile are Basic Red 46, Basic Yellow 28, Disperse Brown 27-1, Disperse Brown 19, Disperse Blue 291, Disperse Blue 79, Disperse Orange 30, Disperse Yellow 27, and Orange 73-1 were examined acute toxicity in *Daphnia Magna* experimental and theoretical by QSAR method. The chemical structures of the studied azo dyes can be seen in Table 1.

Some regulations on human and ecosystem exposure to chemicals (eg REACH legislation) indicate that there are insufficient toxicity and ecotoxicity data for risk assessment of thousands of chemicals produced and used. However, given studies of the long-term and irreversible health effects of many chemicals, there is an increasing incentive to increase regulations and thus controls (Pinheiro et al., 2004).

Along with these incentives, studies examining the toxicological and ecotoxicological properties of azo dyes put on the market and using them in chemical legislation have increased in recent years. However, carrying out studies on human health and harmfulness to the environment, which should be carried out according to the legislation, causes both the use of living things and a great loss of time. For these reasons, the tendency for theoretical studies has increased considerably.

The use of quantitative structure-activity relationship (QSAR) analysis in predicting the toxicity and ecotoxicity of chemicals is extensive. QSAR is the result of a large amount of accumulated experimental data. It also addresses the need for reliable prediction methods, particularly in dye chemistry, due to the wide variety of amine structures involved in the production of chemicals. Studies of the mechanisms of dye toxicity and the use of the QSAR tool have hampered the development of azo dyes with direct toxicity and the production of azo dyes capable of converting to carcinogenic aromatic amines in Europe. Computational analyzes for the prediction of toxicological and ecotoxicological processes are useful for screening a wide range of chemicals and predicting the effects of functional groups (Benigni et. al., 2002).

The read-across method is widely used by industry to support the safety assessment of chemicals. The toxicological and ecotoxicological read-across method is based on the principle that chemicals grouped according to the similarity in the molecular structure show compatibility in some toxicodynamic and some toxicokinetic properties (Kutsarova et al., 2021). The concepts of grouping items and read-across describe concepts for estimating a value for an item using experimental data for the same value from previously studied items considered "similar" to the target item. This similarity, which is a prerequisite for any read-across method, is a concept based on the similarity between the target and the chemicals under investigation and the commonality of their metabolic consequences (Kuseva et. al., 2019).

According to the work of the Organization for Economic Co-operation and Development (OECD) (OECD, 2020), the increasing reliance on read-across as a data gap-filling method has brought appropriate approaches to establishing chemical categories and analog identification. In this study, the QSAR Toolbox 4.2 program, downloaded from ECHA's "http://www.oecd.org/chemicalsafety/oecd-qsar-toolbox.htm" website, which is also frequently used by the chemical industry, was used. OECD QSAR Toolbox is a software created to perform hazard assessment of chemicals used/to be used and to effectively evaluate mechanical and existing information about chemicals used/to be used. As a free-to-use theoretical program, it creates an alternative to animal testing and encourages the use of these methods. It prevents unnecessary animal testing by reducing human health and environmental risks. It is software created for use by governments, the chemical industry, and stakeholders. In addition, these Toolboxes reduce the cost of testing in laboratory tests and increase the number of chemicals evaluated. The toxicity and ecotoxicity of chemicals can be predicted before they are produced, supporting sustainable product development and green chemistry (Wexler, 2014; Keşkek Karabulut, 2020).

MATERIALS AND METHODS

Theoretical

In this study, OECD QSAR Toolbox 4.2 program was used. Toolbox is an in silico application designed to perform chemical hazard assessment. It can be used to scan current experimental results, evaluate category similarity, and fill in data gaps for the target chemical by cross-reading. The Toolbox consists of six basic tabs that can be used to create estimates and report results.

The OECD QSAR Toolbox's workflow starts with the profiling and classification steps. These steps can be intervened by the user and the program automatically calculates according to the data obtained from the previous studies. The profiling step is a process for obtaining appropriate information about the chemical to be scanned in databases (Keşkek Karabulut, 2020; Dimitrov et al., 2016).

The azo dyes used in this study are Disperse Brown 27-1, Disperse Brown 19, Disperse Orange 30, Disperse Blue 291, Disperse Yellow 27, Disperse Blue 823, Disperse Blue 79, Basic Red 46, Orange 73-1, Basic Yellow 28. Daphnia Magna data include short-term aquatic toxicity expressed in mol/l and as the negative logarithmic of LC50, i.e. the chemical concentration at which 50% lethality is observed in a test batch of Daphnia Magna within a 48 h exposure period.

The relevant azo dyes were calculated by cross-reading method using OECD QSAR Toolbox. Considering the experimental results of approximately 50-150 conformation in the theoretical calculation for all azo dyes, the read-across method was used. When QSAR results were examined, it was seen that the first three azo dyes with the highest toxicity on Daphnia Magna were Disperse Blue 291, Disperse Brown 27-1, and Basic Yellow 28 (Table 2) (Keşkek Karabulut, 2020).

Experimental

Daphnia Magna was cultured in a standard freshwater solution and the pH of the medium was kept constant at 7.5 [19]. Test compounds were provided by Setaş Kimya San. A.Ş. Chemicals and other chemicals of the test were obtained from Sigma Chemical Company, St. Louis MO, USA. Stock solutions were prepared by dissolving the appropriate amount of azo dyes in the M7 medium.

At the start of the test, the animals were less than 24 hours old and the second generation was used to reduce deviations within the test. The toxicity of related azo dyes was measured using 24 h Daphnia Magna (OECD, 2004). All organisms used for a particular test were origin from cultures established from the same stock of Daphnids. Toxicity tests were performed four times using five Daphnids in each 10 ml effective volume test beaker. Daphnia Magnas were grown within the range of 18 ^{0}C and 22 ^{0}C, and for every single test, it was constant within 1 ^{0}C in the laboratory. The experiment was followed at the end of 24 hours and the final 48 hours. At the end of 48 hours, the experiment was terminated. The values at the end of 48 hours were taken into account while plotting the mobile and immobile Daphnia Magna count and percent immobility curve (Table 2).

RESULTS AND DISCUSSION

Theoretical and experimental acute toxicity tests were performed on Daphnia Magna. Both tests were performed by the protocols. The EC50 value indicates the maximum concentration at which the relevant structure is toxic. It can be said that the lower the EC50 value, the more toxic the relevant aromatic amine is.

In the theoretical calculation for all azo dyes, considering the experimental results of approximately 50-150 conformation the read-across method was used by OECD QSAR. As a result of the theoretical calculation, it was seen that the EC50 values of the toxicities found varied between 1-150 mg/L. It was observed that the three most toxic azo dyes are Disperse Blue 291, Disperse Brown 27-1, and Basic Yellow 28.

A control group was formed in the experimental acute toxicity test. No change in oxygen content and pH value was observed during the test. The toxicity EC50 value range of a total of 9 azo dyes studied was between 1-130 mg/l. When the experimental EC50 values are examined, it can be said that the three most toxic azo dyes are Basic Yellow 28, Disperse Blue 291 and Disperse Brown 27-1 (Table 2).

Table 2: Theoretical and experimental (with *Daphnia Magna)* acute aquatic toxicity values of azo dyes

Azo Dye	Theoretical EC_{50} (mg/L)	Experimental EC_{50} (mg/L)
Basic Red 46	10.8	3.17
Basic Yellow 28	**3.37**	**1.1**
Disperse Blue 79	145	126
Disperse Blue 291	**1.02**	**1.55**
Disperse Brown 19	23.9	14.36
Disperse Brown 27-1	**2.77**	**3.15**
Disperse Orange 30	37.08	67.72
Disperse Yellow 27	36.9	75.28
Orange 73-1	50.3	95.5

According to some studies, toxicologists have attempted to understand the structure-toxicity relationship of azo dyes. Appropriate interactions of chemical components and environmental factors responsible for toxicological effects should be considered before formulating regulatory policies for their industrial use. Moreover, it can be used as a preliminary criterion for the identification of toxic dyes for which ecotoxicological evaluation should be made. The chemical structure of azo dyes is the most important criterion for their ecotoxic potential. With the same point of view, groups that increase toxicity were tried to be determined in this study (Rawat et al., 2016).

In another study on azo dyes, ecotoxicity testing was performed on the azo dyes CI Disperse Violet 93, CI Disperse Blue 291, and CI Disperse Blue 373 in Daphnia Magna. As a result of the study, it was found that disperse dyes showed very low ecotoxicity and the EC50 value of Disperse Blue 291 azo dye was higher than 0.02 mg/L. Similarly, in this study, the ecotoxicity of the Disperse Blue 291 dye was found to be higher than the specified value (Umbuzeiro et al., 2017).

In some studies, it has been observed that metals and nonmetals in azo dyes increase ecotoxicity and make the azo dye more toxic. It has been suggested that copper molecules in dye structures play an important role in the assessment of the aquatic toxicity of dye solutions. In parallel with these data, the effect of halogen presence in azo dyes on toxicity was also considered in this study (Bae et al., 2006; Bae et al., 2007).

According to many studies to date, azoic dyes have azo groups and aromatic rings that stabilize these water-resistant compounds. Some of these dyes are associated with mutagenic, cytotoxic, and carcinogenic behavior. As seen in the results of this study, the position of the aromatic ring and the number and position of both amino and nitro groups were found to affect potential toxicity (Chung, 2016; Jaafarzadeh et al., 2018).

In 2011, the textile dye red 1 ((N-ethyl-N-(2-hydroxyethyl)-4-(4-nitrophenylazo)aniline) was found to be highly toxic to aquatic invertebrates as well as being mutagenic (Ferraz et al., 2011).

According to a 2013 study examined a commercial red dispersion dye product and suggested that the ecotoxicity of the commercial preparation was similar to the dye. In this study, the effective concentration (EC50) for Daphnia was found to be 0.1 mg/L. Therefore, this dye can be classified as highly toxic to aquatic organisms according to the Global System for Classification and Labeling of Chemicals (GHS) (Vacchi et al., 2013). In this study, it was observed that dyes similar to the structure of Red 1 dye were toxic.

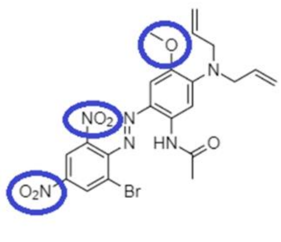
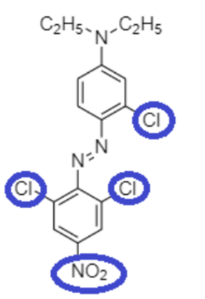
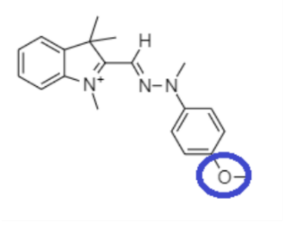
Minor differences in the molecular structures of azo dyes could greatly alter their toxicity activities and carcinogenic potential, so it is critical to adequately test each marketed azo dye (Umbuzeiro et al., 2005). Disperse azo dyes are poorly soluble or insoluble in water. Structures also play a big role in resolution values. The presence of ester in the structure and solubility in water, biodegradation, and sorption may affect the results of aquatic toxicity tests. According to a study to examine the effect of esters on toxicity on Daphnia, only low molecular weight esters were found to be acutely toxic. It was observed that as the molecular weight of chemicals containing ester in their structure increased, the acute effect levels decreased. Among the azo dyes examined in this study in terms of toxicity, it was observed that the structures of Disperse Blue 79 and Disperse Blue 291 azo dyes were very similar to each other, but their toxic effect levels were different. As mentioned in the literature, it can be said in this study that the ester groups present in the Disperse Blue 79 azo dye reduce toxicity (Staples et al.,2009).

Recent studies have shown that daphnias are highly sensitive to aniline and the species susceptibility distribution of aniline is complex to the sensitive side. These data indicate that anilines may act with a different mode of action in daphnias. Furthermore, it is not yet known whether water fleas are the only species sensitive to aromatic amines (Ramos et al., 2002).

CONCLUSION

As a result of *Daphnia Magna* Acute Aquatic toxicity studies, it was seen that theoretical calculations and experimental test studies were parallel to each other.

Table 5: Structures and active groups of Disperse Blue 291, Disperse Brown 27-1, and Basic Yellow 28

Disperse Blue 291	Disperse Brown 27-1	Basic Yellow 28
		

In experimental tests and theoretical calculations, Disperse Blue 291, Disperse Brown 27-1, and Basic Yellow 28 azo dyes were found to be the three most toxic azo dyes among the azo dyes studied. When the molecular structures of these three azo dyes were examined, it was seen that the nitro group and the ether group were present in the structures. In addition, it can be said that the presence of halogens in the structure of azo dyes with high aquatic toxicity is effective in toxicity (Table 5).

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Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

REFERENCES

Achwal WB, 1997. Problems during analysis of textile as per ecostandards and the customer articles ordinance (Part I). Colourage 44(5):29-31.

Bae JS, Freeman HS, 2007. Aquatic toxicity evaluation of new direct dyes to the *Daphnia Magna*. Dyes and Pigments 73(1):81-85. DOI: 10.1016/j.dyepig.2005.10.015

Bae JS, Freeman HS, Kim SD, 2006. Influences of new azo dyes to the aquatic ecosystem. Fibers and polymers 7:30-35. DOI: 10.1007/BF02933599

Benigni R, Passerini L, 2002. Carcinogenicity of the Aromatic Amines: From Structure-Activity Relationships to Mechanisms of Action and Risk Assessment. Mutation Research 511(3):191-206. DOI: 10.1016/s1383-5742(02)00008-x

Bulut C, Çetinkaya O, Kubilay A, Akçimen U, Ceylan M., 2013. Acute Toxicity of Zinc (Cu+2) on Sand Smelt. Süleyman Demirel University Journal of Natural and Applied Science 17(3): 32-38. DOI: 10.22392/actaquatr.874241

Brüschweiler BJ, Merlot C, 2017. Azo Dyes In Clothing Textiles Can Be Cleaved Into A Series of Mutagenic Aromatic Amines Which Are Not Regulated Yet. Regulatory Toxicology and Pharmacology 88: 214-226. DOI: 10.1016/j.yrtph.2017.06.012

Carliell CM, Barclay SJ, Naidoo N, Buckley CA, Mulholland DA, Senior E, 1995. Microbial Decolourisation of A Reactive Azo Dye Under Anaerobic Conditions. Water SA 21(1): 61-69.

Chung KT, 2016. Azo dyes and human health: A review. Part C: Environ. Carcinog. Ecotoxicol. Rev. 34:233-261. DOI: 10.1080/10590501.2016.1236602

Correia VM, Stephenson T, Judd SJ, 1994. Characterisation of Textile Wastewaters - A Review. Environmental Technology 15:917-929. DOI:10.1080/09593339409385500

Dimitrov SD, Diderich R, Sobanski T, Pavlov TS, Chankov GV, Chapkanov AS, Karakolev YH, Temelkov SG, Vasilev RA, Gerova KD, Kuseva CD, Todorova ND, Mehmed AM, Rasenberg M, Mekenyan OG, 2016. QSAR Toolbox – workflow and major functionalities. Environmental Research 27(3): 203-219. DOI: 10.1080/1062936X.2015.1136680

Ekici P, Leupol G, Parlar H, 2001. Degradability of selected azo dye metabolites in activated sludge systems. 44(4):721-728. DOI: 10.1016/S0045-6535(00)00345-3

Ferraz ERA, Umbuzeiro GA, De Almeida G, Caloto Oliveira A, Chequer FMD, Dorta DJ, Oliveira DP, 2011. Differential toxicity of disperse red 1 and disperse red 13 in the Ames test, HepG2 cytotoxicity assay, and Daphnia acute toxicity test. Environ Toxicol. 26(5):489–497. DOI: 10.1002/tox.20576

Hildenbrand S, Schmahl FW, Wodarz R, Kimmel R, Dartsch PC, 1999. Azo Dyes and Carcinogenic Aromatic Amines in Cell Cultures. International Archives of Occupational and Environmental Health 72:M052-M056.

Jaafarzadeh N, Takdastan A, Jorfi S, Ghanbari F, Ahmadi M, Barzegar G, 2018. The performance study on ultrasonic/Fe_3O_4/H_2O_2 for degradation of azo dye and real textile wastewater treatment. J. Mol. Liq. 256:462-470. DOI: 10.1016/j.molliq.2018.02.047

Jarosz-wilkolazka A, Kochmanska-Rdest J, Malarczyk E, Wardas W, Leonowicz A, 2002. Fungi and their ability to decolorize azo and anthraquinonic dyes. Enzyme and Microbial Technology 30(4):566-572. DOI: 10.1016/S0141-0229(02)00022-4

Jillella GK, Roy K, 2021. QSAR modelling of organic dyes for their acute toxicity in Daphnia Magna using 2D-descriptors. SAR and QSAR in Environmental Research 33(2):111-139. DOI: 10.1080/1062936X.2022.2033318

Keşkek Karabulut Y, 2020. Bazı Aromatik Aminlerin Toksikolojik Ve Ekotoksikolojik Özelliklerinin Qsar Yöntemi ile İncelenmesi. Doctoral Thesis, Institute of Science, Namık Kemal University, Tekirdağ, Türkiye.

Kuseva C, Schultz TW, Yordanova D, Tankova K, Kutsarova S, Pavlov T, Chapkanov A, Georgiev M, Gissi A, Sobanski T, Mekenyan OG, 2019. The implementation of RAAF in the OECD QSAR Toolbox. Regulatory Toxicology and Pharmacology 105:51-61. DOI: 10.1016/j.yrtph.2019.03.018

Kutsarova S, Schultz TW, Chapkanov A, Cherkezova D, Mehmed A, Stoeva S, Kuseva C, Yordanova D, Georgiev M, Petkov T, Mekenyan OG, 2021. The QSAR Toolbox Automated Read-across Workflow for Predicting Acute Oral Toxicity: II. Verification and Validation. Computational Toxicology 20:2468-1113. DOI: 10.1016/j.comtox.2022.100219

Majcen-Le MA, Slokar YM, Taufer T, 1997. Decoloration of chlorotriazine reactive azo dyes with H2O2/UV. Dyes Pigments 33(4):281-298. DOI:10.1016/S0143-7208(96)00057-5

Nam S, Renganathan V, 2000. Non enzymatic reduction of azo dyes by NADH. Chemosphere 40(4):351-357. DOI: 10.1016/S0045-6535(99)00226-X

OECD, 2004. Test No. 202: Daphnia sp. acute immobilisation test. In OECD Guidelines for the Testing of Chemicals, OECD Publishing, Paris.

Ohe T, Watanabe T, Wakabayashi K, 2004. Mutagens in Surface Waters: A Review. Mutation Research, 567(2-3):109-149. DOI: 10.1016/j.mrrev.2004.08.003

Organization for Economic Cooperation and Development (OECD), 2020. Overview of Concepts and Available Guidance related to Integrated Approaches to Testing and Assessment (IATA). OECD Series on Testing and Assessment No. 329, OECD, Paris.

O'Neill C, Hawkes FR, Hawkes DL, Lourenco ND, Pinheiro HM, Delée W, 1999. Colour in textile effluents – sources, measurement, discharge consents, and simulation: a review. Journal of Chemical Technology and Biotechnology 74(11):1009-1018.DOI:10.1002/(SICI)1097-4660(199911)74:11<1009::AID-JCTB153>3.0.CO;2-N

Pearce CI, Lloyd JR, Guthrie JT, 2003. The removal of color from textile wastewater using whole bacterial cells: a review. Dyes and Pigments 58(3):179-196. DOI:10.1016/S0143-7208(03)00064-0

Pinheiro HM, Touraud E, Thomas O, 2004. Aromatic Amines from Azo Dye Reduction: Status Review with Emphasis on Direct UV Spectrophotometric Detection in Textile Industry Wastewaters. Dyes and Pigments 61(2):121–139. DOI: 10.1016/j.dyepig.2003.10.009

Puvaneswari N, Muthukrishnan J, Gunasekaran P, 2006. Toxicity Assessment And Microbial Degradation Of Azo Dyes. Indian Journal of Experimental Biology 44(8):618-626. PMID: 16924831

Ramos EU, Vaal MA, Hermens JLM, 2002. Interspecies Sensitivity in the Aquatic Toxicity of Aromatic Amines. Environmental Toxicology and Pharmacology 11(3-4):149-158. DOI:10.1016/s1382-6689(02)00004-2

Rawat D, Mishra V, Sharma RS, 2016. Detoxification of azo dyes in the context of environmental processes. Chemosphere 155:591-605. DOI: 10.1016/j.chemosphere.2016.04.068

Staples CA, Adams WJ, Parkerton TF, Gorsuch JW, Biddinger GR, Reinert KH, 2009. Aquatic toxicity of eighteen phthalate esters. Environmental Toxicology and Chemistry 16(5):837-1086. DOI:10.1002/etc.5620160507

Umbuzeiro GA, Szymczyk M, Li M, Chen Y, Vendemiatti JAS, Albuquerque AF, Santos A, Maselli BS, Kummrow F, Vinueza NR, Freeman HS, 2017. Purification and characterization of three commercial phenylazoaniline disperse dyes. Coloration Technology 133(6):513-518. DOI: 10.1111/cote.12307

Umbuzeiro GA, Freeman HS, Warren SH, Oliveira DP, Terao Y, Watanabe T, Claxton LD, 2005. The contribution of azo dyes to the mutagenic activity of Cristais River. Chemosphere 60(1):55–64.DOI: 10.1016/j.chemosphere.2004.11.100

Umbuzeiro GA, Roubicek DA, Rech CM, Sato MIZ, Claxton LD, 2004. Investigating the sources of the mutagenic activity found in a river using the Salmonella assay and different water extraction procedures. Chemosphere 54(11):1589–1597. DOI: 10.1016/j.chemosphere.2003.09.009

Umbuzeiro GA, Freeman H, Warren SH, Kummrow F, Claxton LD, 2005. Mutagenicity evaluation of the commercial product CI Disperse Blue 291 using different protocols of the Salmonella assay. Food and Chemical Toxicology 43(1): 49-56. DOI: 10.1016/j.fct.2004.08.011

Vacchi FI, Albuquerque AF, Vendemiatti JAS, Morales DA, Ormond AB, Freeman HS, Zocolo GJ, Zanoni MVB, Umbuzeiro GA, 2013. Chlorine disinfection of dye wastewater: Implications for a commercial azo dye mixture. Science of the Total Environment 442:302–309.DOI:10.1016/j.scitotenv.2012.10.019

Ventura-Camargo BC, Marin-Morales MA, 2013. Azo Dyes: Characterization and Toxicity A Review. Textiles and Light Industrial Science and Technology (TLIST) 2(2):85-103.

Wexler P, 2014. Encyclopedia of Toxicology, 3rd edn, US National Library of Medicine, Bethesda, MD, USA.