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# INVESTIGATION OF TOXICOLOGICAL PROPERTIES OF SOME AZO DYES BY OECD QSAR METHOD

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## Abstract

In this study, the toxicological properties of some of the aromatic amine raw materials commonly used in textile dyes and the dyestuffs produced from these raw materials were examined with the OECD QSAR method and their toxicological effects on human health were investigated. In the literature review conducted with the relevant structures, it was seen that no study set was found in terms of toxicology, considering the comparison of raw materials and products.

Studies have found that some aromatic amine raw materials and azo dyes produced from these raw materials are generally moderately irritating. When the results were examined, it was seen that the raw materials studied had eye irritant properties. When looking at the products, no irritating properties were observed. It can be said that 12 aromatic amine raw materials and 13 azo dyes have skin sensitizing properties. Raw materials, that are 2,6-dibromo-4-nitro aniline, 6-methoxybenzothiazole-2-ylamine, 2-Bromo-4,6-dinotroaniline and azo dyes, that are Disperse Brown 27-1, Disperse Brown 19 and Disperse Blue 291 were found to be mutagenic.

**Keywords:** Toxicity, Aromatic Amines, Azo Dyes, OECD QSAR

# BAZI AZO BOYALARIN TOKSİKOLOJİK ÖZELLİKLERİNİN OECD QSAR YÖNTEMİYLE ARAŞTIRILMASI

## Öz

Bu çalışmada tekstil boyalarında yaygın olarak kullanılan bazı aromatik amin hammaddelerin ve bu hammaddelerden üretilen boyarmaddelerin toksikolojik özellikleri OECD QSAR yöntemi ile incelenmiş ve insan sağlığı üzerindeki toksikolojik etkileri araştırılmıştır. İlgili yapılarla yapılan literatür taramasında, hammadde ve ürün karşılaştırmasına bakıldığında toksikolojik açıdan herhangi bir çalışma setine rastlanmadığı görülmüştür.

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Yapılan araştırmalar bazı aromatik amin ham maddelerinin ve bu ham maddelerden üretilen azo boyaların genel olarak orta derecede tahriş edici olduğunu bulmuştur. Sonuçlar incelendiğinde çalışılan hammaddelerin gözü tahriş edici özelliklere sahip olduğu görülmüştür. Ürünlere bakıldığında herhangi bir tahriş edici özelliği gözlenmemiştir. 12 aromatik amin hammaddesi ve 13 azo boyanın cildi hassaslaştırıcı özelliği olduğu söylenebilir. 2,6-dibromo-4-nitro anilin, 6-methoxybenzothiazole-2-ylamine, 2-Bromo-4,6-dinotroanilin hammaddeleri ve Dispers Brown 27-1, Dispers Brown 19 ve Dispers Blue 291 azo boyaları mutajeniktir.

**Anahtar Kelimeler:** Toksisite, Aromatik Aminler, Azo Boyaları, OECD QSAR

## 1. INTRODUCTION

Azo dyes obtained from aromatic amines constitute the majority of all dyestuffs used in textile production. Studies on the effects of this type of dyestuff on human health are increasing day by day. Dermal and bacterial biotransformation of azo dyes can cause the release of aromatic amines and are largely absorbed dermally upon contact with the skin. Aromatic amines are the most commonly used intermediates in the synthesis of azo dyes. According to many studies, it has been shown that aromatic amines transported to consumer products, especially aromatic amines, pose a risk to human health due to their toxicological, mutagenic and/or carcinogenic properties. The toxicity of aromatic amines results from metabolic activation of the amino group, which can produce reactive intermediate hydroxylamine [1].

According to the studies, it has been determined that there are more than 3000 types of dyes in the market and half of them are in the azo dye class. These dyes are also frequently used in the dyeing of leather and plastics obtained from petroleum, especially in the textile industry [2].

While azo dyes meet the need for coloring in industry, they cause toxicological damage to hydraulic resources, soil, and atmosphere. The amount of dyes in wastewater causes toxicity in the body and adversely affects public health [3]. Aromatic amines are chemical compounds that carry one or more amino groups in their molecular structure and have one or more aromatic rings. According to recent studies, the possibility of decomposition into aromatic amines used as raw materials during the use of the produced azo dyes in the dyeing process poses a great danger to health [4].

Azo dyes are toxic because they produce aromatic amines after degradation and degradation of the azo bond in their chemical structure, usually with the help of intestinal anaerobic bacteria. Aromatic

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amines are highly reactive electrophiles that covalently bind to DNA. Various studies have shown that the release of azo dyes into the environment and organisms exposed to azo dyes form biotransformation products that can cause different types of damage. These products, on the other hand, have shown to be of concern due to their toxic, mutagenic, and carcinogenic properties [5]. The reduction of azo bonds is sufficient for some of the azo dyes to exhibit mutagenic activity. The aromatic amines formed by this reduction may be more carcinogenic and/or mutagenic than the azo dye, of which they are the raw material, depending on their chemical structure. Furthermore, these amines have been shown to be carcinogenic in some studies [6]. One of the most important criteria defined for classifying a dye as harmful to humans is its ability to decompose and therefore to produce aromatic amines when in contact with sweat, saliva, or gastric juices [7]. These aromatic amines are carcinogenic and are very likely to accumulate in food chains [8].

Quantitative structure-activity relationship (QSAR) analysis is based on a relationship between the structure of the molecule and its biological activity and is widely used to predict the toxicity of aromatic amines. It also meets the need for reliable estimation methods by comparing the amine structures of various raw materials involved in the production of chemicals in dye chemistry with empirical data [9].

In the chemical industry market, the dye industry is one of the sectors where a wide variety of products and intermediate products are processed. For this reason, textile and dyestuff manufacturers associations such as EURATEX and ETAD have been established and EU strategies have been given importance to control the release of used chemicals into the environment. In particular, the need for registration of products and raw materials used and marketed in the EU market in quantities exceeding a certain tonnage has arisen [10], [11]. The use of the QSAR method has improved existing knowledge of the mechanisms of dye toxicity and has allowed the development of low-toxicity azo dyes. Thus, it helped to largely eliminate in Europe azo dyes, which can decompose into carcinogenic aromatic amines [3].

The legislation known by the acronym "REACH" (Registration, Evaluation and Authorization of Chemicals) defines the management of risks related to chemicals in the European Union (EU). The proposed legislation is used to regulate new and existing products and their raw materials used in the relevant industry. A European Chemicals Agency (ECHA) has been established to manage the

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technical, scientific and administrative aspects of the REACH regulatory system. REACH consists of four main elements; Record; Evaluation; Authority; and Restriction. During the registration phase, manufacturers and importers are asked to collect information on the properties of chemicals and submit the information in the form of a registration file to a central database managed by ECHA [11]. First of all, all available information on the natural properties of the substance, regardless of production and use, should be collected by the manufacturer/importer, who is responsible for registering it, either internally, using different sources or through various means of sharing. It includes physicochemical properties, epidemiological information, in vitro and in vivo test data, data obtained through models such as QSAR, and other data on the risky properties of the substance. The extent of the data gaps identified should be defined and how they could be achieved should be discussed. Missing information about registration is available and acceptable through in vivo and in vitro testing, modeling tests such as QSAR, or data collection [12].

The OECD QSAR Toolbox is software designed to perform hazard assessment of chemicals and evaluate other chemical-related information in a cost-effective and efficient manner. The OECD QSAR Toolbox, available free of charge, encourages the use of alternative modeling methods to animal testing and minimizes animal testing. It is software designed for use by the chemical industry and other stakeholder industries. In addition, such modeling methods reduce the cost of testing and increase the number of chemicals evaluated. Predicting the toxicity of substances before they are produced facilitates sustainable product development and green chemistry. OECD QSAR Toolbox uses Microsoft's. NET framework and its database engine is PostgresSQL14 from Firebird [13].

## 2. MATERIALS AND METHOD

The toxicological properties of raw materials commonly used in textile dyes were investigated using OECD QSAR Toolbox. In addition, the toxicological properties of the dyestuffs produced from these raw materials were studied by the OECD QSAR Toolbox.

Skin irritation/corrosion, eye irritation, skin sensitization, and mutagenicity tests were performed using OECD QSAR Toolbox software to examine the toxicological properties of 25 substances consisting of raw materials and products.

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OECD QSAR Toolbox is free software designed for researchers interested in chemical hazard assessment, as well as for the private sector. It can be used to screen available experimental data for its target chemical structure, to evaluate other chemicals of similar nature, and to fill in the data gap by read-across. The software consists of six basic modules that can be used to make forecasts and report forecasts.

In the method, for the aromatic amine raw materials to be studied and the azo dyes produced from these raw materials, first of all, basic information about their chemical structures (structural, toxicological and ecotoxicological) was collected by using the "Profile" module. This information obtained from the database was evaluated and used to search for similar chemicals that share the same functions (in the "Category Definition" module) in the selected databases (in the "Data" module). Potential azo dyes and raw materials identified by experimental data were used in "Data gap filling" to predict the relevant property for the target construct. Azo dyes analyzed in the "Report" module and raw materials of these dyes were evaluated together with similar structures, and a report was created for estimations. In addition, the OECD QSAR Toolbox and the existing connection to the IUCLID database15 [14] allow data transfer between both systems. The OECD QSAR Toolbox is constantly updated and expanded with new data while maintaining the workflow core structure.

In this study, skin irritation/corrosion, eye irritation, skin sensitivity, and mutagenicity tests, which are among the toxicological properties, were theoretically performed on selected azo dyes and raw materials of these azo dyes (total 25 aromatic amines) using OECD QSAR Toolbox.

Attempts have been made in many countries to reduce skin sensitization issues through the implementation of chemical legislation. All these regulations are currently limited to a simple dual hazard identification (i.e. whether the chemical is a skin sensitizer), but efforts are underway to make improvements to allow differentiation of skin sensitizers of different strengths [15]. The Skin irritation/corrosion test calculation results of azo dyes and their raw materials are shown in Table 1.

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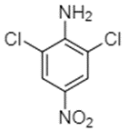
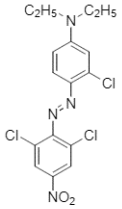
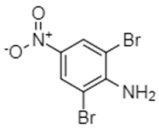
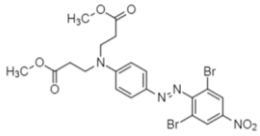
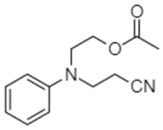
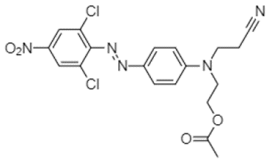
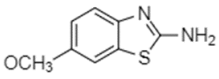
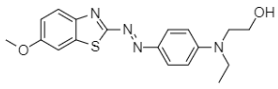
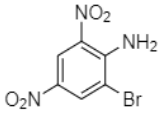
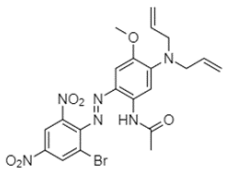
**Table 1.** Skin irritation/corrosion calculation results of azo dyes and their raw materials

| Raw Material | Skin irritation/corrosion | Azo Dye | Skin irritation/corrosion |
|---|---|---|---|
|  **2,6-dichloro-4-nitroaniline** | Moderate Irritant |  **Disperse Brown 27-1** | Moderate Irritant |
|  **2,6-dibromo-4-nitro aniline** | Moderate Irritant |  **Disperse Brown 19** | Moderate Irritant |
|  **N-Cyanoethylacetoxyethylaniline** | Moderate Irritant |  **Disperse Orange 30** | Moderate Irritant |
|  **6-Methoxy Benzothiazole-2-ylamine** | Moderate Irritant |  **Red BS P/C** | Moderate Irritant |
|  **2-bromo-4,6-dinitroaniline** | Moderate Irritant |  **Disperse Blue 291** | Moderate Irritant |

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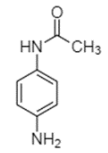
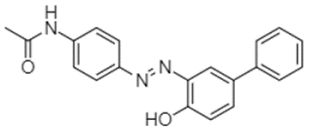
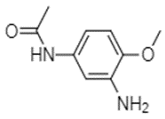
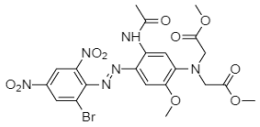
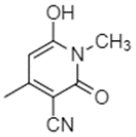
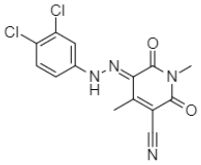
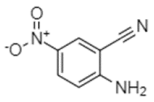
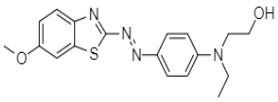
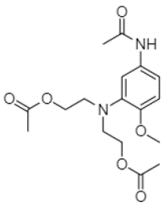
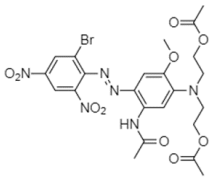
|---|---|---|---|
|  **4'-Aminoacetanilide** | Moderate Irritant |  **Disperse Yellow 27** | Irritating |
|  **p-Acetanisidide, 3'-amino -** | Moderate Irritant |  **Disperse Blue 823** | Corrosive |
|  **3-Pyridinecarbonitrile,1,2-dihydro-6-hydroxy-1,4-dimethyl-2-oxo** | Moderate Irritant |  **Disperse Yellow 241** | Not irritating or corrosive to the skin |
|  **2-cyano-4-nitroaniline** | Moderate Irritant |  **Red BS P/C** | Moderate Irritant |
|  **N, N-Dihydroxyethyl-3-amino-4-anisidine** | Non-Irritating |  **Disperse Blue 79** | Irritating |

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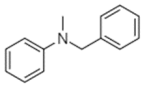
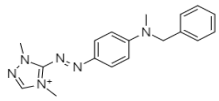
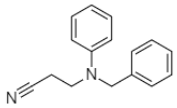
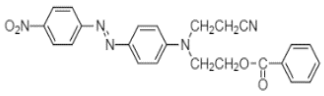
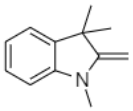
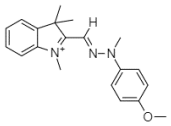
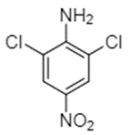
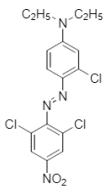
|---|---|---|---|
|  | Irritating |  | Corrosive |
| **N-Benzyl, N-Methylaniline** | | **Basic Red 46** | |
|  | Moderate Irritant |  | Moderate Irritant |
| **N-Cyano Ethyl N-Benzyl Aniline** | | **Orange 73-1** | |
|  | Moderate Irritant |  | Irritant to skin |
| **1,3,3-Trimethyl-2-Methyleneindoline** | | **Basic Yellow 28** | |

Some approaches to eye irritation assessments have been adopted by international organizations to reduce the need for animal testing. Quantitative structure activity relationship (QSAR) analysis is one such approach and allows to estimate the magnitude of a particular feature, such as an eye irritation score, by correlating one or more physicochemical and/or structural parameters of a molecule [16]. The Eye irritation test calculation results of azo dyes and their raw materials are shown in Table 2.

**Table 2.** Eye irritation calculation results of azo dyes and their raw materials

| Raw Material | Eye Irritation | Azo Dye | Eye Irritation |
|---|---|---|---|
|  | Irritant or Corrosive to Eyes |  | Non-Irritating or Corrosive to Eyes |
| **2,6-dichloro-4-nitroaniline** | | **Disperse Brown 27-1** | |

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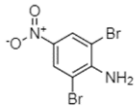
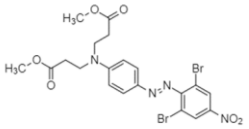
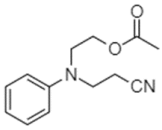
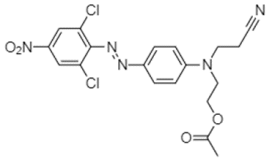
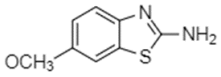
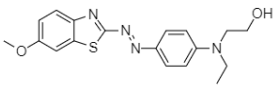
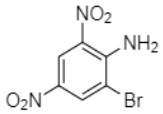
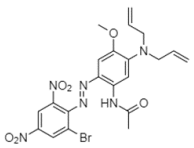
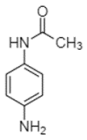
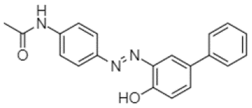
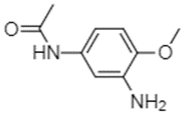
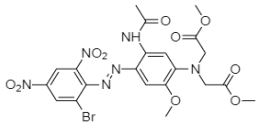
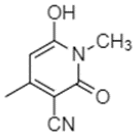
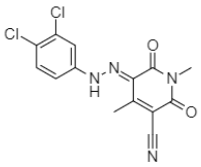
|---|---|---|---|
|  **2,6-dibromo-4-nitro aniline** | Unknown |  **Disperse Brown 19** | Non-Irritating or Corrosive to Eyes |
|  **N-Cyanoethylacetoxyethylaniline** | Irritant or Corrosive to Eyes |  **Disperse Orange 30** | Not Corrosive to Eyes |
|  **6-Methoxy Benzothiazole-2-ylamine** | Non-Irritating or Corrosive to Eyes |  **Red BS P/C** | Non-Irritating or Corrosive to Eyes |
|  **2-bromo-4,6-dinitroaniline** | Unknown |  **Disperse Blue 291** | Non-Irritating or Corrosive to Eyes |
|  **4'-Aminoacetanilide** | Irritant or Corrosive to Eyes |  **Disperse Yellow 27** | Unknown |
|  **p-Acetanisidide, 3'-amino -** | Irritant or Corrosive to Eyes |  **Disperse Blue 823** | Not Corrosive to Eyes |
|  **3-Pyridinecarbonitrile,1,2-dihydro-6-hydroxy-1,4-dimethyl-2-oxo** | Irritant or Corrosive to Eyes |  **Disperse Yellow 241** | Unknown |

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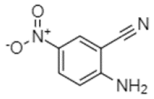
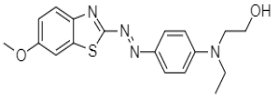
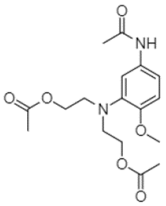
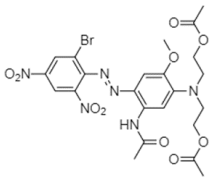
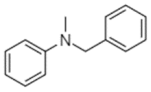
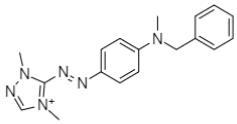
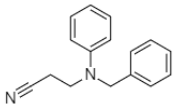
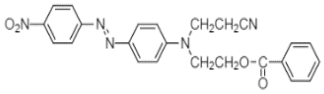
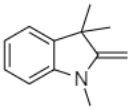
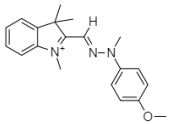
| | | | |
|---|---|---|---|
|  **2-Cyano-4-nitroaniline** | Irritant or Corrosive to Eyes |  **Red BS P/C** | Non-Irritating or Corrosive to Eyes |
|  **N, N-Dihydroxyethyl-3-amino-4-anisidine** | Unknown |  **Disperse Blue 79** | Non-Irritating or Corrosive to Eyes |
|  **N-Benzyl, N-Methylaniline** | Unknown |  **Basic Red 46** | Non-Irritating or Corrosive to Eyes |
|  **N-Cyano Ethyl N-Benzyl Aniline** | Unknown |  **Orange 73-1** | Non-Irritating or Corrosive to Eyes |
|  **1,3,3-Trimethyl-2-Methyleneindoline** | Unknown |  **Basic Yellow 28** | Unknown |

Many chemicals cause adverse effects in contact with the skin; The hazard-related phenomenon includes skin sensitization, skin penetration, and skin irritation. Each of these phenomena has mostly been studied independently, although there are links between them. In these conditions, theoretical calculation methods emerge as a practical solution for the evaluation of items without experimental data [16]. The Skin sensitization test calculation results of azo dyes and their raw materials are shown in Table 3.

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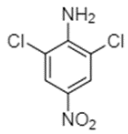
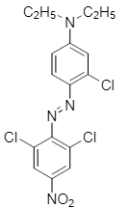
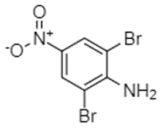
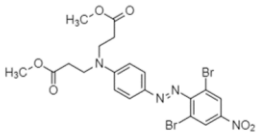
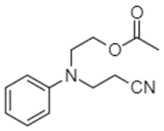
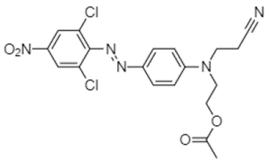
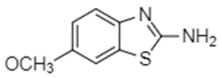
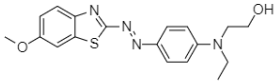
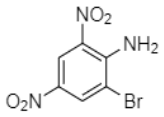
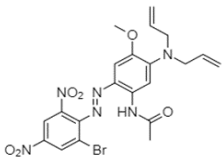
**Table 3.** Skin sensitization calculation results of azo dyes and their raw materials

| Raw Material | Skin Sensitization | Azo Dye | Skin Sensitization |
|---|---|---|---|
|  | Positive |  | Positive |
| **2,6-dichloro-4-nitroaniline** | | **Disperse Brown 27-1** | |
|  | Positive |  | Positive |
| **2,6-dibromo-4-nitro aniline** | | **Disperse Brown 19** | |
|  | Positive |  | Positive |
| **N-Cyanoethylacetoxyethylaniline** | | **Disperse Orange 30** | |
|  | Negative |  | Positive |
| **6-Methoxy Benzothiazole-2-ylamine** | | **Red BS P/C** | |
|  | Positive |  | Positive |
| **2-bromo-4,6-dinitroaniline** | | **Disperse Blue 291** | |

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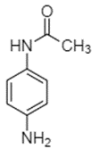
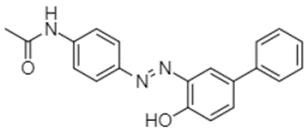
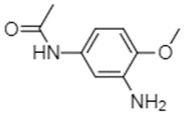
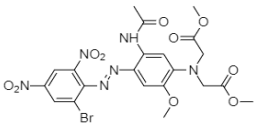
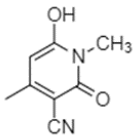
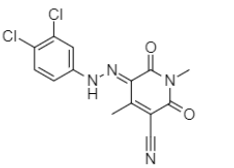
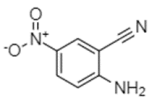
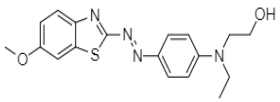
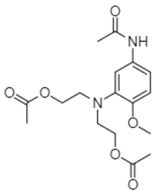
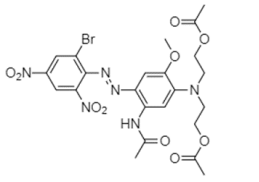
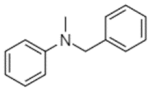
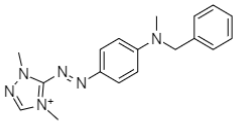
| | | | |
|---|---|---|---|
|  | Positive |  | Positive |
| **4'-Aminoacetanilide** | | **Disperse Yellow 27** | |
|  | Positive |  | Positive |
| **p-Acetanisidide, 3'-amino -** | | **Disperse Blue 823** | |
|  | Positive |  | Positive |
| **3-Pyridinecarbonitrile,1,2-dihydro-6-hydroxy-1,4-dimethyl-2-oxo** | | **Disperse Yellow 241** | |
|  | Negative |  | Positive |
| **2-cyano-4-nitroaniline** | | **Red BS P/C** | |
|  | Positive |  | Positive |
| **N, N-Dihydroxyethyl-3-amino-4-anisidine** | | **Disperse Blue 79** | |
|  | Positive |  | Positive |
| **N-Benzyl, N-Methylaniline** | | **Basic Red 46** | |

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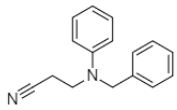
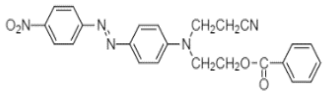
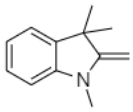
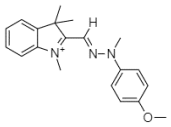
| | | | |
|---|---|---|---|
|  | Negative |  | Positive |
| **N-Cyano Ethyl N-Benzyl Aniline** | | **Orange 73-1** | |
|  | Positive |  | Positive |
| **1,3,3-Trimethyl-2-Methyleneindoline** | | **Basic Yellow 28** | |

Most of the chemical substances have genotoxic and carcinogenic effects that cause changes in the hereditary structure of living things. These chemicals, which have a direct or indirect carcinogenic effect, can also be considered mutagenic. It is thought that 90% of carcinogens are mutagens. Chemical substances known to be mutagenic are known to cause birth defects, heart diseases, and aging, but it is thought that they induce the formation of cancer and cause reproductive disorders by damaging some cell lines [17]. To detect premutagenic/precancerous substances that require metabolic activation with this test, liver microsomes are prepared and metabolic activations of chemicals are determined. Microsomal enzyme extract (S9 Fraction) is used to investigate whether the metabolic products of the test substance are mutagenic [18]. According to a study conducted in 2019, it was seen that phenolic compounds have important antimutagenic properties. The most important feature of many phenolic compounds is that they are known to have antioxidant effects; In the related study, it was thought that the antimutagenic activity observed was due to the antioxidant properties of the studied substances [19]. Furthermore, according to studies conducted in recent years, it has been found to affect mutagenicity at high concentrations [20-21]. The Mutagenicity calculation results in the presence and absence of the S9 fraction of azo dyes and their raw materials are shown in Table 4.

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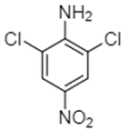
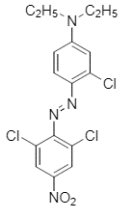
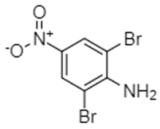
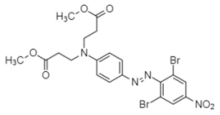
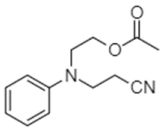
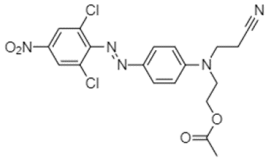
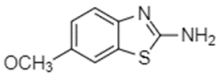
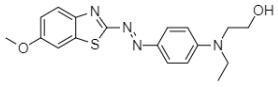
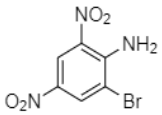
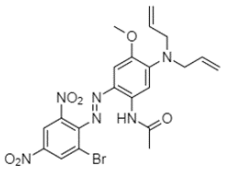
**Table 4.** Mutagenicity calculation results in the presence and absence of the S9 fraction of azo dyes and their raw materials

| Raw Material | With S9 | Without S9 | Azo Dye | With S9 | Without S9 |
|---|---|---|---|---|---|
|  **2,6-dichloro-4-nitroaniline** | Negative | Negative |  **Disperse Brown 27-1** | Positive | Negative |
|  **2,6-dibromo-4-nitro aniline** | Positive | Positive |  **Disperse Brown 19** | Positive | Positive |
|  **N-Cyanoethylacetoxyethylaniline** | Negative | Unknown |  **Disperse Orange 30** | Negative | Negative |
|  **6-Methoxy Benzothiazole-2-ylamine** | Positive | Negative |  **Red BS P/C** | Negative | Negative |
|  **2-bromo-4,6-dinitroaniline** | Positive | Positive |  **Disperse Blue 291** | Positive | Negative |

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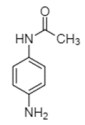
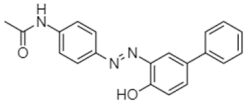
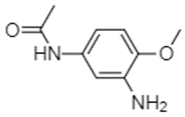
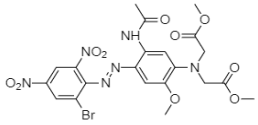
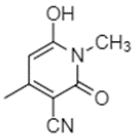
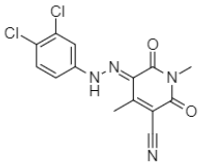
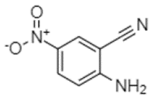
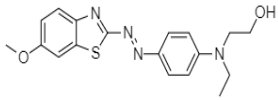
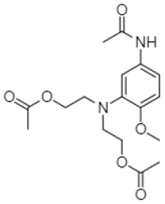
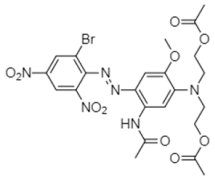
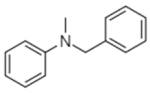
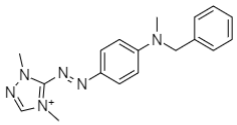
| | | | | | |
|---|---|---|---|---|---|
|  **4'-Aminoacetanilide** | Negative | Negative |  **Disperse Yellow 27** | Negative | Negative |
|  **p-Acetanisidide, 3'-amino -** | Positive | Negative |  **Disperse Blue 823** | Negative | Negative |
|  **3-Pyridinecarbonitrile,1,2-dihydro-6-hydroxy-1,4-dimethyl-2-oxo** | Negative | Negative |  **Disperse Yellow 241** | Negative | Positive |
|  **2-cyano-4-nitroaniline** | Negative | Positive |  **Red BS P/C** | Negative | Negative |
|  **N, N-Dihydroxyethyl-3-amino-4-anisidine** | Negative | Negative |  **Disperse Blue 79** | Negative | Negative |
|  **N-Benzyl, N-Methylaniline** | Negative | Negative |  **Basic Red 46** | Negative | Negative |

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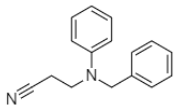
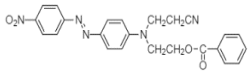
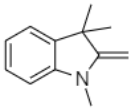
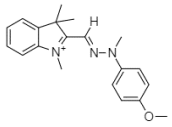
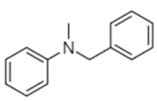
| | | | | | |
|---|---|---|---|---|---|
|  **N-Cyano Ethyl N-Benzyl Aniline** | Negative | Negative |  **Orange 73-1** | Negative | Negative |
|  **1,3,3-Trimethyl-2-Methyleneindoline** | Negative | Negative |  **Basic Yellow 28** | Negative | Positive |

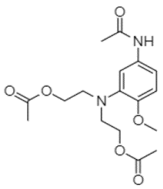
## 3. RESULT AND DISCUSSION

In this study, skin irritation/corrosion, eye irritation, skin sensitization, and mutagenicity tests were performed with OECD QSAR management to examine the toxicological properties. When azo dyes and their materials were examined in the skin irritation/corrosion test, they were generally found to be moderate irritants.

Among the 13 raw materials examined, N, N-Dihydroxyethyl-3-amino-4-anisidine was found to be non-irritating. Contrary to this raw material, N-Benzyl, N-Methylaniline was found to be irritating (Fig. 1.).



**N-Benzyl, N-Methylaniline**



**N, N-Dihydroxyethyl-3-amino-4-anisidine**

**Figure 1.** Molecular structures of N-Benzyl N-Methylaniline and N, N-Dihydroxyethyl-3-amino-4-anisidine

When azo dyes were examined, Disperse Blue 79 and Disperse Yellow 27 azo dyes showed irritating properties. Disperse Blue 823 and Basic Red 46 azo dyes were found to be corrosive. Another remarkable result of the skin irritation/corrosion study is that while the Disperse Yellow

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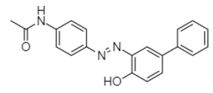
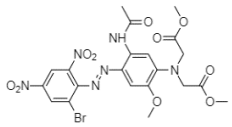
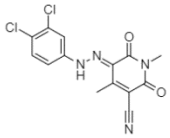
241 azo dye does not show any irritating properties, its raw material, 3-pyridinecarbonitrile, 1,2-dihydro-6-hydroxy-1,4-dimethyl-2-oxo aromatic amine is highly irritating (Fig. 2.)



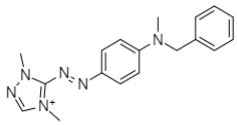
**Disperse Yellow 27**



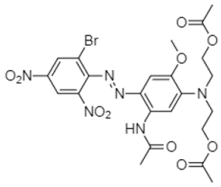
**Disperse Blue 823**



**Disperse Yellow 241**



**Basic Red 46**



**Disperse Blue 79**

**Figure 2.** Molecular structures of Disperse Yellow 27, Disperse Bue 823, Disperse Yellow 241, Disperse Blue 79, and Basic Red 46

It was found that the raw materials studied generally exhibited eye irritant properties, however the products did not exhibit irritating features, when the findings of eye irritation, another toxicological examination, were evaluated.

When 13 raw materials and 12 azo dyes are examined with the OECD QSAR method in terms of skin sensitization, it can be mentioned that aromatic amines have skin sensitizing properties. Studies have shown that 6-methoxybenzothiazole-2-ylamin, 2-cyano-4-nitroaniline, and N-cyano ethyl N-benzyl aniline aromatic amine raw materials do not have skin sensitizing properties, but when they are converted into Red BS P/C and Orange 73-1 products, exhibit sensitizing properties (Fig.3.)

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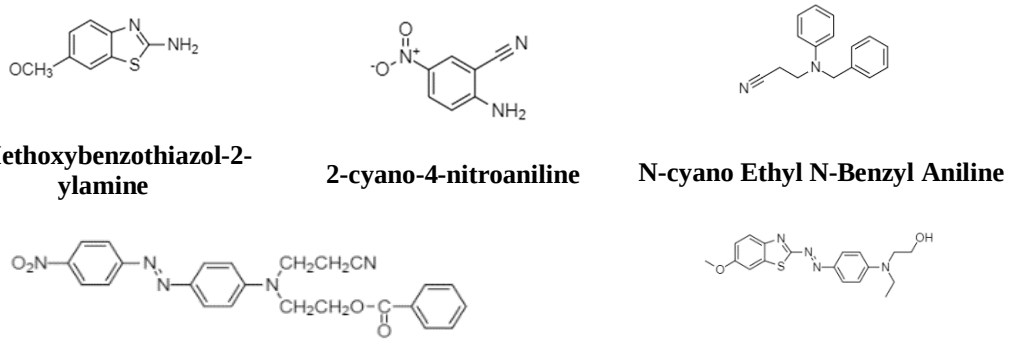
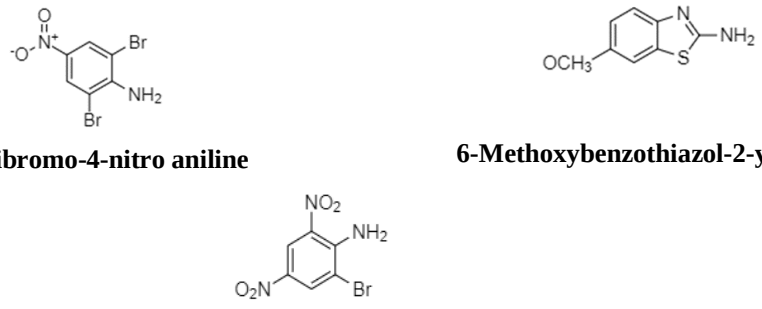



**6-Methoxybenzothiazol-2-ylamine** **2-cyano-4-nitroaniline** **N-cyano Ethyl N-Benzyl Aniline**

**Orange 73-1** **Red BS P/C**

**Figure 3.** Molecular structures of 6-Methoxybenzothiazol-2-ylamine, 2-cyano-4-nitroaniline, N-cyano Ethyl N-Benzyl Aniline, Red BS P/C and Orange 73-1

In the last study to investigate the toxicological effects of azo dyes and their raw materials, the mutagenicity test was calculated in the presence and absence of the S9 fraction. Fraction S9 defines the microsomal enzyme extract. Mutagen can be defined as a physical or chemical factor that changes the molecular structure of cellular information and management chains such as DNA or RNA of biological living organisms, causing the organism in question to naturally mutate at a much higher level than expected [18]. Once raw materials' mutagenic potential was investigated, it was found that they generally exhibited no mutagenic characteristics. Calculations revealed that 2,6-dibromo-4-nitro aniline, 6-methoxybenzothiazol-2-ylamine and 2-Bromo-4,6-dinotroaniline compounds from 13 aromatic amines, which are raw materials, are mutagenic (Fig. 4.)




**2,6-dibromo-4-nitro aniline** **6-Methoxybenzothiazol-2-ylamine**

**2-bromo-4,6-dinitroaniline**

**Figure 4.** Molecular structures of 2.6-dibromo-4-nitro aniline, 6-Methoxybenzothiazol-2-ylamine and 2-Bromo-4,6-dinitroaniline

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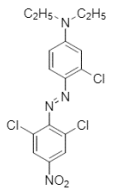
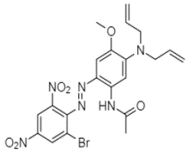
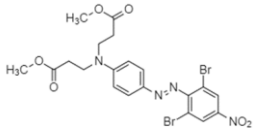
It has been observed that the dyes Disperse Brown 27-1, Disperse Brown 19, and Disperse Blue 291 have mutagenic effects (Fig. 5.)



**Disperse Brown 27-1**



**Disperse Blue 291**



**Disperse Brown 19**

**Figure 5.** Molecular structures of Disperse Brown 27-1, Disperse Brown 19 and Disperse Blue 291

## 4. CONCLUSION

In this study, skin irritation/corrosion, eye irritation, skin sensitization, and mutagenicity tests were performed with OECD QSAR management to examine the toxicological properties. Among the 13 raw materials examined, N, N-Dihydroxyethyl-3-amino-4-anisidine was found to be non-irritating. Contrary to this raw material, N-Benzyl, N-Methylaniline was found to be irritating. It can be said that the ratio of the benzene ring in the compound affects the skin irritation feature. When skin irritating results are taken into account, it can be said that the presence of the ester group increases the irritating feature, as well as the importance of the benzene ring in skin irritating properties. Another remarkable result of the skin irritation/corrosion study is that the presence aromatic amine increased irritating properties. Attention can be drawn to the effect of the presence of the amine double bond, which is characteristic of azo dyes, between the benzene rings. It can be mentioned that the absence of the double bond between the amines affects reducing the irritant feature.

When the results of eye irritation, it can be said that contrary to the skin irritant feature, the increase in the benzene ratio and the double bonds in the amines between the benzenes reduce the eye irritant

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feature. In terms of skin sensitization, it can be concluded that the presence of hydroxyl groups in azo dyes and the rise in the proportion of amine-linked methyl groups in the benzene ring increase the skin sensitivity feature.

When the mutagenic effect of raw materials was examined, it can be said that the nitro and amine groups attached to the benzene ring, as well as the bromine molecule attached to the benzene ring, increase the mutagenic effect. Furthermore, it was observed that the presence of nitro and amine groups, as well as the presence of electronegative groups such as chlorine and bromine, increased the mutagenic effect.

## 5. ACKNOWLEDGEMENT

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