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Investigation of Ecotoxicological Properties of Some Azo Dyes by OECD QSAR Method

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In this study, the effects of some azo dyes, which are frequently used in the industry, and aromatic amines, which are used as raw materials in their synthesis, on the environment were investigated with the OECD QSAR model. When the literature was searched, it was seen that no studies were found in comparison with ecotoxicologically related dyes and raw materials.

As a result of the studies showed that 2-Bromo-4,6-dinitroaniline aromatic amine, which is accepted as a raw material in the acute water toxicity test, is the most toxic substance in *Daphnia Magna*. It was determined that Disperse Blue 291 dye showed the highest toxicity in the acute aquatic toxicity test performed on fish with an LC50 value of 0.0675 mg.L^{-1}. In addition, when the molecular structure of azo dyes and their raw materials was examined, it was observed that some functional groups such as nitro group and ether group presence were quite effective in toxicity.

Keywords: *Azo Dyes; Aromatic Amines; OECD QSAR; Ecotoxicology*

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1. Introduction

Generally, dyes are an application tool used to change the color properties of different substrates. In previous years, substances with coloring properties were obtained from natural sources such as animals or vegetables. In recent years, such natural dyes have been replaced by synthetic dyes and the use of synthetic dyes has increased considerably. Hundreds of new synthetic colored compounds are introduced every year and take place in the market [1]. 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 the azo dye group [2]. These dyes are used in the textile industry to color fibers with various raw materials. Azo dyes are easily synthesized with excellent fixing and holding properties. It also has a wide range of colors compared to natural dyes [3].

Substances defined as azo dyes are synthetic dyes known as amines or phenol diazotized amines containing an azo group (N=N-) in their structure and are often used in the textile industry [4]. 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 [5].

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 [6]. Dye residue is either discharged into waters passing through environmental treatment residues or directly diffused and is more commonly observed in the presence of a large number of textile industries, causing serious pollution of water bodies [7]. Polluting dye residues include azo dyes discharged directly into water bodies in large environments, which is a major cause of pollution [8]. According to some studies, about 10-15% of the dyes used by industries are lost during the dyeing process and are thus released [9-10]. However, the exact data of the activation-released dye data are not yet fully known [11]. In addition, azo dyes do not degrade readily under natural conditions and are typically not removed from wastewater by conventional wastewater treatment systems [12]. Studies evaluating the toxicity of azo dyes and their metabolites due to their degradation are important in terms of establishing strategies to reduce the harmful effects of these chemicals [13].

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 [14]. 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, make decisions accordingly, and predict limitations. In these tests, while all other conditions are kept constant, trials are carried out by changing only the factor level and the toxic substance's concentration [15].

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 [16].

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 [17].

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 paint 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 [18,19].

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 [20]. 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 [21].

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, 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 [22].

The target chemical can be used to screen available experimental data for profile structures, examine analogues, assess category consistency, and also to supplement the data gap through QSAR and read-across. Toolbox has a workflow of six core modules that can be used to make a forecast and report forecasts. This workflow is illustrated in Figure 1.

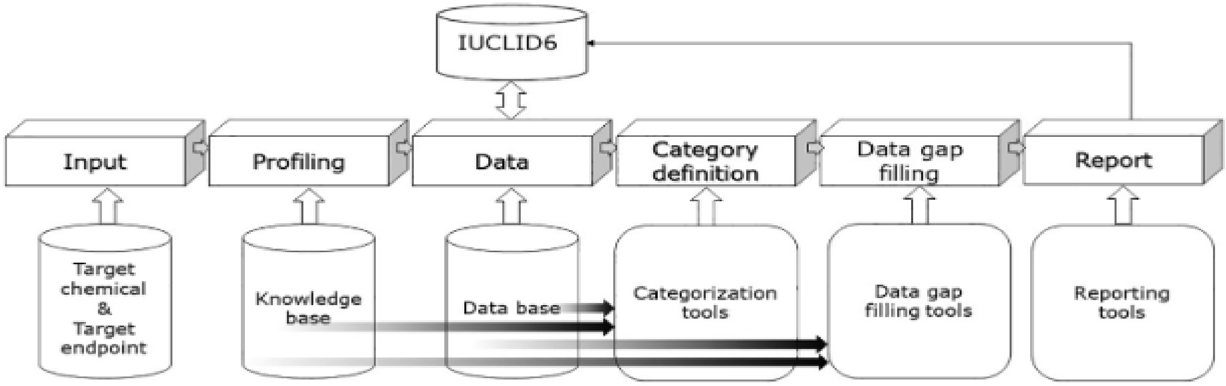


Figure 1: Scheme of the OECD QSAR Toolbox Workflow

2. Theoretical Calculations

Raw materials commonly used in textile dyes (2,6-dichloro-4-nitroaniline, 2.6-dibromo-4-nitroaniline, N-Cyanoethylacetoxyethylylanil, 6-Methoxybenzothiazol-2-ylamine, 2-Bromo-4,6-dinitroaniline, 4' - Aminoacetanilide, p-Acetanilide, 3'-amino-, 3-Pyridinecarbonitrile, 1,2-dihydro-6-hydroxy-1,4-dimethyl-2-oxo, 2-cyano-4-nitroaniline, N, N- Dihydroxyethyl -3-amino-4-anisidine, N-Benzyl, N-Methylaniline, N-Cyano Ethyl N-Benzyl Aniline, 1,3,3-Trimethyl-2-Methylenenindoline) ecotoxicological properties were investigated using OECD QSAR Toolbox.

In addition, the dyestuffs produced from these raw materials (Disperse Brown 27-1, Disperse Brown 19, Disperse Orange 30, Red BS P/C, Disperse Blue 291, Disperse Yellow 27, Disperse Blue 823, Disperse Yellow 241, Disperse Blue 79, Basic Red 46, Orange 73-1, Basic Yellow 28) ecotoxicological properties were studied by the same method. Acute aquatic toxicity (daphnia and fish), tests were performed using OECD QSAR Toolbox software to examine the ecotoxicological properties of 25 substances consisting of raw materials and products.

The azo dyes to be studied and the raw materials of these azo dyes were entered into the system. Basic information about the aromatic amine to be studied (called the target chemical based on the Program) was obtained with the "Profile" module. Target azo dyes and their raw materials were screened for their relevance to existing predefined structural, toxicological, and ecotoxicological functions. The information obtained from the database was evaluated and used to search for analogues in selected databases (in the "Data" module) that share the same functions (in the "Category Definition" module).

The Toolbox profile module is a set of algorithms that allow the identification of certain properties of chemicals and the mechanical validation of results. Potential azo dyes and their raw materials (analogues) identified by experimental data can be used in "Data gap filling" to predict the trait of interest for the target construct.

In the “Report” module, a report was created for estimations by evaluating the analyzed azo dyes and the raw materials of these dyes together with their analogues.

In addition, the existing connection to the OECD QSAR Toolbox and the IUCLID database15 [23] allows data transfer between both systems. The OECD QSAR Toolbox is constantly updated and expanded with new data while maintaining the workflow core structure.

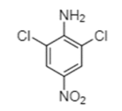
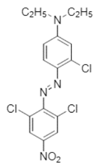
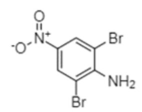
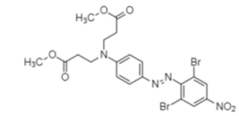
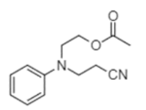
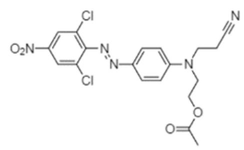
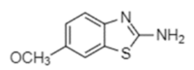
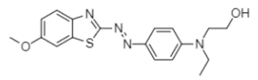
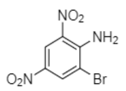
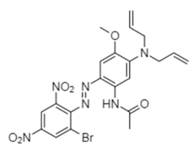
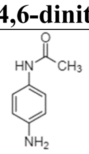
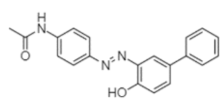
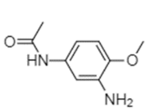
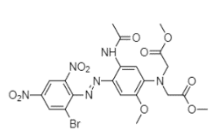
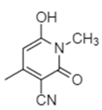
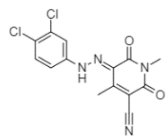
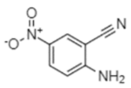
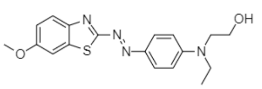
2.1. Acute Aquatic Toxicity Test on Daphnia Magna

Acute aquatic toxicity calculation of Daphnia was made using OECD QSAR software for selected azo dyes and their raw materials, which are aromatic amines. The relevant azo dyes and their raw materials 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 and their raw materials, the read-across method was used. Since the theoretical calculation is based on acute toxicity, it was designed as 48 hours and EC50 values were taken into account. The theoretical results are shown in Table 1.

2.2. Acute Aquatic Toxicity Test on Fish

Acute aquatic toxicity calculation of Fish was made using OECD QSAR software for selected azo dyes and their raw materials, which are aromatic amines. Considering the experimental results of approximately 700-900 conformation in the theoretical calculation for all azo dyes and their raw materials, the read-across method was used. The test was based on one of the fish species, Pimephales promelas. This is because the breed is relatively hardy and produces a large number of offspring. This species is used as an indicator in acute toxicity tests. In this study, the LC50 value was calculated with 98 hours of exposure to this species. The theoretical results are shown in Table 2.

Table 1: Theoretical Calculation Results of Acute Aquatic Toxicity of Azo Dyes and Raw Materials in Daphnia Magna

Raw Materials	EC50-48h-*Daphnia* Magna (mg.L^{-1})	Azo Dyes	EC50-48h-*Daphnia Magna* (mg.L^{-1})
 **2,6-dichloro-4-nitroaniline**	21.2 mg.L^{-1}	 **Disperse Brown 27-1**	2.77 mg.L^{-1}
 **2.6-dibromo-4-nitro aniline**	1.79 mg.L^{-1}	 **Disperse Brown 19**	23.9 mg.L^{-1}
 **N-Cyanoethylacetoxyethylaniline**	90.4 mg.L^{-1}	 **Disperse Orange 30**	37.8 mg.L^{-1}
 **6-Methoxybenzothiazol-2-ylamine**	618 mg.L^{-1}	 **Red BS P/C**	23.1 mg.L^{-1}
 **2-bromo-4,6-dinitroaniline**	7.3E3 µg.L^{-1}	 **Disperse Blue 291**	1.02 mg.L^{-1}
 **4'-Aminoacetanilide**	244 mg.L^{-1}	 **Disperse Yellow 27**	36.9 mg.L^{-1}
 **p-Acetanisidide, 3'-amino-**	244 mg.L^{-1}	 **Disperse Blue 823**	0.0820 mg.L^{-1}
 **3-Pyridinecarbonitrile,1,2-dihydro-6-hydroxy-1,4-dimethyl-2-oxo**	46.1 mg.L^{-1}	 **Disperse Yellow 241**	58.6 mg.L^{-1}
 **2-cyano-4-nitroaniline**	61.7 mg.L^{-1}	 **Red BS P/C**	23.1 mg.L^{-1}

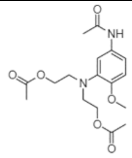
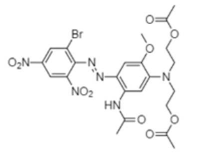
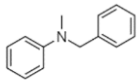
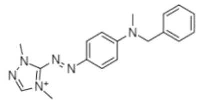
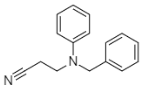
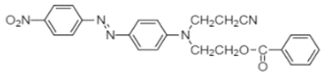
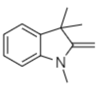
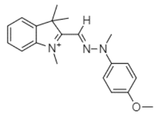
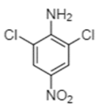
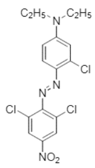
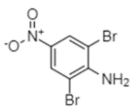
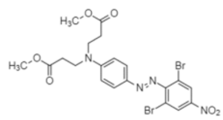
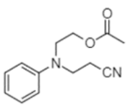
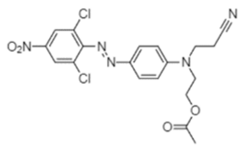
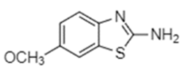
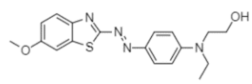
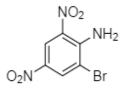
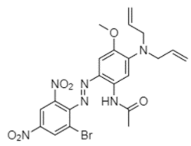
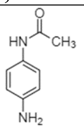
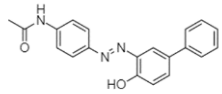
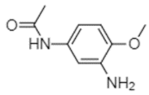
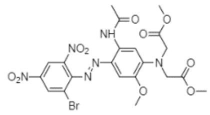
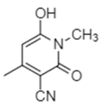
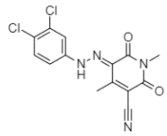
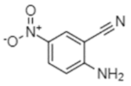
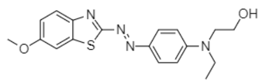
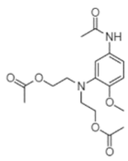
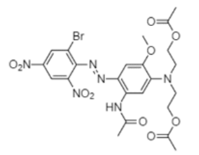
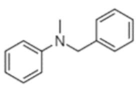
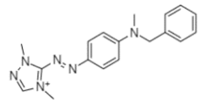
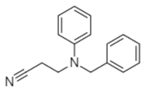
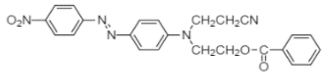
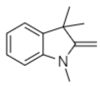
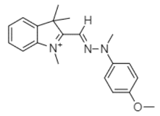
 **N, N-Dihydroxyethyl-3-amino-4-anisidine**	526 mg.L^{-1}	 **Disperse Blue 79**	145 mg.L^{-1}
 **N-Benzyl,N-Methylaniline**	357 mg.L^{-1}	 **Basic Red 46**	10.8 mg.L^{-1}
 **N-cyano Ethyl N-Benzyl Aniline**	521 mg.L^{-1}	 **Orange 73-1**	37.4 mg.L^{-1}
 **1,3,3-Trimethyl-2-Methyleneindoline**	804 mg.L^{-1}	 **Basic Yellow 28**	5.38E8 mg.L^{-1}

Table 2: Theoretical Calculation Results of Acute Aquatic Toxicity of Azo Dyes and Raw Materials in Pimephales Promelas

Raw Materials	**LC50-96h-** ***Pimephales Promelas*** **(mg.L^{-1})**	**Azo Dyes**	**LC50-96h-** ***Pimephales Promelas*** **(mg.L^{-1})**
 **2,6-dichloro-4-nitroaniline**	12.4 mg.L^{-1}	 **Disperse Brown 27-1**	32.2 mg.L^{-1}
 **2.6-dibromo-4-nitro aniline**	4.50 mg.L^{-1}	 **Disperse Brown 19**	202 mg.L^{-1}
 **N-Cyanoethylacetoxyethylaniline**	97.6 mg.L^{-1}	 **Disperse Orange 30**	203 mg.L^{-1}
 **6-Methoxybenzothiazol-2-ylamine**	104 mg.L^{-1}	 **Red BS P/C**	32.2 mg.L^{-1}

 **2-bromo-4,6-dinitroaniline**	11.5 mg.L^{-1}	 **Disperse Blue 291**	0.0675 mg.L^{-1}
 **4'-Aminoacetanilide**	202 mg.L^{-1}	 **Disperse Yellow 27**	348 mg.L^{-1}
 **p-Acetanisidide, 3'-amino-**	400 mg.L^{-1}	 **Disperse Blue 823**	100 mg.L^{-1}
 **3-Pyridinecarbonitrile,1,2-dihydro-6-hydroxy-1,4-dimethyl-2-oxo**	113 mg.L^{-1}	 **Disperse Yellow 241**	1.93 mg.L^{-1}
 **2-cyano-4-nitroaniline**	59.6 mg.L^{-1}	 **Red BS P/C**	32.2 mg.L^{-1}
 **N,N-Dihydroxyethyl-3-amino-4-anisidine**	0.69 mg.L^{-1}	 **Disperse Blue 79**	100 mg.L^{-1}
 **N-Benzyl,N-Methylaniline**	20.4 mg.L^{-1}	 **Basic Red 46**	3.12 mg.L^{-1}
 **N-cyano Ethyl N-Benzyl Aniline**	11.3 mg.L^{-1}	 **Orange 73-1**	203 mg.L^{-1}
 **1,3,3-Trimethyl-2-Methyleneindoline**	2.95 mg.L^{-1}	 **Basic Yellow 28**	7.84 mg.L^{-1}

3. Results

Firstly, acute aquatic toxicity in *Daphnia Magna* was studied theoretically. This calculation is based on finding the EC50 by focusing on 48 hours of exposure. The Half maximal effective concentration (EC50) value indicates the maximum concentration at which the construct is toxically effective. It can be said that the smaller the EC50 value, the more toxic the relevant aromatic amine is. When the results were examined, the EC50 value of 2-Bromo-4,6-dinitroaniline aromatic amine out of 13 aromatic amines considered as raw material was found as 7.3 e3 µg/L. When azo dyes consisting of aromatic amines were examined, it was seen that the EC50 value of Disperse Blue 823 azo dye was 0.0820 mg.L^{-1}, and the most toxic effect was seen in this dye among the dyes examined (Fig. 2.).

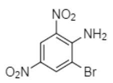
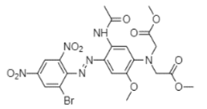
 **2-bromo-4,6-dinitroaniline**	 **Disperse Blue 823**

Figure 2: Structure of 2-Bromo-4,6-dinitroaniline and Disperse Blue 823.

Acute Aquatic Toxicity on fish was calculated by the same method in order to examine the ecotoxicological feature. In this calculation, the LC50 value was taken into account after 96 hours of exposure to *Pimephales Promelas*. Lethal concentration 50 (LC50) is the amount of a substance suspended in the air required to kill 50% of a test animal during a predetermined observation period. The smaller this value, the more toxic the structure could be said to be. It was found that N, N-Dihydroxyethyl-3-amino-4-anisidine had the lowest LC50 value of 0.69 mg.L^{-1} among 13 aromatic amines defined as raw materials. When azo dyes were examined, it was found that Disperse Blue 291 dye showed the highest toxicity among all aromatic amines, with an LC50 value of 0.0675 mg.L^{-1} (Fig. 3).

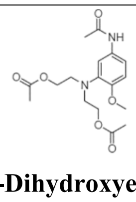
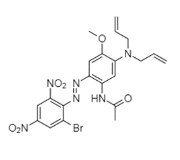
 **N, N-Dihydroxyethyl-3-amino-4-anisidine**	 **Disperse Blue 291**

Figure 3: Structure of N, N-Dihydroxyethyl-3-amino-4-anisidine and Disperse Blue 291.

4. Conclusion

In the study, acute water toxicity tests (in Daphnia Magna and Fish) were examined theoretically using OECD QSAR Toolbox software to predict the ecotoxicological properties of twelve azo dyes, which are widely used in the textile industry, and the raw materials of these dyes. According to the results of the acute aquatic toxicity test in *Daphnia Magna*, it can be said that the presence of nitro groups and ether groups further increases the ecotoxic properties. When the results of acute aquatic toxicity in fish are examined, it can be said that the ester group increases the toxicity according to this theoretical study. In addition, the presence of nitro groups was also found to be effective in showing toxic properties.

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