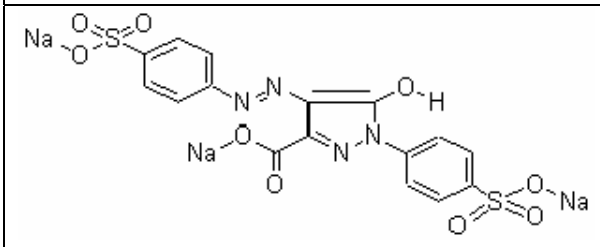
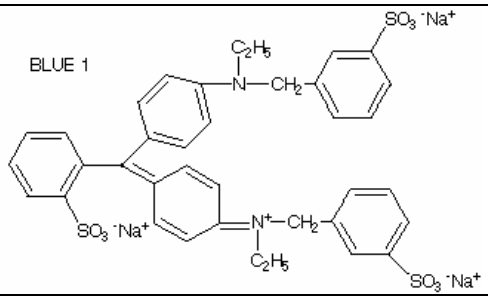
# ECOLOGICAL RISK ASSESSMENT

**Re-registration**

"Acid Blue 9"(Erioglaucine) and "Acid Yellow 23" (Tartrazine ) Dyes Used together in the End-use Products Aquashade, Aquashade OA, Admiral Liquid, Admiral WSP and Pond Care AlgaBlocker for Control of Algal Growth and Other Undesirable aquatic Plants

| Acid Yellow 23: | Acid Blue 9 (trisodium salt) |
|---|---|
| PC Code Number:110302<br>CAS Registry No.:1934-21-0 | PC Code Number: 110301<br>CAS Registry No.:<br>Trisodium salt: 2650-18-2<br>Triammonium salt: 3844-45-9<br>FD&C Blue 1<br>In theTriammonium salt. the counter cation is ammonium instead of sodium. The nature of the counter cation is not relevant because it is the anion what is responsible for color of the dyes |
|  |  |

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## I. **Executive Summary**

### A. Nature of the Chemical Stressor

The chemical stressors are the dyes Acid Yellow 23 and Acid Blue 9, which are used together in several end-use products intended to control algae and other undesirable aquatic plants in artificial, confined water bodies (ornamental ponds, golf course lakes, fountains; hatcheries; swimming pools). The dyes block sunlight energy necessary for algal growth and, at the same time, impart a greenish blue coloration to the water. The two dyes are also regulated under FIFRA as "inerts" used as colorants in some pesticide formulations. In addition, they are regulated under the Food, Drug, and Cosmetic Act as colorants for food (food additives), drug formulations, and cosmetics[1].

The specific end-use products containing Acid Blue 9 and Acid Yellow 23 are: (a) Pond Care Algae Blocker (2.36% Acid Blue 9 and 0.24% Acid Yellow 23; EPA Reg. No. 8709-6); (b) Aquashade (23.6% Acid Blue 9 and 2.39% Acid Yellow 23, EPA Reg. No. 3306-1); (c) Aquashade OA (2.36% Acid Blue 9 and 0.24% Acid Yellow 23; EPA Reg. No. 33068-2); (d) Admiral Liquid (15.31% Acid Blue 9 and 1.00 % Acid Yellow 23; EPA Reg. No. 67064-2); (e) Admiral WSP (49.72% Acid Blue 9 and 3.27% Acid Yellow 23; EPA Reg. No. 67064-1). Except for Admiral WSP, all the products are liquid formulations.

The Agency had assigned the PC 110301 to Acid Blue 9, 110302 to Acid Yellow 23, and 110303 to Aquashade, the mixture of 23.6% Acid Blue 9 and 2.39% Acid Yellow 23 (an end-use product). In this document, the names Acid Blue 9 and Acid Yellow 23 were used when the percentage of each dye was not specified or when individual dyes were described separately. "Aquashade" was used when referring to the test substance used in the ecological toxicity studies.

### B. Potential Risks to Non-target Organisms

Based on available toxicity data and the exposures expected when used according to the label, the Environmental Fate and Effects Division (EFED) does not believe that "Aquashade" and other products containing Acid Blue 9 and Acid Yellow 23 [2]

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[1] The specific names used for "Acid Blue 9"under the FDCA are "FD&C Blue No.1" for the trisodium salt and "D&C" "Blue No.4" for the triammonium salt (also known as erioglaucine). Both are salts of the same anion. Acid Yellow 23 is FD&C "Yellows", also known as Tartrazine**.**

[2] The ecological toxicity studies were conducted with an Aquashade end-use product containing 23.6% Acid Blue 9 and 2.365 Acid Yellow 23. The PC Number assigned to the product is 110303, which has created some confusion because this product is a combination of two dyes, each with and assigned PC Number,

pose a direct acute risk to freshwater fish and invertebrates, birds, amphibians, reptiles or mammals, including endangered species. These dyes are not expected to harm terrestrial animals that drink treated water. Note that even though we do not have toxicity data on amphibians and reptiles, aquatic phase amphibians are represented by fish, and reptiles and terrestrial phase amphibians are represented by birds. It is possible that endangered terrestrial animals may be affected indirectly by loss of food in water bodies where treatment occurs. The extremely limited area in which this may occur, given the label-specified water bodies and the method of application (i.e. by hand) may allow a further analysis to reduce this apparent possibility to be almost nonexistent. However, this analysis has not been completed.

Aquashade is an aquatic herbicide and will kill algae and nontarget submerged aquatic plants. If any treated habitats contained endangered aquatic plants at the time of application, effects are possible. Exposure to emerged plants and terrestrial plants is assumed to be low, resulting in little or no risk.

The Environmental Fate and Effects Division is unable to assess the potential chronic risk to animals due to lack of data and therefore cannot conclude that potential chronic risk to these taxa (and the taxa for which they are surrogates) does not exist. However, because of its low acute toxicity, mode of action which is not toxicological, and because Acid Yellow 23 dye is shown not to be chronically toxic to mammals, EFED does not believe that chronic effects are likely and therefore, chronic studies are not needed.

C. Conclusions- Exposure Characterization

*Status of Data Requirements*

All of the environmental fate data requirements were placed in "Reserved" in 1993, depending on the results of the required ecological toxicity studies. Because the risk assessment did not identify risks to fish, aquatic invertebrates, or mammals, the environmental fate studies may be "Waived". The environmental fate in this assessment is qualitative, based mostly on data from the open literature on structurally related dyes. Although structure-activity relationship (SAR) estimates using EPI Suite/EPIWIN[3] were performed, these programs do not adequately estimate physical and chemical properties of salts, particularly those of a large anion such as in Acid Blue 9 and Acid Yellow 23. For this reason, the qualitative assessment relies on open literature information.

No environmental fate data from Subdivision N guideline studies are required for the present uses. If new uses and/or changes in rate or method of application or products are proposed, the need for new data will be reevaluated.

[3] EPI (Estimation Programs Interface) Suite™ (formerly known as EPIWIN)

*Exposure in the Environment*

Unlike the uses on food, drugs and cosmetics, the dyes are exposed to an open (but contained) aquatic environment when used as herbicides. Because the concentrated products are added **directly** to a water body, the dyes (which do not react chemically with each other) become diluted in the treated water body. Neither runoff nor spray drift are routes of exposure because a specified amount of product is directly applied to the water body to attain recommended target concentrations of the product of either "1 ppm or 2 ppm", depending on the weed to be controlled. To attain these target concentrations, the labels specify the volume of product to be added per volume of water to be treated. These maximum, target concentrations were assumed to be maintained after treatment. That is, no degradation was assumed.

The major route of dissipation of the dyes in an aquatic environment is likely indirect photolysis, which depends of on the nature and concentration of natural photosensitizers as well as on the geographical location where and season when the products are used. However, biotransformation under anaerobic conditions may also contribute to the dissipation of each dye. The specific chemical nature of photoproducts and metabolites is not known.

The dyes are predominantly associated with the water column and have no potential to volatilize from water. Although the dyes are not applied to soils, they are unlikely to volatilize from soils. Acid Blue 9 and Acid Yellow 23 do not have the potential to bioaccumulate in fish.

D. Conclusions- Effects Characterization

Aquashade is a formulation that contains, as its active ingredients, a yellow and a blue dye. These dyes, Acid Yellow 23 and Acid Blue 9 are also used as food drug and cosmetic colorants for human consumption. Their mode of herbicidal action is not toxicological, therefore little toxicity is expected. The toxicity information available to characterize the toxicity to wildlife includes testing with formulations such as Aquashade and Admiral WSP and also testing with the individual dyes. Data used to characterize effects include studies conducted by a registrant (testing with Aquashade and Admiral WSP) and studies reported in published literature. Published literature were obtained by the Office of Research and Development's (ORD) Middle Ecological Division in Duluth through their literature searched conducted as part of the ECOTOX program. All testing indicates that these dyes, whether alone, or in formulations cause no toxicity to fish, aquatic invertebrates, birds or mammals at doses and concentrations far above those expected in the environment. No studies were required or found that tested the dyes or formulations on aquatic or terrestrial plants. In as much as this is a control agent for submerged aquatic vegetation and algae, it is assumed to have an adverse effect on aquatic plants. The label also warns that if it gets on emergent vegetation, some burning will occur. This indicates that these dyes and formulations may be toxic to terrestrial plants. However, the method of application would preclude exposure to terrestrial plants, so toxicity to this group of organisms is not considered necessary. If treated waters are used for irrigation, exposure to terrestrial plants may be possible and toxicity testing for terrestrial plants would be needed to assess risk.

E. Uncertainties and Data Gaps

1. Exposure

Exposure to the dyes were based on the target dilution concentration of a directly applied volume of product to a volume of water body to attain the concentrations specified in the labels. (1 or 2 ppm; 1 or 2 mg/L). Thus, the exposure concentrations in water are the same as the target concentrations in the labels. It is assumed that the dyes are stable in the water and that the target concentration remains constant. That is, routes and rates of dissipation were not taken into account, as data are not available.

Indirect photolysis has been identified as the major route of dissipation of the dyes in aquatic systems. However, the specific nature of transformation products for Acid Blue 9 and Acid Yellow 23 is not known. Although no kinetics data are available to assess how fast each dye photodegrades, the geographical location, season, and nature/concentration of natural photosensitizers would control the rate of photolysis. Therefore, an uncertainty exists on the chemical nature and concentration of photoproducts. In addition, anaerobic biotransformation may also be a route of dissipation, but the chemical identity of the metabolites specific to each dye is not known.

*Purity of the Dyes*

Three major issues have been identified that are associated with the purity of the dyes and/or test substance used in the ecological toxicity studies.

a. The experimental characterization of the physical and chemical properties required under FIFRA was not apparently done with a 100% pure dye. The Acid Blue 9 is reported to be 50% pure (43503401) and Acid Yellow 23 as being 28% pure (43503402). Although the presence of chemical impurities affect the physical and chemical properties of a chemical substance, the extent by which they affect those of the dyes is unknown. Some of the physical and chemical properties reported in these studies are not consistent with those expected for dyes and these differences may be related to the unspecified impurities. The high vapor pressure reported in the studies may be that of a volatile impurity.

b. The above studies were submitted in support of Aquashade (23.6% Acid Blue 9 and 2.39 Acid Yellow 2.39% registration It is unclear if this percent composition take into account the purity of each dye. In addition, it is not known if the impurities in the test substances used in the characterization of physical and chemical properties are the same as those declared as impurities in the technical and/or end-use product (Confidential Business Information). It is unclear if the purity of the dyes in other products is the same as in Aquashade.

c. The Aquashade product that was used in the “basic six” studies was labeled as containing 23.63 % Acid Blue 9 and 2.39% Acid Yellow 23. The study authors stated that the test substance characterization provided by the sponsor indicated a purity of 13.9% “azure blue dye”. The use of the term “azure” is unclear, as it could describe a color or a series of structurally

related “azure dyes”. These azure dyes are not structurally related to Acid Blue 9 or Acid Yellow 23. Therefore the exact composition and purity of the toxicant that was used in the studies is not certain. The registrant must clarify what is meant by “azure blue dye”.

2. Effects

EFED has toxicity data for both formulations containing the yellow and blue dye, plus testing with the dyes separately. Tests with the Aquashade product containing 23.6% Acid Blue 9 and 2.36% Acid Yellow 23 as the test substance were conducted on birds, fish and aquatic invertebrates. The Health Effects Division (HED) has a rat study conducted with Admiral WSP, which showed that the $LD_{50}$ was equal or higher than 5,000 mg/kg. There are also mammal acute toxicity data with both the blue and yellow dyes also indicating low toxicity.

No chronic studies using aquashade or other formulations with animals have been submitted, so it is not known for certain what would happen over an extended exposure period. However, there is mammal chronic testing with the Acid Yellow 23 dye which indicates it does not have chronic effects on mammals. Furthermore, these dyes are used as colorants in human foods drugs and cosmetics, so long term effects from either the yellow or blue dye to mammals are not expected. While directly correlating lack of long-term mammal toxicity to birds is uncertain, given the mode of herbicidal action and low level of exposure it is considered unlikely that long-term exposures would have adverse effects on birds. The effects of chemical impurities in the dyes is not known.

No studies on aquatic plants have been required, because they are the target of Aquashade and other end-use products and it is assumed that all submerged aquatic plants in the treated pond will be killed. No terrestrial plant studies have been required, because there is no expected exposure unless treated water are used for irrigation.

Because of the mode of action (blocking sunlight energy that enters bodies of water) and the low acute toxicity of this pesticide, it is believed that additional ecological toxicity studies are not needed unless new uses, methods of applications or new products are proposed.

## II. Problem Formulation

### A. Stressor Source and Distribution

#### 1. Source and Identity of the Stressor

Pond Care Algae Blocker, Aquashade, and Admiral are trade names for products containing the two dyes[4] Acid Blue 9 and Acid Yellow 23 which do not react chemically with each other. Each product has a different ratio of the dyes (see below), but in all of the product formulations the percent of Acid Blue 9 is higher than that of Acid Yellow 23. The use pattern of the products are essentially the same in the sense that all of the products are applied **directly** (as a volume of product or number of packets) to artificial (or natural), confined water bodies, including swimming pools (provided that chlorine disinfectants are not used). Minimal or no outflow are specified in the labels because the mode of action is such that allowing treated water to outflow would eliminate the effectiveness of the products. These dyes must remain in the water column and block the sunlight energy for a sufficient time to inhibit photosynthesis and cause the plants to die. They are not intended for use in non-confined natural water bodies, drinking water sources, or artificial water bodies with outflow. Use of the treated water for irrigation is not precluded on the label, and this is an uncertainty because irrigation could result in exposure to nontarget terrestrial plants, and there are no tests with terrestrial plants.

The specific names of the end-use products containing Acid Blue 9 and Acid Yellow 23 as the active ingredients are AlgaeBlocker and Aquashed OA (both 2.36% Acid Blue 9 and 0.24% Acid Yellow 23), Aquashade (23.63% Acid Blue 9 and 2.39% Acid Yellow 23), Admiral Liquid (15.31% Acid Blue 9 and 1.00% Acid Yellow23), and Admiral WSP (49.72% Acid Blue 9 and 3.27% Acid Yellow 23). Additional information on these end-use products is presented within the "Use Characterization" section.

The dyes Acid Blue 9 and Acid Yellow 23 are known by several different names, which may cause confusion about the chemical identity of the dye. For this reason, each one is also identified by the chemical name used by the Office of Pesticide Programs, the Chemical Abstracts Registry Number, and their synonyms (Table 1). "Acid Blue 9" could refer to either the trisodium and triammonium salts, each one having a different Chemical Abstracts Registry Number. In most of the product labels, the blue dye is identified as "Acid Blue 9" alone, without indicating which salt is used in the formulation. Throughout the document, the names "Acid Blue 9" and "Acid Yellow 23" are used, as they are identified as such in the product labels. In the Admiral products, they are identified as "tartrazine" for the yellow dye and as "erioglaucine" for the blue dye. The nature of the counter cation for Acid Blue 9 in the products is unclear, although the Color Index International identifies it as the triammonium salt. It is likely that the blue dye used in the products is the triammonium salt. Further information on the chemical identity of the dyes are included in Table 2. However, because it is the anion of the blue dye what exerts herbicidal activity by blocking sunlight energy, the identity of the counter cation is not relevant to the exposure and risk assessment when used as herbicides.

[4] A dye is defined as a chemical compound that is capable of imparting color and that is soluble in the vehicle in which it is applied.

In addition to being regulated under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), the two dyes are regulated by the Food and Drugs Administration (FDA) under the Food, Drugs, and Cosmetics Act (FDCA) as color additives (colorants) in food, drugs and cosmetics . Acid Yellow 23 (FD&C Yellow 5) is a food, drug, and cosmetics colorant. The trisodium salt of Acid Blue 9 is a FD&C colorant under the name "Blue 1", whereas the triammonium salt (Blue 4; erioglaucine) is a colorant in drugs and cosmetics (D&C). Use on foods, drugs, and cosmetics require batch[5] certification prior to use. While the nature of the counter cation is important for regulation under FDCA, under FIFRA the pesticide active species is the anion. It is the anion what gives color to the treated water. Therefore, for the use of these blue dyes as an algicide and aquatic herbicide for submerged plants, the nature of the counter cation is irrelevant. While the use of the dyes in food, drug formulations and cosmetics require a certification of their purity, the purity of the dyes used as an algicide/ aquatic herbicide is unclear.

Acid Blue 9 and Acid Yellow 23 are also regulated under FIFRA as "Inerts" used to impart color to pesticide formulations. On December 21, 2004 the Agency completed the "Reassessment of the Exemptions from the Requirement of a Tolerance for the FDA-Certified FD&C Additives Blue No.1 [Acid Blue 9]; erioglaucine] and Yellow 5 [Acid Yellow 23; tartrazine]" [6]. This reassessment was based on structure-activity relationships (EPISuite) and does not include a discussion of transformation products.. However, physical and chemical properties and environmental fate estimates using EPIWIN are uncertain because salts of large anions are not adequately handled by EPIWIN.

The Society of Dyers and Colourists and the American Association of Textile Chemists and Colorists have developed a Color Index International[7] standard for identification of pigments and dyes. The Color Index classifies dyes and pigments by their composition. Individual pigment or dye are identified by a unique Color Index Generic Name (C.I. Name) and a Color Index Constitution Number (C.I. Number), although the C.I. Generic Name is most commonly used..

Expanded listings of other names used for Acid Yellow 23 and Acid Blue 9 can be found from the Unilever Center for Molecular Informatics, Cambridge University,

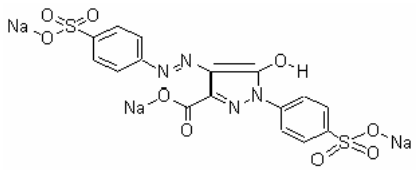
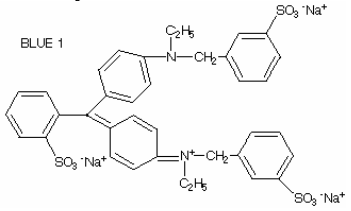
[5] "Batch" is defined as "an homogeneous lot of color additive or color additive mixture produced by an identified production operation, which is set apart and held as a unit for the purpose of obtaining certification of such quantity". The "Batch Number" is the number assigned to a batch of colorant by the person who requests certification of the batch. A "Lot Number" is the identifying number or symbol assigned by the FDA to a batch of color additive after certification. Note than only the trisodium salt (Blue 1) can be used in food.

[6] "Reassessment of the Exemption from the Requirement of a Tolerance for the FDA-Certified Color Additives FD&C Blue No.1, FD&C Red No. 40, and FD&C Yellow No. 5 (Tartrazine)". U.S. Environmental Protection Agency, Office of Pesticides Programs. December 21, 2004.

[7] http://www.colour-index.org/

Acid Blue 9: http://www.dspace.cam.ac.uk/handle/1810/5713
Acid Yellow 23: http://www.dspace.cam.ac.uk/handle/1810/6131

Table 1 Chemical Nomenclature and Structure of the Dyes

| | Acid Yellow 23 | Acid Blue 9 |
|---|---|---|
| Common Name as Appearing in the labels of the Admiral Products | Tartrazine | Erioglaucine (diammonium salt) |
| Chemical Name(s) | 4,5-Dihydro-5-oxo-1-(4-sulfophenyl)-4-((4-sulfophenyl)azo)-1H-pyrazole-3-carboxylic acid trisodium salt<br><br>3-carboxy-5-hydroxy-1-p-sulfophenyl-4-p-sulfophenylazopyrazole trisodium salt<br><br>5-hydroxy-1-(p-sulfophenyl)-4-((p-sulfophenyl) azo) pyrazole-3-carboxylic acid trisodium salt | *N*-Ethyl-*N*-(4-[(4-{ethyl[(3-sulfophenyl)methyl]amino}phenyl)(2-sulfophenyl)methylene]-2,5-cyclohexadien-l-ylidene)-3-sulfobenzenemethanaminium hydroxide inner salt, **trisodium salt**<br>*N*-Ethyl-*N*-(4-[(4-{ethyl[(3-sulfophenyl)methyl]amino}phenyl)(2-sulfophenyl)methylene]-2,5-cyclohexadien-l-ylidene)-3-sulfobenzenemethanaminium hydroxide inner salt, **triammonium salt** |
| Molecular Structure |  | Only the structure of the disodium salt is represented. The diammonium salt has ammonium instead of sodium as the counter cation. It is the organic anion what is responsible for the color of the dye.<br>  |

| | | |
|---|---|---|
| Synonyms | FD & C Yellow No. 5:<br>21CFR74.705 (Foods)<br>21CFR74.1705 (Drugs)<br>21CFR74.2705 (Cosmetics)<br>These FD&C dyes are of high purity and require batch certification analysis when used in food, drugs, or cosmetics.<br><br>CI Food Yellow 4<br>CI Number 15985<br><br>CAS Registry No. 1934-21-0 | Sodium Salt:<br>Brilliant Blue<br>CI Food Blue 2<br>Erioglaucine trisodium salt<br>CI Food Blue 2.<br>CI Number 42090<br>CAS Registry No. 2650-18-2<br><br>Triammonium salt<br>Erioglaucine diammonium salt<br>Alphazurine<br>CI Blue 8<br>CI 42090<br>CAS Reg. No. 3844-54-9<br><br>FD&C Blue 1<br>21CFR74.101 (Foods)<br>21CFR74.1101 (Drugs)<br>21CFR74.2101 (Cosmetics)<br><br>D&C Blue 4<br>21CFR74.1104 (Drugs)<br>21CFR.74.2104 (Cosmetics) |
| Chemical Family | Azo (monoazo) dye; Contains one azo group, - N=N-) | Aminotriphenylmethane dye |

Other uses of the dyes include;
Acid Yellow 23- Dye for wool and silk; As indicator for chlorine estimations in biochemistry
Acid Blue 9- Biological stain; Textile dye; Wood stain; Indicator

## 2. Physical and Chemical Properties and Environmental Fate of the Stressor

*Physical and Chemical Properties*

The most distinct characteristic of dyes is that they absorb energy[8] strongly within the wavelength range of the visible spectrum (360 to 750 nm) and that they are highly soluble in the vehicle in which they are used, in this case water. Dyes are highly conjugated systems (i.e., multiple double bonds), for which multiple resonance structures can be written. These resonance structures cause shifting or appearance of absorption bands into the visible spectrum of electromagnetic radiation. Multiple resonance structures increase the stability of a molecule. The color of a dye or other colored materials is determined by the energy (wavelength) of maximum absorption (electronic absorption) of incident light by the molecule. The observed color is determined by the wavelength region in which the substance does not absorb light (i.e., the reflected color). In an "acid dye", the chromophore is part of a negative ion (anion)[9] . Therefore, the color is independent of the counter cation. Table 2 summarizes information on the light absorption characteristics of each dye.

[8] Light is energy. The color observed in dyes (or in gems) is caused by electronic transitions between energy levels of a molecule. The color-giving absorption band is known as a chromophore ("color bearer")

[9] Although Acid Blue 9 and Acid Yellow 23 belong to different chemical families, both dyes contain sulfonate groups.

Table 2- Electronic Absorption Spectra of Acid Yellow 23 and Acid Blue 9 in Water

| Electronic Absorption Spectra | Acid Yellow 23 | Acid Blue 9 |
|---|---|---|
| Wavelength of Absorption Maximum ($\lambda_{max}$, in nm) in water | 420- 430 range, $\lambda_{max}$, 425 | 620- 639 range; $\lambda_{max}$, 630 |
| Reflected Color | Yellow | Blue |

The physical and chemical properties of Acid Yellow 23 and Acid Blue 9 are summarized in Table 3.

Table 3- Physical and Chemical Properties of Acid Yellow 23 and Acid Blue 9 (Estimated); Intrinsic properties

| Information | Acid Yellow 23 | Acid Blue 9 |
|---|---|---|
| Empirical Formula | $C_{16}H_9N_4Na_3O_9S_2$ | $C_{37}H_{34}N_2Na_2\ O_9\ S_3$ (disodium salt)<br><br>$C_{37}H_{34}N_2(NH_4)_2\ O_9\ S_3$ (diammonium salt) |
| Molecular Weight | 534.37 (Marmion) | 792.84 (disodium salt) (Marmion)<br>782.96 (diammonium salt) |
| Physical State, as pure chemicals | Bright orange-yellow powder (Marmion) | Reddish-violet powder (Marmion) |
| Vapor pressure,at 25° C, in mmHg | $7.43 \times 10^{-22}$<br>there is some uncertainty in these estimated values, however they do not significantly impact the assessment as the vapor pressure are very low<br>EPI Suite, 2004<br><br>25.0 (Reported in 43503402) | $2.97 \times 10^{-42}$<br>there is some uncertainty in these estimated values, however they do not significantly impact the assessment as the vapor pressure are very low<br>EPI Suite, 2004<br><br>28.6 (Reported in 43503401) |
| Solubility in Water, 25°C in mg/L | $1 \times 10^6$; EPI Suite, 2004<br>"Completely soluble" (Reported in 43503402) | 1.45 mg/L; EPI Suite, 2004<br>"Completely soluble" (Reported in 43503401) |
| Log *n*-Octanol/Water Partition Coefficient | -10.7<br>EPI Suite, 2004<br>and SRC | -1.50<br>EPI Suite, 2004<br>and SRC |

Data Source

Marmion= **Handbook of U.S. Colorants- Food, Drugs**, **and Medical Devices**, Third Edition., 1991.

EPI Suite, 2004= Tolerance Reassessment Document

Submitted studies= 40503401 (Acid Blue 9) and 40503402 (Acid Yellow 23); According to the studies, the purity of Acid Blue 9 was 50% (43503401) and that of Acid Yellow 23 was 28%. Thus, the reported physical chemical properties do not reflect those of the pure materials.. The high vapor pressure reported for the two dyes are likely to be associated with volatile impurities.

SRC= 2004. Syracuse Research Corporation. Interactive Physical Properties (PHYSPROP) Database Demo. Search terms: FD&C Blue. No. 1, FD&C Red No. 40, FD&C Yellow No. 5. (November 28, 2004); http://www.syrres.com/esc/physdemo.htm

Dissociation Constant (pKa): Acid dyes are weak acids. The $pK_a$ values for Acid Blue 9 and Acid Yellow 23 are below 4. Therefore, they are completely dissociated in the environmentally significant pH range of 5 to 9.

*Environmental Fate Parameters (Extrinsic Properties)*

Dyes such as Acid Yellow 23 and Acid Blue 9 are not readily biodegradable under aerobic conditions nor degrade via abiotic hydrolysis (no hydrolyzable groups)[10], Although they absorb energy strongly in the visible range of sunlight, they appear to undergo slow direct photolysis[11] Direct photolysis would be only significant in clear, shallow water. But dyes have varying susceptibility to fading when exposed to sunlight. Light fastness[12] is the degree to which a dye resists fading, which varies from dye to dye. Indirect photolysis is likely to be the cause of fading in water and one of the major routes of transformation in the environment. Unlike uses in food, drugs, and cosmetics, the dyes when used as algicides, are exposed to sunlight in an open aquatic environment. Given that sunlight energy varies according to latitude and time of the year, their rate of fading are expected to vary depending on geographical location and season. Biotransformation under anaerobic conditions has as also been documented for other structurally related dyes. Acid Blue 9 and Acid Yellow 23 do not react chemically with each other.

Although environmental fate properties for Acid Blue 9 and Acid Yellow 23 were estimated with Structure-Activity Relationships using the EPI (Estimation Programs Interface) Suite™ (formerly known as EPIWIN)[13] , it was considered that the estimates are not reliable because EPIWIN is not suitable for handling salts, particularly of those with large anions. Therefore, EPIWIN introduces a high degree of uncertainty for dyes. In addition, EPIWIN does not provide any information on the products that may form as the result of photoreactions or anaerobic biotransformation.

The vapor pressure and the Henry's Law Constants of dyes are very low and it is unlikely that they volatilize from soil or water. Dyes such as Acid Blue 9 and Acid Yellow 23 are very hydrophilic (Log Kow << 1) and, therefore , they are not likely to bioaccumulate in fish.

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[10] Lynch, D.G. "Estimating the Properties of Synthetic Organic Dyes", in **Handbook of Property Estimation Methods for Chemicals- Environmental Health Sciences**, Edited by Robert S. Boethling and Donald Mackay. Published by Lewis Publishers, Boca Raton, FL, 2000; Pages 447- 467. And pertinent references therein.

[11] Even though it has the necessary condition (i.e., absorb energy within the spectrum of sunlight) to undergo direct photolysis that process is slow. However, they can degrade via indirect photolysis**.** For further information on indirect photolysis refer to Footnote 17.

[12] In artist's color language, a dye or pigment that fades is said to be "fugitive" and those that do not fade are called "permanent colors".

[13] http://www.epa.gov/opptintr/exposure/docs/episuite.htm

3. Pesticide Type, Class, and Mode of Action

There are five products that contain both Acid Blue 9 and Acid Yellow 23 in their formulation: (1) Care Pond AlgaeBlocker (one label); (2) Aquashade (two labels) and (3) Admiral (two labels). The products are used to control the growth of algae in ornamental ponds, recreational man-made (closed-system ponds, such as golf course lakes), rearing lakes for non-edible fish., and fountains containing fish. The dyes do not directly kill the algae, but prevent growth by blocking sunlight energy necessary for photosynthesis and survival of the algal and other undesirable aquatic plants. Thus, these products serve as algicides or as herbicides for undesirable aquatic plants. In addition, the dyes confer a bluish green coloring to the water body.

4. Overview of Pesticide Usage

These products are to be applied directly to the water body by adding a recommended amount of the product to a specified volume of water to attain 1 or 2 ppm according to the target weed. The extent of use, timing of application, and where it is used is not known, but geographical location and season will affect the rate of fading and frequency of application. The dose to be apply varies with the products and is specified in the labels and takes into account the volume and depth of the water to be treated. The specific times of application are not included in the labels. However, some of the labels recommend that the product be applied "early in the season" (presumably Spring) and also indicate that the product can be added to an ice surface of the water body (i.e., before melting).

B. Receptors

1. Aquatic Effects

For aquatic ecosystems, ecological receptors include all aquatic life (fish, amphibians, invertebrates, plants) and those terrestrial animals (e.g., birds and mammals) that consume water and/or aquatic organisms. Since these products are directly applied to contained water bodies, any effects on aquatic life and terrestrial animals would come from consumption of water or fish in rearing lakes or fountains. Immersion in the treated water may also have effects, such as irritation or changes in color caused by the dyes. Terrestrial animals may also be affected indirectly if they depend on the aquatic plants in the treated water bodies for food.

The labels for these products state that non-target aquatic plants (water lilies, hyacinths, cattails) may suffer contact burns if the chemicals are accidentally poured directly into them and that desirable submerged plants may also be affected due to reduced levels of sunlight.

Aquatic organisms that depend on sunlight for survival may be affected by the reduction of sunlight. Examples include non-target plants, animals that consume non-target or target plants or prey on the animals that consume aquatic plants.

Risk to aquatic animals is based on registrant submitted acute, dietary laboratory studies with aquatic vertebrates (rainbow trout and bluegill sunfish) and invertebrates (water fleas).

The taxa evaluated for ecological effects are presented in Table 4.

Table 4. Taxa evaluated for ecological effects in screening level risk assessments.

| Taxon | Surrogate Species Used in Risk Assessment |
|---|---|
| Birds [a] | Mallard duck (*Anas playtrhynchos*)<br>Bobwhite quail (*Colinus virginianus*) |
| Mammals | Laboratory rat (*Rattus norvegicus)* |
| Freshwater fish [b] | Bluegill sunfish (*Leopomis macrochirus*)<br>Rainbow trout (*Oncorhynchus mykiss*)) |
| Freshwater invertebrates | Water flea (*Daphnia magna*) |
| Estuarine/marine fish | Not Required because estuarine exposure is considered unlikely |
| Estuarine/marine invertebrates | Not Required because estuarine exposure is considered unlikely |
| Terrestrial plants | Not Required because exposure to terrestrial plants is not expected providing treated water is not used for irrigation |
| Aquatic plants and algae | Not Required because aquatic plants are the target and assumed to be controlled by use of aquashade |

[a] Birds are used as surrogates for amphibians (terrestrial phase) and reptiles.
b Freshwater fish may be surrogates for amphibians (aquatic phase).

Because of the very low exposure potential and mode of action and very restricted use pattern, only the avian oral, avian dietary, and acute freshwater studies were required. An acute rat study was submitted to OPP's Health Effects Division (HED) and EFED used their review as a surrogate for wild mammals.

2. Terrestrial Effects

Terrestrial plants are not receptors for the direct application of Aquashade to contained water. Terrestrial animals (e.g. birds and mammals) may become receptors if they consume or immerse in the water treated with these dyes.

Risk to terrestrial animals will be based on registrant submitted acute laboratory tests with birds (bobwhite quail and mallard duck) and acute and chronic tests with mammals to represent all terrestrial vertebrates. In addition, effects data from open literature will also be considered.

3. Ecosystems at Risk

All of the products are directly applied to relatively small, ornamental or decorative water bodies without outlets and are not to be used in or discharged to streams or rivers. The ecosystems at risk are those aquatic habitats in the treated water bodies and the areas occupied by terrestrial animals that might consume, or immerse themselves in, the treated water. The labels allow treatment of ponds in golf courses, which suggests that "natural looking" ponds may be treated. However, it is assumed that these water bodies are managed regularly for esthetic

reasons (i.e. to control weed population and to add color to the water body). The terminology "relatively small" is intended to differentiate the potential use areas from extremely large water bodies (e.g. large lakes and rivers). These products are used to curtail photosynthesis in weeds and algae by blocking sunlight energy and therefore, use of these products to control aquatic plants and algae in extremely large or flowing water bodies is impractical.

## C. Assessment Endpoints

*Ecological Effects*

Assessment endpoints are the "explicit expressions of the environmental value that is to be protected." Defining an assessment endpoint involves two steps: 1) identifying the valued attributes of the environment that are considered to be at risk, and 2) operationally defining the assessment endpoint in terms of an ecological entity (*i.e.*, a community of fish and aquatic invertebrates) and its attributes (*i.e.*, survival and reproduction). Therefore, selection of the assessment endpoints is based on valued entities (*i.e.*, ecological receptors), the ecosystems potentially at risk, the migration pathways of pesticides, and the routes by which ecological receptors are exposed to pesticide-related contamination. The selection of clearly defined assessment endpoints is important because they provide direction and boundaries in the risk assessment for addressing risk management issues of concern.

**Table 5. Summary of assessment and measurement endpoints.**

| Assessment Endpoint | Measurement Endpoint |
|---|---|
| 1. Abundance (*i.e.*, survival and growth and reproduction) of individuals and populations of birds. | 1a. Bobwhite quail and mallard duck acute oral $LD_{50}$.<br>1b. Bobwhite quail and mallard duck subacute dietary $LC_{50}$.<br>1c. Avian reproductive NOAEL (in this case, no avian chronic data are available, low chronic toxicity is inferred based on low chronic toxicity to mammals) |
| 2. Survival of individual mammals and reproductive effects. | 2a. Laboratory rat acute oral $LD_{50}$ and reproduction test NOAEL. |
| 3. Survival and reproduction of freshwater fish and invertebrates | 3a. Rainbow trout and bluegill sunfish acute $LC_{50}$.<br>3c. Water flea acute $LC_{50}$.<br>3d. Fish and invertebrate chronic toxicity NOAEL. |
| 4. Survival and Growth of Aquatic Plants | 4. Algae and vascular plant EC50 and NOAEL |
| 5. Survival and Growth of Terrestrial Plants | 5. Terrestrial monocot and dicot vegetative vigor and seedling emergent EC25 and NOAEL |

$LD_{50}$ = Lethal dose to 50% of the test population.
$LC_{50}$ ($EC_{50}$) = Lethal (effective) concentration to 50% of the test population.
$EC_{25}$ = Estimated concentration at which 25% response in tested population would be expected
NOAEL = Highest dose or test level at which no statistically significant effects were observed.

## D. Conceptual Model

### 1. Risk Hypothesis

Risk hypotheses are specific assumptions about potential adverse effects (i.e., changes in assessment endpoints) and may be based on theory and logic, empirical data, mathematical

models, or probability models (EPA, 1998). For this assessment, the risk is stressor-linked, where the stressors are the release of Acid Yellow 23 and Acid Blue 9 by direct application into artificial and confined water bodies (i.e. no outlets) and in accordance . The following hypotheses are presumed for this screening level assessment when the products are used in accordance with instructions and the precautions stated in the labels.

Aquashade represents a group of end-use products containing the two dyes Acid Blue 9 and Acid Yellow 23. They are used to control weeds in contained water bodies (no outflow) by blocking sunlight to aquatic weeds and thereby inhibiting photosynthesis. They are applied directly to these contained water bodies. The risk hypothesis is that when used as directed, Aquashade and the other end-use products may represent a risk to aquatic and terrestrial organisms."

2. Conceptual Model

Water is treated directly either by pouring the product directly, or by dropping packets.

Aquatic plants and animals are exposed directly.

Terrestrial animals may be exposed by drinking the treated water or immersing in it.

Terrestrial plants are unlikely to be exposed unless treated water is used for spray irrigation.

E. Analysis Plan

1. Preliminary Identification of Data Gaps and Methods

*Environmental Fate*

As a first step, the current status of FIFRA's Subdivision N Data Requirements was identified for Acid Blue 9 and Acid Yellow 23. The 161-1 (Abiotic Hydrolysis), 161-2 (Direct Photolysis in Water), 162-3 (Anaerobic Aquatic Metabolism), and 162-4 (Aerobic Aquatic Metabolism) guideline studies were "Reserved" based on low-volume of use of these chemicals (when use as pesticides)[14], but pending the results of the ecological toxicity studies (Guidelines 71 through 72). Physical and chemical properties were submitted in 1994 for the individual dyes (MRID Numbers 43503401 and 43503402), reviewed, and deemed acceptable However, these two studies were not conducted with a high purity dye (50% for Acid Blue 9 and 28% for Acid Yellow 23)). These two studies were submitted in support of the Aquashade product of 23.6% Acid Blue 9 and 2.39% Acid Yellow 23.

In December 2004, the Agency completed a tolerance reassessment for these two chemicals when used as inert components in pesticide formulations. Most of the environ mental

[14] Memorandum: "Review of Phase 4 List D Package for Aquashade" (EFGWB # 93-0119, 93-0120; Chemical # 110301 and 110302; Case # 819437 and 819438; DP Barcode D184289 and D184278). Dated February 4, 1993.

fate information in the document come from structure-activity relationships using computational tools (EPIWIN). In addition, a "Robust Summary for Acid Yellow 23 (tartrazine)[15] was submitted to the Agency under the High Production Volume (HPV) Challenge Program", which also relies on EPIWIN estimates. As already indicated, EPIWIN is not a suitable estimation program for salts, particularly for salts of a large anion. Physical and chemical properties estimated by EPI Suite/EPIWIN have been included in Table 3 and come from the 2004 tolerance reassessment document.

Information from the open literature was included to present a more comprehensive, but qualitative, assessment of the environmental fate of the two dyes beyond structure-activity estimates (Refer to the Exposure Characterization- Environmental Fate and Transport section of this document).

Given that the ecological risk assessment concluded that products containing Acid Blue 9 and Acid Yellow 23 did not pose risk to non-target organisms, no further environmental fate data are required unless new uses or products are proposed.

*Ecological toxicity*

Measures of effects are obtained from a suite of registrant-submitted guideline studies conducted with a limited number of surrogate species and from open literature. The test species are not necessarily intended to be representative of the most sensitive species but rather were selected based on their ability to thrive under laboratory conditions. Acute measures of effect are the concentrations that produce 50% mortality or growth reduction in the test organisms ($LC_{50}$s and $EC_{50}$s, respectively). Chronic measures are the no observed adverse effect level or NOAEL which is the highest test level at which significant effects were not observed.

2. Measures to Evaluate Risk Hypotheses and Conceptual Model

a. Measures of Exposure from Contaminated Water

For these products, which are **directly** applied to the water body in a specified volume of the liquid product or number of packets, (no spraying), the concentration of each dye depends on the amount added and the ratio of each dye in the products. The amount to be added depends on where it is used and the size and depth of the water body. Neither runoff nor spray drift are routes of exposure. Therefore, simulation models (GENEEC; PRZM and EXAMS) are not applicable to estimate environmental exposure concentrations in the treated water.

To estimate exposure to terrestrial animals, estimates of water consumption were used to derive daily doses of the formulation aquashade and compared to acute toxicity endpoints.

b. Measures of Effects

[15] http://www.epa.gov/chemrtk/ciacdylo/c15133tc.htm

"Robust Summaries & Test Plans: C.I. Acid Yellow 23 (FD&C Yellow 5)", Submitted ti the Agency by the International Association of Color Manufacturers on March 10, 2004.

Since Aquashade (23.6% Acid Blue 9 and 2.39% Acid Yellow 23) is composed of two dyes that are never used individually as algicide/aquatic herbicide, all of the studies submitted were conducted with the end-use product (Refer to the Memorandum cited in Footnote 13). Studies submitted to fulfill the "basic six" requirements (71-1b avian oral, 71-2b avian dietary, 72-1b and 72-1d Freshwater fish acute, and 72-2b Aquatic invertebrate acute toxicity). Based on low toxicity and no effects observed at the highest test level in the acute toxicity test, the mode of action, the low chronic toxicity of the yellow dye demonstrated to mammals in chronic testing, and the fact that both of the dyes are used as food coloring for human foods and often used in other animal foods with no long-term effects detected there is no reason to believe that other effects (reproductive, estuarine, marine, *etc.*) are likely. Chronic studies are not required.

For the present ecological risk assessment, EFED has used the ecological toxicity data from the studies conducted with together with an acute laboratory rat toxicity conducted with Admiral WSP submitted to HED.

In addition EFED has made the assumption that the dyes do not represent chronic risks based on a mammalian chronic study with the Acid Yellow dye 23.

c. Measures of Ecosystem and Receptor Characteristics

Aquashade's and the labels of the other end-use products specifically state that treated water bodies are not to be drained into streams, rivers or other water systems, it is assumed that exposure to these other water bodies will not occur. The aquatic organisms that occur in the treated water bodies are generally assumed to have been placed there by people that manage these decorative ponds and containers. The submerged plants in the ponds are assumed to be the target organisms, so the risk to them is not assessed.

Since it is never applied to terrestrial environments, exposure to terrestrial animals assessment estimating residues on terrestrial vegetation and invertebrates was inappropriate. Instead calculations of the amount in water consumed by birds or mammals drinking from the treated body of water were done to derive a dose of Aquashade to birds and mammals in three size classes.

The receptors addressed by the aquatic and terrestrial risk assessments for Aquashade are summarized in Table 4. For aquatic assessments, freshwater fish and invertebrates were studied. Estuarine/marine animals are not represented, because Aquashade is not applied to these environments and is not expected to drain into them.

## III. Analysis

A. Use Characterization

The use characterization of the products containing Acid Blue 9 and Acid Yellow 23 is based on the current labels for these products. Table 7 identifies these end-use products with

their EPA Registration Numbers, and label date. Except for Admiral WSP, all of the products are liquid formulations. The products are directly poured or droppen into water in a specified volume of the liquid formulation or number of packets. No spraying is involved which could lead to spray drift or inadvertent exposure to terrestrial habitats.

Table 7 Identification of the End-use Products Containing Acid Blue 9 and Acid Yellow 23 as the Active Ingredients

| Product | EPA Reg. No<br>Registrant<br>Label Date | Composition (by weight)<br>Type of Formulation | Target pests |
|---|---|---|---|
| Pond Care Algae Blocker | 8709-6<br>Aquarium Pharmaceuticals, Inc.<br>February 6, 2004 | 2.36 % Acid Blue 9<br>0.24 % Acid Yellow 23<br>Liquid formulation | Control of growth of many algae and underwater aquatic weeds |
| Aquashade OA | 33068-2<br>Aquashade , Inc.<br>February 23, 1993 | 2.36 % Acid Blue 9<br>0.24 % Acid Yellow 23<br>Liquid formulation | Control of growth of many algae and underwater aquatic weeds |
| Aquashade | 3306-1<br>Aquashade, Inc.<br>1981 | 23.63% Acid Blue 9<br>2.39% Acid Yellow 23<br>Liquid formulation | Control of growth of many algae and underwater aquatic weeds |
| Admiral Liquid | 67064-2<br>Becker Underwood<br>March 14, 2000 | 15.31% Acid Blue 8<br>1.00% Acid Yellow 23<br>Liquid formulation | Control algae and aquatic vegetation and color the water |
| Admiral WSP | 67064-1<br>Becker Underwood<br>August 17, 2001 | 49.72% Acid Blue 9<br>3.27% Acid Yellow 23<br>Concentrated blend in pre-measured water soluble packages | Control algae and aquatic vegetation and color the water |

The use sites and timing of application are summarized in Table 8. Note that the uses are limited to confined water bodies with minimal or no outflow, but that the Admiral products has a wider range of use sites than AlgaeBlocker and Aquashade. Only the Admiral products have uses on swimming ponds, but treated swimming ponds must not be treated with a chlorine disinfectant to avoid fading of the dyes. Likewise, no carbon filters should be used to prevent removal of the dyes from water.

Table 8- Use Sites and Timing of Application for the End-use Products Containing Acid Blue 9 and Acid Yellow 23 as the Active Ingredient

| Product | Use Sites | Time of Application |
|---|---|---|
| Pond Care Algae Blocker | Ornamental Ponds and recreational man-made, closed system ponds (Golf course lakes);<br>Rearing lakes for non-edible fish;<br>Fountains containing fish. | Not specified<br>“Reapply as needed” |

| | | |
|---|---|---|
| Aquashade OA<br><br>Aquashade | Fountains<br>Aquarium<br>Ornamental Ponds<br>Re-circulated or artificial waterscapes<br><br>Ideal Settings:<br>Shopping malls<br>Executive office parks<br>Botanical gardens<br>Recreational and amusement parks.<br><br>Natural and manmade contained ponds and lakes including ornamental, recreational, fish rearing and fish farming ponds, golf course ponds<br><br>Do not apply directly to streams, other natural bodies, or any body of water not under control of the user. Do not apply to water that will be used for human consumption. | Not specified<br><br>Recommends application before weed growing season.<br><br>May be applied while ice is still covering the water body |
| Admiral Liquid | Natural or manmade ponds, lakes, fountains, fish farms, fish hatcheries, golf courses and swimming ponds | Prior to or early in the weed growing season for optimum results<br><br>May be applied while ice is still covering the water body |
| Admiral WSP | Natural or manmade ponds, lakes, fountains, fish farms, fish hatcheries, golf courses and swimming ponds | Prior to or early in the weed growing season for optimum results<br>Reapply "as needed" |

The liquid concentrates AlgaeBlocker, Aquashade, Aquashade OA, and Admiral liquid are added directly to water in a specified volume of product according to the volume and depth of the water body to be treated. to reach a target concentration. Admiral WSP is also directly applied to water in a prescribed number of packets for specific volume of the water bodies. The resulting concentration of the products in water is fixed to "1 or 2 ppm", depending on the target weed.. The dosing specified in the labels are summarized in Table 9 .

Table 9.- Dose Recommended for the End-Use Products Containing Acid Blue 9 and Acid Yellow 23 as the Active Ingredients

| Product | Dose |
|---|---|
| Pond Care Algae Blocker | Ornamental ponds and fountains less than 2 feet deep: 8 mL of product per every 100 gallons of pond water (378 L);<br>Fish rearing ponds and large ornamental ponds over 2 feet deep: 4 mL for every 100 gallons of water<br>Maximum application rate, 1 ppm |

| | |
|---|---|
| Aquashade OA<br><br><br><br><br><br><br>Aquashade | "Small water bodies" (unspecified)<br>One drop for each gallon of water<br>'Larger water bodies"<br>1 oz per 1,000 gallons of water<br>For controlling submerged weed, the depth of water must be 2 feet<br>Maximum application rate, 1 ppm<br><br>One gallon of product (1 ppm) per acre with 4 foot average depth when submerged weeds and algae are growing at depths > 2 feet. target pests are leafy pondweed, Chara, slender naiad, filamentous green and blue algae, spyrogyra-Water milfoil.<br><br>2 ppm to prevent production of Hydrilla's tubers. |
| Admiral Liquid | The amount of the product added depends on the volume of the water body. Amount to be added (volume) per volume of water body is specified in the label.<br><br>Regardless of the amount added and volume of the water body, the concentration regime in the water is :<br><br>1 ppm for filamentous blue and blue algae, Chara, Leafy Pond weed, spirogyra, slender naiad, and water milfoil<br><br>2 ppm is used to prevent Hydrilla tuber production after a herbicide treatment |
| Admiral WSP | Same as Admiral Liquid |

Most of the labels contain precautionary language and/or environmental hazard statements, but the language is not consistent across the labels. Table 10 summarizes the "Precautionary Language", whereas Table 11 summarizes the "Environmental Hazard Statements" as they appear in each label.

Table 10 Precautionary Language Included in the End-use Products Containing Acid Blue 9 and Acid Yellow 23 as the Active Ingredients

| Products | Precautionary Language Included in the Labels |
|---|---|
| Pond Care Algae Blocker | Non-target plants (water lilies, hyacinths, cattails) may suffer contact burns if material is accidentally poured directly into them<br>Desirable submerged plants may also be affected due to reduced levels of sunlight |
| Aquashade OA<br><br>Aquashade | None<br><br>None |
| Admiral Liquid | Water must be under the complete control of the user and have little or no outflow (dye concentration must be maintained).<br>Do not apply directly to streams, or any body of water that may be used for human consumption |
| Admiral WSP | Same as for Admiral Liquid |

Table 11 Environmental Hazard Statements Included in the Labels of the End-use Products Containing Acid Blue 9 and Acid Yellow 23 as the Active Ingredients

| Products | Environmental Hazard Statements Included in the Labels |
|---|---|
| Pond Care Algae Blocker | Do not contaminate domestic livestock, irrigation water and streams with outflow. Use only in impounded water with no outlet and under the total control of the applicator. Do not use in recreational waters intended for swimming or in waters intended for edible fish |
| Aquashade OA<br><br>Aquashade | Desirable plants such as water lilies may suffer contact burn if material is accidentally poured on them directly<br><br>Shoreline non-target plants (cattails, water lilies) may suffer contact burn if material is accidentally poured on them. Do not contaminate water by cleaning of equipment or disposal of wastes. |
| Admiral Liquid | Shoreline non-target plants (cattails; water lilies) may suffer contact burn if the material is accidentally poured on them. Apply this product only as specified in the label. Do not make applications when weather conditions favor drift from non-target areas. Do not apply where runoff is likely to occur. |
| Admiral WSP | None specified |

B. Exposure Characterization

1. Environmental Fate and Transport

No experimental environmental fate, Subdivision N Guideline studies were conducted with each individual dye as the test substance or an end-use product. These studies were placed in "Reserved" pending the results of the required ecological toxicity studies, as indicated in the "Analysis Plan" section. For consistency with the tolerance reassessment for inerts, the environmental fate assessment originally based findings on estimates from Structure-Activity Relationship (SAR) using EPISUITE (EPIWIN). However, these estimates are considered uncertain because EPIWIN is not suitable for salts, particularly those of large anions, such as in dyes. A literature search for environmental fate studies conducted with Acid Yellow 23 and Acid Blue 9 as the test substances did not produced sufficient experimental data specific to these two dyes. Therefore, most of the environmental fate information comes from dyes structurally related to Acid Yellow 23 (azo dyes) and Acid Blue 9 (triphenylmethane dyes). Further information was obtained from the "Handbook of U.S. Colorants- Food, Drugs, and Medical Devices" (Marmion, 1991). Thus, most of the present environmental fate assessment uses published information about the properties of synthetic dyes and other relevant, open literature sources (Lynch, 2000; Lynch, D., OPPTS. private communication, August 2005). The environmental fate assessment is only qualitative in nature.

Acid Blue 9 and Acid Yellow 23 belong to different chemical families but both contain sulfonate groups. Because none of these two dyes contain hydrolyzable groups, abiotic hydrolysis is not a degradation pathway for these two dyes.

As chemical substances absorbing sunlight energy, photodegradation in water can occur via direct or indirect photolysis. The direct photolysis of most dyes appears to be slow and would be only significant in clear, shallow water (which could be the case for ornamental uses, such as in fountains exposed to sunlight). Because the presence of natural photosensitizers in the environment[16], indirect photolysis has been identified as a major transformation pathway for dyes in environmental water. Besides the concentration of photosensitizers in water, the rate of photolysis would also be a function of the solar photon flux, which depends on latitude and season. For example, photolysis would be faster in the Summer than in the Spring and faster in the Southern than in the Northern parts of the country. Acid Yellow 23 appears to be more photolytically stable than Acid Blue 9, as the latter fades faster (Marmion, 1991). Photo-degradation of dyes can also occur on soil surfaces.

There are no experimental data that identifies the photoproducts of Acid Blue 9 or Acid Yellow 23. However, for azo dyes (Acid Yellow 23 is an azo dye), azo bond cleavage and photoredox reactions occur in most cases. Potential products are aminobenzyl sulphonic acids, but their specific chemical identity and toxicity are not known.

In general, most azo dyes appear to resist biodegradation under aerobic conditions. However, microbes living in an anaerobic environment can reduce azo bonds resulting in significant loss of color (Brown and Laboureur, 1983; Weber and Adams, 1995; Jank, et al., 1998; Baughman, 1995). Some of the products of the anaerobic transformation of azo dyes are aromatic amines. Therefore, in the environment azo dyes can lose their color via photoredox reactions and by anaerobic biodegradation. Anaerobic biodegradation is most likely to occur in sediments.

Fading can also occur by reaction with trace metals, such as zinc, tin, aluminum, iron, and copper, mostly via redox reactions. However, fading is primarily a photochemical process.

Dyes such as Acid Blue 9 and Acid Yellow 23 are completely dissociated in the environmentally significant pH range of 5 to 9. Because the chromophoric species is the anion, the dyes do not adsorb strongly to soils or sediments and are predominantly associated with the water column. Acid Blue 9 has been long used as a hydrological tracer because it does not adsorb strongly on soil particulates (Mon et al., 2005; Tsai, et al., 2004)
.

The very low vapor pressure and Henry's Law Constant of the days indicate that volatilization would not be a significant process. As highly hydrophilic chemicals with Log Kow << 1, they are not likely to bioaccumulate in fish.

2. Measures of Aquatic Exposure

a. Aquatic Exposure Modeling

[16] Natural photosensitizers in environmental waters include dissolved organic matter (DOM), singlet oxygen ($^1O_2$), and hydroxy radicals (·OH). These species are photooxidants. In additions, surfaces of semiconducting metal oxides (such as titanium dioxide or zinc oxide) may serve as heterogeneous photooxidants and it is a potential technology to treat wastewater containing . dye residues. (Baran, et al., 2003). In addition, photoreactions at the surface of iron oxides/hydroxides may also be involved.

All of these products are to be applied directly to a confined or mostly confined water body. Therefore, the concentration of each dye in water is only the result of direct application to water and depends on the relative ratio of the two dyes for each product and the recommended dose. Neither spray drift nor runoff are routes of exposure for Acid Yellow 23 and Acid Blue 9 in the water. A Drinking Water Assessment was performed.and it was concluded that there was no exposure from drinking water. This assessment was based on the label restrictions, which indicate that treated water is not to be used as a drinking water source for humans.

For the aquatic assessment, the concentrations of Acid Yellow 23 and Acid Blue 9 were those target concentrations specified in the labels (1 ppm or 2 ppm, depending on the target weed). These concentrations are attained by directly adding a specified volume of product per specified volume of water to be treated (i.e., by dilution of the product into a larger volume of water). It should be noted that the dosing language in some labels (e.g., Aquashade OA ) are vague ("one drop") in indicating the volume of product to be added to the water body. It is unclear if the specified concentration is in terms of percent of product (i.e., the two dyes and inerts) nor the purity of the dyes are taken into account..

The concentrations resulting from direct application are summarized in Table 12. Because there are no kinetics data for indirect photolysis or anaerobic biodegradation, it is assumed that these concentrations remain constant. For Acid Blue 9, the estimated concentrations do not take into account the type of counter cation (i.e., no molar fraction correction, given the uncertainty in the identity of the salt). Even though the environmental concentrations were estimated for each individual dye, there are no ecological toxicity for the individual dyes because the test substance in all of the ecological toxicity studies was an end-use Aquashade product containing 23.6% Acid Blue 9 and 2.39% Acid Yellow 23 of uncertain purity. Therefore, an assessment based on each individual dye was not possible. The ecological toxicity of products with a higher percentage of each individual dye and/or different ratio of the dyes than Aquashade may potentially be under represented. These products are Admiral Liquid and Admiral WSP.

Table 12 Maximum Concentrations of Acid Blue 9 and Acid Yellow 23 Expected from Direct Application of Each Product at the Label at Rates Set in the Labels and assuming 100% purity of each dye (Source: Product labels)

| Product | Application Rate of Product as target concentration, ppm; mg/L | Concentration of Acid Blue 9, in mg/L | Concentration of Acid Yellow 23, in mg/L |
|---|---|---|---|
| Aquashade OA | 1<br>2 | 0.024<br>0.048 | 0.0024<br>0.0048 |
| Aquashade | 1<br>2 | 0.24<br>0.48 | 0.024<br>0.048 |
| Pond Care AlgaeBlocker | 1 | 0.024 | 0.0023 |
| Admiral Liquid | 1<br>2 | 0.15<br>0.3 | 0.01<br>0.02 |

| | | | |
|---|---|---|---|
| Admiral WSP | 1<br><br>2 | 0.497 (0.5)<br><br>0.99 | 0.033<br><br>0.066 |

b. Aquatic Exposure Monitoring and Field Data

The use of monitoring data and/or field data is not applicable to these dyes when used to control algal growth or other undesirable aquatic plants in confined, with no or minimal outlet water bodies. However, these dyes also have industrial applications and have been detected in waste water, sewage systems, and water bodies[17]. Ecological risk from those sources of potential exposure are not addressed in this document.

c. Measures of Terrestrial Exposure

3. Terrestrial Exposure Modeling

Routinely, exposure to birds and mammals feeding on a treated field is estimated by modeling residues on terrestrial vegetation and invertebrates using the Terrestrial Residue EXposure (TREX) simulation model. Water treated with Aquashade or any of the other products is not expected to reach terrestrial vegetation or invertebrates. Because exposure to the dyes from residues on terrestrial food items is not expected, the use of terrestrial exposure models is not applicable. An exception could be if treated water was used for irrigation. If this happened, it is possible the dyes in these formulations would get on terrestrial food items. However, given the low toxicity, it is unlikely to result in direct acute risk to terrestrial animals.

a. Terrestrial Exposure via Consumption of Contaminated Water Modeling

To determine the exposure of mammals and birds to "Aquashade" *via* consumption of contaminated water, a single daily dose of Aquashade was estimated using the calculated volume of water that birds and mammals are expected to consume per day and the concentration of Aquashade in water as follows:

**Daily Exposure (mg) = Daily Water Consumption (L) × Water Concentration (mg/L).**

To estimate the volume of water that mammals and birds are expected to consume per day, allometric equations from the EPA Wildlife Exposure Factors Handbook (US EPA 1993) were used. For birds, the daily water consumption (L) was calculated using the equation (US EPA 1993, Equation 3-15, p. 3-8, for all birds):

$$\mathbf{L = \alpha\ (body\ weight\ in\ kg)^{\beta},\ \ where\ \alpha = 0.059\ and\ \beta = 0.67}$$

[17] See, for example, "Background Document for Identification and Listing of Deferred Dye and Pigment Wastes, U.S. Environmental Protection Agency, Office of Solid Waste, Washington, D.C., June 1999.

For mammals, the daily water consumption (L) was calculated as using the equation (US EPA 1993, Equation 3-7, p. 3-10, for all mammals):

**$L = \alpha$ (body weight in kg) $^{\beta}$, where $\alpha = 0.099$ and $\beta = 0.9$**

The maximum concentration (weight to volume) of Aquashade in water after application at the labeled rate is 2 mg/L. As summarized in Table 13, a single daily exposure via drinking water containing 2 mg/L Aquashade was calculated for three weight classes for birds (20, 100, and 1000g) and mammals (15, 35, and 1000g).

**Table 13. Exposure estimates for birds and mammals via consumption of water contaminated with Aquashade at a maximum calculated concentration of 2 mg/L.**

| Taxon | Body Weight (g) | Estimated Water Consumption (L) | Estimated Exposure[a] (mg) |
|---|---|---|---|
| Birds | 20 | 0.0043 | 0.008 |
| | 100 | 0.013 | 0.026 |
| | 1,000 | 0.059 | 0.119 |
| Mammals | 15 | 0.0023 | 0.004 |
| | 35 | 0.0048 | 0.01 |
| | 1,000 | 0.099 | 0.1 |

[a] Estimated Daily Exposure (mg) = Daily water consumption (L) × Water concentration 2 mg/L).

b. Residue Studies

There is no terrestrial exposure from this use pattern. Therefore, residue data are not necessary.

C. Ecological Effects Characterization.

This document is a screening level evaluation of the potential ecological risk from the end-use products that contain Acid Yellow 23 and Acid Blue 9 dyes, and are represented by a formulation referred to as Aquashade. All of the products are composed of two dyes that are never used individually as an algicide/aquatic herbicide. These products are applied directly to confined water bodies and never to terrestrial or aquatic ecosystems.

Available data are from tests conducted with either a formulation containing Acid Yellow 23 and Acid Blue 9, or with the dyes separately.

All of the toxicity studies on birds, fish and aquatic invertebrates submitted by the registrant were conducted with the end-use product Aquashade (23.6% Acid Blue 9 and 2.39% Acid Yellow 23) and not with the individual dyes. The studies were submitted to support the registration of this "Aquashade" end-use product (EPA Reg. No. 33068-1). Through searches conducted by the ORD Middle Ecological Division at Duluth under their ECOTOX literature

search program, ecological toxicity studies conducted with the individual dyes were located. The results of these published literature data supported the results of, and did not indicate greater toxicity than, or effects other than the registrant submitted tests, therefore, they were not used to estimate risk in this assessment. A laboratory rat study was conducted with the Admiral WSP product as the test substance (49.62% Acid Blue 9 and 3.05% Acid Yellow 23).

There is an uncertainty in the purity of the test substance used in the ecological toxicity studies. However, considering the low exposure level relative to even the lowest assumed purity, this uncertainty does not cause doubt in the conclusions.

The studies submitted to fulfill the "basic six" requirements (an avian oral, two avian dietary, two fish acute, and one aquatic invertebrate acute toxicity) found very low toxicity (practically nontoxic and slightly toxic). A study on the laboratory rat found an LD50 equal to or higher than 5,000 ppm. Table 14 summarizes the acute data for the tested species used to assess risk.

**Table 14. Summary of most sensitive acute data. No chronic data were required for birds, fish or aquatic invertebrates.**

| Species | Study Type[1] | Source | Results[2] |
|---|---|---|---|
| Test Material Aquashade | | | |
| Bobwhite quail | Acute Avian Oral | 433367-01 | LD50 & acute NOAEC ≥ 2250 mg/kg |
| Mallard duck | Acute Avian Oral | 433367-02 | LD50 & acute NOAEC ≥ 2250 mg/kg |
| Bobwhite quail | Avian Dietary | 435034-03 | LC50 & acute NOAEC ≥5, 620ppm |
| Mallard duck | Avian Dietary | 435034-04 | LC50 & acute NOAEC ≥ 5,620ppm |
| Bluegill sunfish | Fish Toxicity Bluegill | 432975-02 | LC50 & acute NOAEC ≥ 96 mg/L |
| Rainbow trout | Fish Toxicity Rainbow Trout | 432975-01 | LC50 & acute NOAEC ≥ 96 mg/L |
| *Daphnia magna* | Invertebrate Toxicity | 432975-03 | LC50 & acute NOAEC ≥ 97 mg/L |
| Test Material Admiral WSP | | | |
| Laboratory rat | Wild Mammal Acute | 452811-01 | LD50 ♂ & ♀ ≥ 5,000 mg/kg |

[1] All were performed with the Typical End-use Product, Aquashade or Admiral WSP
[2] The results refer to the concentration of the end-use product.

Birds and mammals are expected to drink from ponds that have been treated with the end-use products. EFED calculated an estimated dose that they would receive from that water (see Table 10).

1. Terrestrial Effects

*Avian effects*

The avian oral acute toxicity studies for both the bobwhite quail and the mallard duck found $LD_{50}$'s and NOAECs equal to or greater than 2,250 mg/kg. The avian dietary toxicity studies found $LC_{50}$s and NOAELs equal to or greater than 5,620 ppm. Aquashade is considered to be practically nontoxic to birds. Higher tiered studies are not required.

*Mammalian effects*

An acute toxicity study that used Admiral WSP with the laboratory rat was reviewed by OPP's Health Effects Division found an $LD_{50}$ equal to or greater than 5,000 mg/kg. EFED

considers this to be practically nontoxic. The Health Effects Division wrote that, ". . . this formulation contained 49.62 % Acid Blue dye and 3.05% Acid Yellow dye (presumably Acid Blue 9 [Erioglaucine], and Acid Yellow 23 [Tartrazine], respectively). A chronic mammal toxicity test with Acid Yellow 23 indicated low likelihood of adverse chronic effects at environmentally relevant exposure levels.

*Calculating Daily Dose*

Terrestrial animals drink water from ponds and other bodies and may be affected by the Aquashade or any of the other end-use products in the water. Exposure estimates for terrestrial animals via drinking contaminated water were expressed in terms of a single daily dose (mg) for three different body weight classes. All of the exposures were less than 0.2 mg a.i./day.

*Birds*

The weight classes for birds were 20, 100, and 1,000 g. To obtain the toxicity value for a daily dose (mg of Aquashade) for each weight class for birds termed the "derived median dose," the acute oral $LD_{50}$ ($\geq$ 2,250 mg/kg-bw) was multiplied by body weight (kg). The calculated daily doses for birds for each weight class are summarized in Table 13.

*Mammals*

Exposure estimates for terrestrial animals via drinking of contaminated water were expressed in terms of a single daily dose (mg) for three different body weight classes (15, 25, and 1,000 g). Therefore, to obtain the lethal dose (mg of Aquashade) for each weight class for mammals, the acute rat oral $LD_{50}$ value of $\geq$ 5,000 mg/kg-bw (MRID 452811-01) was multiplied by body weight (kg). The calculated daily doses for mammals for each weight class are summarized in Table 15.

**Table 15. Acute toxicity values for birds and mammals drinking from contaminated water, expressed in terms of a single Aquashade dose (mg).**

| Animals | Body Weight (kg) | $LD_{50}$ (mg/kg-bw) | Derived Median Lethal Dose (mg) a |
|---|---|---|---|
| Birds | 0.020 | $\geq$ 2,250 | $\geq$ 45 |
| | 0.100 | $\geq$ 2,250 | $\geq$ 225 |
| | 1.000 | $\geq$ 2,250 | $\geq$ 2,250 |
| Mammals | 0.015 | $\geq$ 5,000 | $\geq$ 75 |
| | 0.035 | $\geq$ 5,000 | $\geq$ 175 |
| | 1.000 | $\geq$ 5,000 | $\geq$ 5,000 |

**a** Derived Median Lethal Dose (mg) = body weight (kg) $\times$ $LD_{50}$ (mg/kg body weight).

*Terrestrial Plant effects*

The method of application, i.e. pouring and dropping specific volumes or packets into the target water body, is not expected to result in drift or runoff resulting in exposure to terrestrial plants. Since it will not be applied to terrestrial plants no plant studies were required. One uncertainty exists, however, in that not all the labels preclude use of treated water for irrigation. If it was used for irrigation, the potential routes of exposure would be drift and/or runoff. To assess risk to terrestrial plants, tier 1 or tier 2 terrestrial plant testing would be used. If the labels stated that treated water must not be used for irrigation, such testing would be unnecessary. Some of the labeling indicates that emergent plants may experience burning if exposed to the dye formulations. Based on this, it is assumed that any inadvertent exposure to terrestrial plants might cause adverse effects.

2. Aquatic Effects

The registrant submitted studies that determined the toxicity of Aquashade to aquatic animals. The bluegill sunfish and rainbow trout studies found $LC_{50}$s and NOAECs equal to or greater than 96 mg/L. This level is considered by EFED to be slightly toxic. The level for being considered practically nontoxic is 100 mg/L for aquatic animals.

The aquatic invertebrate (*Daphnia magna*) study found that the $LC_{50}$ and the NOAEC were equal to or greater than 97 mg/L. This level is considered to be slightly toxic. No further studies are required.

Aquashade, Admiral, and AlgaBlocker are algicides or herbicides for undesirable aquatic plants. They are not applied to terrestrial environments nor if treated water used for irrigation. Because submerged aquatic plants are the target species and it is assumed that all submerged plants will be killed, no aquatic plant studies were required.

3. ECOTOX and other Published information

The Agency also obtained summary toxicity information from published literature found as part of the ECOTOX program maintained by the EPA Office of Research and Development Middle Ecological Division (MED) at Duluth. This program regularly searches open literature for toxicity information. OPP requested of MED Duluth to search its holdings for toxicity information on aquashade and the dye components of the formulations being assessed for reregistration. Toxicity information was located on mammals and aquatic animals from tests with either of the dyes. In all cases, the test results located through ECOTOX corroborated the findings of the registrant submitted data, and did not indicate effects unforeseen from the registrant studies. Neither did these published literature studies indicate adverse effects to organisms other than those tested by the registrant. None of the data located through ECOTOX were used in the assessment. See Appendix F for a summary table of data located from ECOTOX.

## IV. Risk Characterization

Acid Yellow 23 and Acid Blue 9 are applied together via the end-use products Aquashade Admiral Liquid. Admiral WSP, and AlgaBlocker. These products are applied to confined water bodies or water bodies with minimal outflow. None of the products have terrestrial uses and would not directly contact terrestrial food items for birds and mammals. Therefore, the RQs based on dietary exposure from Aquashade residues on food items were not calculated. However, it could affect them *via* drinking water.

Thus, all of the potential risks of Acid Yellow 23 and Acid Blue 9 are associated with direct exposure of terrestrial animals through drinking water or immersion in the treated water. In addition, aquatic organisms dwelling in treated water are also exposed to the dyes (refer to "Conceptual Model") Risks were estimated based on "environmental" concentrations in a water body resulting from direct application of the product(s) that is, a dilution concentration based on the application rates indicated in the labels (see Table 10). It must be kept in mind that the characterization of effects was based in terms of the combined two dyes for the Aquashade end-use product (23.6% Acid Blue 9 and 2.36% Acid Yellow 23) used in the ecological toxicity studies and not for the individual dyes.

### A. Risk Estimation - Integration of Exposure and Effects Data

#### 1. Birds and Mammals

Exposure to birds and mammals was expected primarily through consumption of drinking water treated with the dyes. Birds are surrogates for reptiles and terrestrial phase amphibians because it is assumed for screening level assessment purposes that reptiles and terrestrial phase amphibians are not more sensitive to oral toxicity than birds.

*Acute Risk Quotients (RQs) via Consumption of Contaminated Water*

Acute RQs for birds and mammals exposed to Aquashade via consumption of contaminated water were calculated for each of three body weight classes using the daily exposure value expressed as milligrams of Aquashade (Table 13) and the toxicity value expressed in terms of milligrams of Aquashade (Table 14). The RQs are summarized in Table 16.

**Table 16. Acute Risk Quotients (RQ) for birds and mammals exposed to Aquashade through consumption of contaminated water.**

| Taxa | Body weight (g) | Estimated Environmental Exposure[a] (mg) | Derived Medium Lethal Dose (DMDL)[b] | Acute RQ EEC/DMDL |
|---|---|---|---|---|
| Birds | 20 | 0.01 | ≥ 45 | < 0.01 |
| | 100 | 0.02 | ≥ 225 | < 0.01 |
| | 1,000 | 0.12 | ≥ 2,250 | < 0.01 |
| Mammals | 15 | < 0.01 | ≥ 75 | < 0.01 |
| | 35 | < 0.01 | ≥ 175 | < 0.01 |
| | 1,000 | < 0.10 | ≥ 5,000 | < 0.01 |

[a] Estimated Environmental Exposure (EEC) = Daily water consumption (L) × Water concentration (mg/L). Rounded to two decimal places.
[b] Derived Median Lethal Dose (mg) = body weight (kg) × $LD_{50}$ (mg/kg body weight).
[c] Acute RQ = Estimated Environmental Exposure / Concentration Derived Median Lethal Dose

RQs are below the Levels of Concern (LOCs) for acute risk (LOC 0.5), acute restricted use (LOC 0.2), and acute endangered species (LOC 0.1).

Acute RQs are below the Levels of Concern (LOCs) for endangered species ( 0.1).

*Use of Treated Water for Irrigation*

While it is considered highly unlikely that treated water would be used for irrigation, given the kinds of water bodies treated, if this happened, exposure to birds and mammals might occur through ingesting terrestrial food items that had been exposed to the water. Given the low toxicity of the dyes, and the relative low concentration in the treated water (no more than 2 ppm), it is unlikely that this route of exposure would cause direct toxicity to birds or mammals. If 2 inches of irrigation water containing 2 ppm were sprayed onto terrestrial vegetation, the resulting "application rate" of the dyes would be approximately 1 lb ai/acre.

Applying 1 lb ai/acre to short grass would result in residues no greater than 240 ppm. For a small (15g) mammal that eats close to its body weight this would still result in a dose per animal much less than the lowest toxicity value for mammals. See appendix E for calculations.

2. Non-target Aquatic Animals and Plants

Aquashade and any of the other end-use product containing Acid Blue 9 and Acid Yellow 23 are only applied directly to ponds and other aquatic systems without an outlet. It is not expected to come in contact with non-target aquatic animals outside of those systems.

This is a screening level assessment of the acute risk of using Aquashade, therefore, the highest EECs (2 mg/L; for Admiral Liquid and Admiral WSP) and the lowest toxicity values were used. The concentration in the treated systems will be higher than any body of water that

accidentally receives (by flooding, *etc.*) a dose of Aquashade. A summary of aquatic acute RQs is presented in Table 17.

**Table 17. Estimated acute risk quotients (RQ) for aquatic animals exposed to Aquashade. In this screening analysis only the highest Estimated Environmental Concentration (EEC = 2 mg/L) and lowest NOAECs were used.**

| Animal | EEC (mg/L) | NOAEC | RQ (EEC / NOAEC) | LOCs Exceeded |
|---|---|---|---|---|
| Bluegill sunfish | 2 | 96 mg/L | <0.01 | none |
| Rainbow trout | 2 | 96 mg/L | <0.01 | none |
| *Daphnia magna* | 2 | 97 mg/L | <0.01 | none |

Fish are used as surrogated for aquatic phase amphibians, so lack of risk to fish results in a conclusion of low risk to amphibians.

Aquatic Plants: This is a screening level assessment based on an Aquashade product. Aquashade, AlgaeBlocker, and Admiral products are aquatic algicides or herbicides for undesirable aquatic plants and are expected to kill all submerged aquatic plants. However, since it is only applied to ponds with little or no outlet, it is not expected to come into contact with nontarget aquatic out side of the target pond. All of the submerged plants in the pond are considered to be targets. Therefore, the RQs were not calculated for aquatic plants. If the water treated by these products were used for irrigation, it is unlikely the concentration of dyes in the runoff from such irrigation would be sufficient to cause effects to aquatic plants in receiving water bodies.

3. Non-target Terrestrial Plants in Dry-land and Semi-aquatic Environments

Terrestrial plants growing in dry-land and semi-aquatic environments are not expected to be exposed to the end-use products, since they products are poured, or applied with an eyedropper into confined water bodies or water bodies with minimal outflow. Because the labels do not allow application to terrestrial ecosystems, a terrestrial plant risk characterization was not performed. An uncertainty exists in that the labels do not preclude use of treated water for irrigation. If this happened, there is a potential for adverse effects to terrestrial plants because some labels indicate that if emerged plants are exposed to the formulations, burning would be expected.

B. Risk Description - Interpretation of Direct Effects

Aquashade does not exceed the level of concern for animals with which it is expected to come in contact. Because of the method of application, no drift or runoff onto terrestrial habitats is expected precluding exposure to terrestrial plants, and to food items consumed by terrestrial animals. Thus, the risk hypothesis is proven wrong for these organisms.

The mode of herbicidal action and intended use will result in adverse effects to target aquatic plants, therefore the risk hypothesis that these formulations adversely affect aquatic plants is correct. However, in all cases, the treated water body is treated for the express reason of

controlling (eliminating) submerged aquatic plants therefore, this effect is not interpreted as an adverse ecological effects unless, as discussed below, there are endangered aquatic plants in these treated water bodies.

## C. Endangered and Threatened Species

The use of these dyes may affect endangered aquatic plants possibly occurring in ponds where this might be used. It may also have the potential to affect endangered animal species which depend on aquatic plants in these managed water bodies for food and/or habitat. The potential for this direct effects to listed aquatic plants, and indirect effects to listed animals is very limited because of the method of application (i.e. by hand), and the kinds of water bodies treated (golf course ponds and other managed ponds) where keeping the water free of all vegetation is aesthetically desirable. These water bodies do not typically have outflow to streams or rivers, which would preclude endangered fish and invertebrates from entering. However, endangered amphibians may enter such water bodies, and the loss of plants that serve as food and shelter for these amphibians have the potential indirectly affect these organisms.

### 1. Action Area

For listed species assessment purposes, the "action area" is considered to be the area affected directly or indirectly by the Federal action and not merely the immediate area involved in the action. At the initial Level I screening assessment, broadly described taxa are considered and thus the screening conservatively assumes that listed species within those broad groups are co-located with the pesticide treatment area. This means that terrestrial plants and wildlife are assumed to be located on or adjacent to the treated site, and aquatic organisms are assumed to be located in a surface water body adjacent to the treated site. The assessment also assumes that the listed species are located within an assumed area that has the relatively highest potential exposure to the pesticide, and that exposures are likely to decrease with distance from the treatment area.

Aquashade, Admiral, and AlgaBlocker are used in ornamental ponds, recreational man-made ponds, closed system ponds, golf course lakes, rearing lakes for fish, fountains containing fish, and recirculated or artificial waterscapes. Therefore, these products are only applied to confined water bodies.

### 2. Taxa Potentially at Risk

In this assessment, it is concluded that the only species potentially at risk are aquatic plants from direct effects if they occur in treated ponds and lakes, and aquatic or terrestrial animals that depend on the vegetation in the treated water bodies.

### 3. Discussion of Risk Quotients

No direct effects on listed animal species are predicted. No exposure to terrestrial plants was expected, so no terrestrial plant RQs were calculated. Even though no RQs were calculated

for aquatic plants, it is assumed they will be affected, since they are the target organisms to be controlled.

4. Probit Dose Response Relationship

Since no mortality was observed in the standard studies, no probit analysis was performed.

5. Data Related to Under-represented Taxa

The screening level assessment relies on RQ calculations that use toxicity endpoints selected from the most sensitive species tested within broad taxonomic groups. There may be situations in which additional effects data from one or more sources may suggest that a given suite of listed taxa may be more or less sensitive than suggested by the effects data used for RQ calculations. No such relationships were found in this analysis.

6. Implications of Sublethal Effects

The Level I screening assessment normally relies on the acute mortality endpoint as well as a suite of sublethal responses to the chemical stressor. EFED decided that, based on its mode of action, the low chronic toxicity of at least one of the dyes to mammals, low potential for exposure based on use pattern, and its history as a human food dye, Aquashade did not need chronic toxicity studies. Sublethal effects were not analyzed.

7. Indirect Effects Analysis

This assessment indicates that Aquashade may impact listed invertebrates, fish and amphibians that depend upon submerged aquatic plants and algae. There is also a potential for indirect effects to listed terrestrial birds and mammals that occur near treated water bodies, and that depend on submerged aquatic vegetation or algae for food or other survival or reproductive processes. The likelihood of potential indirect effects has not been quantified or refined. Such refinement could incorporate information on specific use locations relative to specific locations of listed species. Subsequent refinement may also include additional analyses on potential indirect effects to listed species.

8. Critical Habitat

Aquashade may impact invertebrates and fish that depend upon the target species (submerged aquatic plants and algae). The likelihood of potential indirect effects has not been quantified. Subsequent refinement may include additional analyses on potential indirect effects to listed species.

9. Co-occurrence Analysis

The goal of the analysis for co-location is to determine whether sites of pesticide use are geographically associated with known locations of listed species. Listed species potentially at risk include aquatic plants, and animals that depend on aquatic effects from indirect effects. Aquashade's use pattern makes it (potentially) possible that it will be used in every county in the

United States. Therefore, EFED's database to find which counties harbor which listed species ("LOCATES") was not used. However, there are possible effects to

D. Description of Assumptions, Limitations, Uncertainties, Strength, and Data Gaps

1. Environmental fate

*Data Source*

The environmental fate assessment is only at the qualitative level. Structure-activity estimations (EPIWIN) of physical and chemical properties/characteristics are uncertain. EPIWIN is not suitable for estimating properties of salts of large anions, such as in acid dyes. Therefore, this qualitative environmental fate assessment was primarily based on the general, known behavior of dyes in the environment and not on the specific behavior of Acid Blue 9 and Acid Yellow 23.

*Reaction kinetics and transformation products*

Although indirect photolysis was identified as a major route of dissipation of the dyes in aquatic media, the identity of the photoproducts (and hence, their toxicity) is not known. The rate of phototransformation is expected to be a function of geographical location and season. Biotransformation under anaerobic conditions may also contribute to the dissipation of the dyes and can occur predominately in sediment, but the chemical identity of the metabolites is not known.

*Impurities*

The characterization of the physical and chemical properties required under FIFRA were not done with a 100% pure dye. The Acid Blue 9 is reported to be 50% pure and Acid Yellow 23 as being 28%. Although the presence of chemical impurities affect the physical and chemical properties of a chemical substance, the extent by which they affect those of the dyes is not known. The physical and chemical properties were submitted in support of Aquashade (23.6% Acid Blue 9 and 2.39 Acid Yellow 2.39% registration In is unclear if these percentages take into account the purity of each dye. Furthermore, the percent purity of each dye in the other products is unknown.

Given the low exposure of these dyes in the environment, **when used as pesticides**, no Subdivision N guideline studies are needed for the intended uses. However, if new uses are petitioned, the EFED will reevaluate the status of data requirements.

2 Ecological toxicity

The assessment assumes that Aquashade and related products will be used strictly in accordance with label instructions. It assumes that there will be no large spills of the product

The ecological toxicity studies were not conducted with the individual dyes, but with the mixture of the two dyes as the test substance (23.6% Acid Blue 9 and 2.36 % Acid Yellow 23, An

Aquashade end-use product). There are two other products that contain a higher percent of the dyes than the tested substance, namely Admiral Liquid (15.31% Acid Blue 9 and 1.00% Acid Yellow 23) and Admiral WSP (49. % Acid Blue 9 and  % Acid Yellow 23). Therefore, the ecological toxicity of these products is not known. However, the maximum estimated environmental concentration resulting from direct application to a water body is below the Level of Concern.

The chronic toxicity RQs cannot be calculated, because there are no studies on chronic risk with the pesticide. The low acute toxicity values, along with testing with the Acid Yellow dye 23 with mammals, and the mode of action of Aquashade and other products, suggest low chronic toxicity and EFED has not required any chronic studies.

The Aquashade product that was used in the "basic six" studies was labeled as containing 23.63 % Acid Blue 9 and 2.39% Acid Yellow 23. The study authors stated, "The test substance characterization provided by the sponsor indicated a purity of 13.9% azure blue dye." Therefore the exact composition of the toxicant that was used in the studies is not certain. The use of the word "azure" could describe color, but also the word "azure" could be associated with a series of structurally related dyes that are totally different from Acid Blue 9 or Acid Yellow 23.

## V. **Literature Cited**

### E. Open Literature


Books

Lynch, D.G. "Estimating the Properties of Synthetic Organic Dyes", in **Handbook of Property Estimation Methods for Chemicals- Environmental Health Sciences**, Edited by Robert S. Boethling and Donald Mackay. Published by Lewis Publishers, Boca Raton, FL, 2000; Pages 447- 467. And pertinent references therein.

Marmion, D,M. **Handbook of U.S. Colorants- Food, Drugs**, **and Medical Devices**, Third Edition., 1991. Published by John Wiley and Sons, New York

Helz, G.R., Zepp, R.G., and Crosby, D.G, Editors. 1994. **Aquatic and surface photochemistry**. Lewis Publishers, Boca Raton, Florida.

Journal Articles

Jank. M., Köser, H., Lücking, F., Martienssen, M., and Wittchen, S. 1998, *"Decolorization and Degradation of Erioglaucine (Acid Blue 9) Dye in Wastewater"*, Environmental Technology, v.19(7), pp.741-747.

Weber, E.J. and Adams, R.L. 1995. *"Chemical and Sediment-Mediated Reduction of the Azo Dye Disperse Blue 79"*, Environ. Sci. Technol. v.29, pp. 1163-1170.

Baughman, G.L. 1995. *"Fate of azo dyes in aquatic systems. Part 3: The role of suspended sediments in adsorption and reaction of acid and direct dyes*", Dyes and Pigments, v.27, pp. 197-210.

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Tai, W.T., Chang, C.Y, Ing, C.H., and Chang,C.F. 2004. *"Adsorption of acid dyes from aqueous solutions on activated bleaching earth"*, J. Colloid and Interface Science, vol. 275, pp 72-78.

Mon, J., Flury, M., and Harsh, J.B. 2005 ."*Sorption of four triarylmethane dyes in a sandy soil determined by batch and column experiments"*, Geoderma. in press.

Mon, J., Flury. M., and Harsh. 2005 *"A quantitative structure-activity relationship (OSAR) analysis of triarylmethane dye tracers"*, Journal of Hydrology, in press.

Characterization of Physical and Chemical Properties Submitted to the Agency in Support of Aquashade (23.6% and 2.39% Acid Yellow 23:

Ellison. F.E. 1994. "Aqua Shade Blue 9'- Physical and Chemical Characteristics of Aqua Shade Blue 9: Color, Physical State, Odor, Boiling Point, Specific Gravity, Solubility, Vapor Pressure, pH, and Stability". Performed by Case Consulting Laboratories, Inc. Whippany, NJ and sponsored by Applied Biochemistry, Inc., Milwaukee, WI. Report Dated September 12, 1994. **MRID 43503401**

Ellison. F.E. 1994. "Aqua Shade Yellow 23"- Physical and Chemical Characteristics of Aqua Shade Blue 9: Color, Physical State, Odor, Boiling Point, Specific Gravity, Solubility, Vapor Pressure, pH, and Stability". Performed by Case Consulting Laboratories, Inc. Whippany, NJ and sponsored by Applied Biochemistry, Inc., Milwaukee, WI. Report Dated September 12, 1994. **MRID 43503402**

5. Data Generation

Structure-activity Estimates:
EPI (Estimation Programs Interface) Suite™ (formerly known as EPIWIN):
http://www.epa.gov/opptintr/exposure/docs/episuite.htm

SRC. 2004. Syracuse Research Corporation. Interactive Physical Properties (PHYSPROP) Database Demo. Search terms: FD&C Blue. No. 1, FD&C Red No. 40, FD&C Yellow No. 5. (November 28, 2004) http://www.syrres.com/esc/physdemo.htm

E. U.S. Environmental Protection Agency Documents:

"Robust Summaries & Test Plans: C.I. Acid Yellow 23 (FD&C Yellow 5)", Submitted ti the Agency by the International Association of Color Manufacturers on March 10, 2004. http://www.epa.gov/chemrtk/ciacdylo/c15133tc.htm

"Background Document for Identification and Listing of Deferred Dye and Pigment Wastes, U.S. Environmental Protection Agency, Office of Solid Waste, Washington, D.C., June 1999.

US Environmental Protection Agency. 1998. Guidelines for Ecological Risk Assessment. Risk Assessment Forum. EPA/630/R-95/002F, April 1998

U.S. EPA. 1993. U. S. Environmental Protection Agency. Wildlife Exposure Factors Handbook. Volume I of II. EPA/600/R-93/187a. Office of Research and Development, Washington, D. C. 20460.

E. U.S. Environmental Protection Agency, Office of Pesticide Programs Documents:

Memorandum: “Review of Phase 4 List D Package for Aquashade” (EFGWB # 93-0119, 93-0120; Chemical # 110301 and 110302; Case # 819437 and 819438; DP Barcode D184289 and D184278). Dated February 4, 1993.

“Reassessment of the Exemption from the Requirement of a Tolerance for the FDA-Certified Color Additives FD&C Blue No.1, FD&C Red No. 40, and FD&C Yellow No. 5 (Tartrazine)”. U.S. Environmental Protection Agency, Office of Pesticides Programs. December 21, 2004

D. Internet Resources

Food and Drugs Administration:
http://www.cfsan.fda.gov/~dms/opa-col2.html

Color Index International:
http://www.colour-index.org/

Additional information was also obtained from TOXNET.
http://toxnet.nlm.nih.gov/

## Appendix A- Data Gaps

*Environmental Fate*

All of the Subdivision N, Environmental Fate Data Requirements maybe waived because of very low risk potential. They were identified as "Reserved" in 1993 (Memorandum: "Review of Phase 4 List D Package for Aquashade"; EFGWB #93-0119, 93-0120; Chemicals 110301 and 110302; Case # 819437 and 819438; DP Barcode D184289 and D184278(. Dated February 4, 1993), pending on the results of required ecological toxicity studies.

There are two major uncertainties on the environmental fate behavior of Acid Blue 9 and Acid Yellow 23:

(1) Structure-activity estimates of physical, chemical, and environmental fate behavior are not reliable for salts of large anions, such as Acid Yellow 23 and Acid Blue 9.

(2) The major route of transformation of dyes such as Acid Blue 9 and Acid Yellow 23 is indirect photolysis. However, the chemical identity of the photoproducts of these two dyes are not known. Biotransformation under anaerobic conditions may also contribute to the dissipation of the dyes in water-sediments. The chemical identity of the metabolites is not known.

However, after the review of the ecological toxicity studies and the risk assessment, the environmental fate data requirements placed in 1993 as "Reserved' can be waived. The Environmental Fate and Effects Division is not requiring any new environmental fate data for the two dyes individually or for any of the end-use products (Aquashade, Aquashade OA, Pond Care AlgaeBlocker, Admiral Liquid, and Admiral WSP).

*Ecological Toxicity*

b. Effects

The only EFED ecological toxicity data available is for the Aquashade product (23.6% Acid Blue 9 and 2.36% Acid Yellow 23) as the test substance. There are no EFED ecological toxicity data for Admiral Liquid or Admiral WSP, but HED received a study that found a laboratory rat LD50 equal to or more than 5,000 mg/kg. However, since organisms will be only exposed to either 1 or 2 ppm in the environment, this uncertainty is minor.

No chronic studies have been required, so it is not known what would happen over an extended exposure period. No studies on aquatic plants have been required, because they are the targets of Aquashade and it is assumed that all submerged aquatic plants in the treated pond will be killed. No terrestrial plant studies have been required, because there is little expected exposure. An exception would be if any of these products

might be applied to water used for irrigation. If that occurred, exposure to terrestrial plants may occur.

It is not known what would happen to plants on the margins of a treated pond or growing as emergent vegetation in the shallows of the pond if the technical product or the end-use product was applied to them. Some labels warn that directly applying the products to emerged or terrestrial plants may cause burning.

Because of the mode of action (blocking light that enters bodies of water), the low acute toxicity of this pesticide, and the relatively limited area involved in treatment, it is believed that additional studies are not needed.

## Appendix B Environmental Fate

No Subdivision N Environmental Fate studies were submitted, as these data requirements were waived in 1993. Therefore, no data from guideline studies are included in this Appendix.

Although the Environmental Fate and Effects Division does not usually use open literature data in its assessments, published data on the chemistry and behavior of dyes in aquatic systems was used to supplement the environmental fate assessment. Most of the data comes from structurally related dyes. Acid Blue 9 and Acid Yellow 23 are also food, drugs, and cosmetic colorants regulated by the Food and Drugs Administration.

The environmental fate behavior of dyes such as Acid Yellow 23 and Acid Blue 9 can be summarized as follows:

The dyes are fully ionized in the environmentally significant pH range of 5 to 9.

1. There no hydrolyzable groups in these two dyes and, therefore, abiotic hydrolysis is not an important degradation pathway in the environment.

2. Direct photolysis is slow, as indicated by the fading of the dyes in homogeneous aqueous media exposed to sunlight. However, indirect photolysis has been identified as the major route of degradation in environmental media.

3. The dyes are resistant to biodegradation under aerobic conditions. Structural related dyes are difficult to biodegrade in activated sludge. However, redox reactions under anaerobic conditions appear to degrade the dyes, at least to some extent.

4. The two dyes are highly hydrophilic and do not have the potential to bioaccumulate in fish.

## Appendix C Aquatic Exposure and Modeling

The end-use products containing Acid Blue 9 and Acid Yellow 23 are directly applied to a confined water body and at the dose specified in the labels to attain a concentration of 1 ppm or 2 ppm depending on the target pest. Because there are no runoff or spray drift components, the use of the simulation models GENEEC or PRZM and EXAMS is not appropriate.

Aquatic exposure was based on the target concentration specified in the product labels, depending on the weed to be controlled. The maximum target concentration is 2 ppm (2 mg/L) which is the concentration used to estimate risk for both aquatic and terrestrial animals

## Appendix D. Ecological Toxicity and Uncertainty in Ecological Toxicity Data

EFED requires the submission of six studies for all chemicals that are submitted for registration. Which additional data that is required depends upon the circumstances of the chemical and its use pattern. Birds are used as surrogates for terrestrial phase amphibians and reptiles. Freshwater fish are used as surrogates for aquatic phase amphibians.

The end-use products are not applied to a terrestrial environment. However, terrestrial animals (*e.g.*, mammals and birds) are exposed to the dyes when they use treated water as the source of drinking water.

In addition to data that was submitted by the registrants, EFED also reviewed studies from the open literature. These studies were obtained through the ORD Middle Ecological Division's ECOTOX literature search and retrieval program managed in Duluth, Minnesota (http://www.epa.gov/ecotox). These data are consistent with the contention that the dyes found in Aquashade and the other end-use products are practically nontoxic to the organisms tested.

**Table D-1. Summary of most sensitive acute toxicity endpoints multiplied by 13% based on percent purity of the dyes in aquashade.**

| Species | Study Type[1] | Source | Extrapolated Endpoint |
|---|---|---|---|
| Bobwhite quail | Acute Avian Oral | 433367-01 | $LD_{50}$ & NOAEL $\geq$ 2250 x 0.13 $\geq$ 292.5 mg/kg |
| Mallard duck | Acute Avian Oral | 433367-02 | $LD_{50}$ & NOAEL $\geq$ 2250 x 0.13 $\geq$ 292.5 mg/kg |
| Bobwhite quail | Avian Dietary | 435034-03 | $LC_{50}$ & NOAEL5 $\geq$ 620 x 0.13 $\geq$ 80.6 ppm |
| Mallard duck | Avian Dietary | 435034-04 | $LC_{50}$ & NOAEL5 $\geq$ 620 x 0.13 $\geq$ 80.6 ppm |
| Bluegill sunfish | Acute Toxicity | 432975-02 | $LC_{50}$ & NOAEC $\geq$ 96 x 0.13 $\geq$ 12.48 mg/L |
| Rainbow trout | Acute Toxicity | 432975-01 | $LC_{50}$ & NOAEC $\geq$ 96 x 0.13 $\geq$ 12.48 mg/L |
| *Daphnia magna* | Acute Toxicity | 432975-03 | $LC_{50}$ & NOAEC $\geq$ 97 x 0.13 $\geq$ 12.61 mg/L |

[1] All original tests were performed with the Typical End-use Product, Aquashade. No testing was performed on birds, fish and invertebrates with the end-use products Admiral Liquid and Admiral WSP, which have a higher percentage of the dyes than the tested Aquashade product.

In addition to the uncertainty about the possible percent purity adjustment, Admiral WSP (49.27% acid blue 9 and 3.27% acid yellow 23) has about 50% greater concentration of dyes than Aquashade. To take this higher concentration into account, each toxicity endpoint could be multiplied by 2 resulting in an extrapolated avian acute NOAEL $\geq$ 146 mg/kg and aquatic organism NOAEC $\geq$6.3 mg/L.

| Table D-2. EcoEffects Data including Data from Open Literature | | |
|---|---|---|
| **Guideline / MRID or Citation** | **Discussion** | **Results** |
| 71-1a / 433367-01 Acute oral bobwhite quail [1]<br><br>71-1a / 433367-02 Acute oral mallard duck [1] | The formulated product (23.6% acid blue and 2.39% acid yellow dyes) was used. The study concentration was not adjusted for the purity of the active ingredients (the two dyes). The results are milligrams of the end-use product. Five experimental doses were used - 292, 486, 810, 1350, 2250 mg/L. There were no mortalities or other adverse effects. | $LD_{50} \geq$ 2250 mg eup /kg<br><br>NOAEC = 2250 mg eup /kg |
| 71-2b / 435034-03 Acute dietary bobwhite quail [1]<br><br>71-2a / 435034-04 Acute dietary waterfowl- mallard duck [1] | The formulated product (23.6% acid blue and 2.39% acid yellow dyes) was used. The study concentration was not adjusted for the purity of the active ingredients (the two dyes). The results are parts per million of the end-use product. Five experimental doses were used - 562, 1000, 1780, 3160, and 5620 ppm. There were no mortalities or other adverse effects. | $LC_{50}$ eup $\geq$ 5,620 ppm<br><br>NOAEC eup = 5,620 ppm eup |
| 71-3 / 452811-01 Acute Wild Mammal Toxicity using the Laboratory rat [2] | The study used Admiral WSP, a purple powder formulation. The dyes were not otherwise identified. A group of 7-10 week old rats (5/sex) received a single dose of 5000 mg/kg by gavage and were observed for 14 days. There were no mortalities, therefore the chemical was categorized as "Toxicity Category IV." | Oral $LD_{50}$ ♂ & ♀ > 5,000 mg eup /kg<br><br>Oral $LD_{50}$ ♂ & ♀ $\approx$ 2,500 mg a.i. /kg |
| 83-4 / 434109-01 Reproduction study using the laboratory rat [3] | Three generations of Long-Evans rats were given tartrazine (92% a.i.) at 7.5, 75, 225, and 750 mg/kg/day in their diet. This is consistent with no effects and addresses whether acid yellow 23 causes reproductive toxicity. There were no adverse treatment related effects. | NOAEL ♂ & ♀ = 750 mg/kg/day |
| 72-1c / 432975-01 Acute coldwater fish- Rainbow trout [1]<br><br>72-1a / 432975-02 Acute warmwater fish- bluegill sunfish [1] | The formulated product (23.6% acid blue and 2.39% acid yellow dyes) was placed in the water of aquaria. The study concentration was not adjusted for the purity of the active ingredients (the two dyes). The results are milligrams of the end-use product. The only dose used was 96 mg/L. | $LC_{50}$ eup $\geq$ 96 mg/L<br>NOAEC eup = 96 mg/L |

| Table D-2. EcoEffects Data including Data from Open Literature | | |
|---|---|---|
| **Guideline / MRID or Citation** | **Discussion** | **Results** |
| 72-2a / 432975-03<br>Freshwater invertebrate *Daphnia magna* [1] | The formulated product (23.6% acid blue and 2.39% acid yellow dyes) was placed in the water of aquaria. The study concentration was not adjusted for the purity of the active ingredients (the two dyes). The results are milligrams of the end-use product. The only dose used was 97 mg/L. | $LC_{50}$ eup ≥ 97 mg/L<br>NOAEC eup = 97 mg/L |
| 72-1a<br>Warne & Schifko, 1999<br>FW Cladoceran | Freshwater cladocerans (*Ceriodaphnia dubia*) were exposed to a dye for two days. The $EC_{50}$ put the chemical in the category "practically nontoxic." | $EC_{50}$ = 5707 mg/L |
| 72-3a<br>Estuarine/Marine Acute Toxicity with Sheepshead Minnow, Oyster, Mysids | Exposure to marine habitats not expected | N/A |
| 72-4a<br>Freshwater Fish Early Life Stage Fathead minnow | Exposure to marine habitats not expected | N/A |
| 72-4b<br>Freshwater Invertebrate Life Cycle- Water flea | Chronic effects not expected based on mode of action and lack of effects in acute tests. | N/A |
| 123-1a<br>Tier II Terrestrial Plant Seedling Emergence (GF 871) | If the label is changed and the water is not used for irrigation, exposure to terrestrial plants would be unlikely. | Reserved |
| 123-1b<br>Tier II Terrestrial Plant Vegetative Vigor (GF 871) | If the label is changed and the water is not used for irrigation, exposure to terrestrial plants would be unlikely. | Reserved |
| 123-2<br>Tier II Aquatic Plant Studies | Aquatic plants are the targets. Therefore, testing is not required. Release from treated ponds to other water bodies considered a very low probability. | N/A |

| Table D-2. EcoEffects Data including Data from Open Literature | | |
|---|---|---|
| **Guideline / MRID or Citation** | **Discussion** | **Results** |
| 141-1<br>Honey Bee Acute Contact Toxicity | Mode of action suggests low likelihood of toxicity. The method of application minimizes exposure to terrestrial insects. | N/A |
| Nonguideline<br>Spencer, 1984<br>Crayfish | Crayfish were exposed to 3 levels of Aquashade for 5 days. The oxygen use was effected. The study was categorized as "slightly toxic." | NOAEL = 15 mg/L |
| Nonguideline<br>Borzelleca and Hallagan, 1988.<br>Chronic toxicity/ carcinogenicity [3] | Laboratory rats were fed 4 levels of yellow 5 for 900 days. The study was categorized as "practically nontoxic." | NOAEL = 984 mg/kg/day<br>LOAEL = 2641 mg/kg/day |
| Nonguideline<br>Borzelleca, *et al.*, 1990<br>Chronic toxicity/ carcinogenicity in mice [3] | House mice (*Mus musculus*) were given food with 3 levels of dye in their diet for 720 days. The study was characterized as "practically nontoxic." | 7354 mg/kg/day |

[1] The study was done with Aquashade. The doses were calculated with the end-use product. The percentages of the dyes making up liquid is not clear.
[2] The study was done with Admiral Lake Colorant WSP. The $LD_{50}$ is based on the dry weight of the end-use product. The composition of the powder was not determined, but it is usually 49.62% blue dye and 3.05% yellow dye. The $LD_{50}$ a.i. cannot be determined, but is probably 50% of the stated >5,000 mg/kg.
[3] The study was done with FD&C yellow dye (acid yellow 23 or 5), which is not an end-use products.

**Uncertainty in toxicity information**

Most of the toxicity results used for risk assessment were based on effects testing done with Aquashade (23.6% acid blue 9 and 2.39% acid yellow 23) except the acute mammal $LD_{50}$ was done with Admiral WSP and the reproductive mammalian NOAEL was done with acid yellow.

There is some uncertainty in this approach because 1) the percent of the dyes in this formulation is unclear, and 2) some formulations have a higher percent of each dye than Aquashade, and it is not clear if the doses were expressed in terms of end-use product or active ingredients. The dyes in Aquashade are reported as being 13% pure. To demonstrate the degree of uncertainty, the toxicity values may be multiplied by 0.1 to

take these percentages into account. Note that the risk assessment was conducted with actual toxicity test results, not with these adjusted toxicity values.

**Table F-1. Summary of acute toxicity endpoints multiplied by 13% to composite for the possibility of the tested materials being only 13% pure.**

| Species | Study Type[1] | Source | Extrapolated Endpoint |
|---|---|---|---|
| Bobwhite quail | Acute Avian Oral | 433367-01 | $LD_{50}$ & NOAEL ≥ 2250 x 0.13 ≥ 292.5 mg/kg |
| Mallard duck | Acute Avian Oral | 433367-02 | $LD_{50}$ & NOAEL ≥ 2250 x 0.13 ≥ 292.5 mg/kg |
| Bobwhite quail | Avian Dietary | 435034-03 | $LC_{50}$ & NOAEL ≥ 620 x 0.13 ≥ 80.6 ppm |
| Mallard duck | Avian Dietary | 435034-04 | $LC_{50}$ & NOAEL ≥ 620 x 0.13 ≥ 80.6 ppm |
| Bluegill sunfish | Acute Toxicity | 432975-02 | $LC_{50}$ & NOAEC ≥ 96 x 0.13 ≥ 12.48 mg/L |
| Rainbow trout | Acute Toxicity | 432975-01 | $LC_{50}$ & NOAEC ≥ 96 x 0.13 ≥ 12.48 mg/L |
| *Daphnia magna* | Acute Toxicity | 432975-03 | $LC_{50}$ & NOAEC ≥ 97 x 0.13 ≥ 12.61 mg/L |

[1] All original tests were performed with the typical end-use product, Aquashade. No testing was performed on birds, fish and invertebrates with the end-use products Admiral Liquid and Admiral WSP, which have a higher percentage of the dyes than the tested Aquashade product.

In addition to the uncertainty about the possible percent purity adjustment, Admiral WSP (49.27% acid blue 9 and 3.27% acid yellow 23) has about 50% greater concentration of dyes than Aquashade. To take this higher concentration into account, each toxicity endpoint could be multiplied by 2 resulting in an extrapolated avian acute NOAEL ≥ 146 mg/kg and aquatic organism NOAEC ≥6.3 mg/L.

## Appendix E Status of Data Requirements

1. *Environmental Fate*

Subdivision N, Environmental Fate Data Requirements- Status of Data Requirements

| Data Requirement | Status in 1993 | Status after the Risk Assessment for Reregistration | Additional Data Required |
|---|---|---|---|
| 161-1 [Abiotic] Hydrolysis | Reserved | May be Waived | No |
| 161-2 [Direct] Photolysis in Water | Reserved | May be Waived | No |
| 161-3 Photolysis on Soil | Not Required | Not Required | No |
| 162-1 Aerobic Soil Metabolism | Not Required | Not Required | No |
| 162-2 Anaerobic Aquatic Metabolism | Not Required | Not Required | No |
| 162-3 Anaerobic Aquatic Metabolism | Reserved | May be Waived | No |
| 162-4 Aerobic Aquatic Metabolism | Reserved | May be Waived | No |
| 163-1 Mobility in Soils/Sediments | Reserved | May be Waived | No |
| 164-1 Terrestrial Field Dissipation | Not Required | Not Required | No |
| 164-2 Aquatic Field Dissipation | Reserved | May be Waived | No |
| 165-4 Bioaccumulation in Fish | Reserved | May be Waived<br>The Log Kow does not trigger this data requirement | No |

**Ecological effects data requirements for pesticide registration of Aquashade**
PC Code: 110303 (a mixture of 110301 and 110302), only TEPs were studied

| Guideline Number | DATA REQUIREMENTS (Not Reserved) | FULFILLS REQ'S (Y/N) | MRID | DATE | STATUS |
|---|---|---|---|---|---|
| | Acute Avian Oral Quail TEP | Y | 433367-01 | 9-7-94 | Acceptable |
| 71-1(b) | Acute Avian Oral Duck TEP | Y | 433367-02 | 9-7-94 | Acceptable |
| 71-2(b) | Avian Dietary/Quail TEP | Y | 435034-03 | 4-26-95 | Acceptable |
| 71-2(b) | Avian Dietary/Duck TEP | Y | 435034-04 | 4-26-95 | Acceptable |
| 71-3 | Wild Mammal Toxicity[a] | Y | 452811-01 | 12-5-00 | Acceptable |
| 71-4(a) | Avian Reproductive/Quail | Waived | | | |
| 71-4(b) | Avian Reproductive/Duck | Waived | | | |
| 72-1(b) | Acute Toxicity Bluegill TEP | Y | 432975-02 | 4-25-95 | Acceptable |
| 72-1(d) | Acute Toxicity Rainbow Trout TEP | Y | 432975-01 | 4-25-95 | Acceptable |

**Ecological effects data requirements for pesticide registration of Aquashade**
PC Code: 110303 (a mixture of 110301 and 110302), only TEPs were studied

| Guideline Number | DATA REQUIREMENTS (Not Reserved) | FULFILLS REQ'S (Y/N) | MRID | DATE | STATUS |
|---|---|---|---|---|---|
| 72-2(b) | Acute Invertebrate Toxicity TEP | Y | 432975-03 | 4-25-95 | Acceptable |

[a] This study was performed with Admiral WSP

No additional effects data are being required.

## Appendix F Environmental Fate Bibliography


Books

Lynch, D.G. "Estimating the Properties of Synthetic Organic Dyes", in **Handbook of Property Estimation Methods for Chemicals- Environmental Health Sciences**, Edited by Robert S. Boethling and Donald Mackay. Published by Lewis Publishers, Boca Raton, FL; Pages 447- 467. And pertinent references therein.

Marmion, D,M. **Handbook of U.S. Colorants- Food, Drugs**, **and Medical Devices**, Third Edition., 1991. Published by John Wiley and Sons, New York

Helz, G.R., Zepp, R.G., and Crosby, D.G, Editors. 1994. **Aquatic and surface photochemistry**. Lewis Publishers, Boca Raton, Florida.

Journal Articles

Jank. M., Köser, H., Lücking, F., Martienssen, M., and Wittchen, S. 1998, "*Decolorization and Degradation of Erioglaucine (Acid Blue 9) Dye in Wastewater,"* Environmental Technology, v.19(7), pp.741-747.

Weber, E.J. and Adams, R.L. 1995. "*Chemical and Sediment-Mediated Reduction of the Azo Dye Disperse Blue 79,"* Environ. Sci. Technol. v.29, pp. 1163-1170.

Baughman, G.L. 1995. "*Fate of azo dyes in aquatic systems. Part 3: The role of suspended sediments in adsorption and reaction of acid and direct dyes,*" Dyes and Pigments, v.27, pp. 197-210.

Brown, D. and Laboureur, P. 1983, "*The Degradation of Dyestuffs: Part I- Primary biodegradation under anaerobic conditions,"* Chemosphere, v.122, pp. 397-404.

Baran,W., Makowski, A., Wardas, W. 2003. "*The influence of $FeCl_3$ on the photocatalytic degradation of dissolved azo dyes in aqueous $TiO_2$ suspensions,"* Chemosphere, v.53, pp 82-95.

Tai, W.T., Chang, C.Y, Ing, C.H., and Chang,C.F. 2004. "*Adsorption of acid dyes from aqueous solutions on activated bleaching earth,"* J. Colloid and Interface Science, vol. 275, pp 72-78.

Mon, J., Flury, M., and Harsh, J.B. 2005 ."*Sorption of four triarylmethane dyes in a sandy soil determined by batch and column experiments,"* Geoderma. in press.

Mon, J., Flury. M., and Harsh. 2005 "A quantitative structure-activity relationship (OSAR) analysis of triarylmethane dye tracers," Journal of Hydrology, in press.

Appendix G Ecological Toxicity Bibliography

## Appendix H. Endangered and Threatened Species

EFED also assessed the potential for Aquashade and other end-use products containing the Acid Yellow 23 and Acid Blue 9 dyes to potentially cause harm to endangered species.

### Terrestrial Animals

Listed endangered or threatened species of birds, mammals and reptiles are not expected to be affected directly because exposure does not exceed the endangered species LOC. However, terrestrial animals may be affected indirectly if aquatic plants that are necessary for their survival are eliminated. The degree to which this might occur depends on the extent the treated water bodies are a critical component of their food supply.

### Terrestrial Plants

Terrestrial plants have the potential for exposure if water treated with Aquashade or the other dye containing end-use products are used for irrigation. If irrigation is precluded from use on the labels, EFED concludes there is virtually no potential for exposure to terrestrial plants.

### Aquatic Animals

Listed species of fish, invertebrates or amphibians are not expected to be affected directly because the exposure does not exceed the endangered species LOC. However, aquatic animals dwelling in treated ponds might have the potential for harm if the plants on which they depend for food and habitat are eliminated. The likelihood of this occurring depends on if any listed aquatic animal species would occur in these highly managed, often manmade, normally relatively small water bodies with usually no outflow, or if any, a very small amount.

### Aquatic Plants

Listed species of aquatic plants which begin their life stages as submerged might be harmed if water bodies in which they are growing are treated while they are submerged.

EFED has not fully analyzed the potential for endangered species to be harmed through use of Aquashade and other end-use products. Such analysis would focus on the overlap of specific species with potentially treated water bodies, and in the case of indirect effects, the likelihood that the treated water body comprises enough of a component of the listed species food or habitat base to actually impact the listed species, if that food or habitat was lost.

## Appendix K. Risk Quotient Calculation and Interpretation

Risk characterization integrates the results of the exposure and ecotoxicity data to evaluate the likelihood of adverse ecological effects. The means of this integration is called the quotient method. Risk quotients (RQs) are calculated by dividing exposure estimates by acute and chronic ecotoxicity value and, in this case, it is a deterministic approach.

RQ = EXPOSURE/TOXICITY

RQs are then compared to OPP's levels of concern (LOCs). These LOCs are used by OPP to analyze potential risk to nontarget organisms and the need to consider regulatory action. The criteria indicate that a pesticide used as directed has the potential to cause adverse effects on nontarget organisms. LOCs currently address the following risk presumption categories: (1) **acute** -- potential for acute risk; regulatory action may be warranted in addition to restricted use classification, (2) **acute restricted use** -- the potential for acute risk, but may be mitigated through restricted use classification, (3) **acute endangered species** - endangered species may be adversely affected, and (4) **chronic risk** - the potential for chronic risk; regulatory action may be warranted. Currently, EFED does not perform assessments for chronic risk to plants, acute or chronic risks to nontarget insects, or chronic risk from granular/bait formulations to birds or mammals.

The ecotoxicity test values (measurement endpoints) used in the acute and chronic risk quotients are derived from required studies. Examples of ecotoxicity values derived from short-term laboratory studies that assess acute effects are: (1) $LC_{50}$ (fish and birds), (2) $LD_{50}$ (birds and mammals), (3) $EC_{50}$ (aquatic plants and aquatic invertebrates) and (4) EC25 (terrestrial plants). Examples of toxicity test effect levels derived from the results of long-term laboratory studies that assess chronic effects are: (1) LOAEC (birds, fish, and aquatic invertebrates), and (2) NOAEC (birds, fish and aquatic invertebrates). For birds and mammals, the NOAEC generally is used as the ecotoxicity test value in assessing chronic effects, although other values may be used when justified. However, the NOAEC is used if the measurement end point is production of offspring or survival

**Risk presumptions and the corresponding RQs and LOCs, are tabulated below.**

**Table K-1: Risk presumptions for terrestrial animals**

| Risk Presumption | RQ | LOC |
|---|---|---|
| Birds | | |
| Acute High Risk | EEC[1]/LC50 or LD50/sqft[2] or LD50/day[3] | 0.5 |
| Acute Restricted Use | EEC/LC50 or LD50/sqft or LD50/day (or LD50 < 50 mg/kg) | 0.2 |
| Acute Endangered Species | EEC/LC50 or LD50/sqft or LD50/day | 0.1 |
| Chronic Risk | EEC/NOAEC | 1 |
| Wild Mammals | | |
| Acute High Risk | EEC/LC50 or LD50/sqft or LD50/day | 0.5 |
| Acute Restricted Use | EEC/LC50 or LD50/sqft or LD50/day (or LD50 < 50 mg/kg) | 0.2 |
| Acute Endangered Species | EEC/LC50 or LD50/sqft or LD50/day | 0.1 |
| Chronic Risk | EEC/NOAEC | 1 |

[1] abbreviation for Estimated Environmental Concentration (ppm) on avian/mammalian food items

[2] $mg/ft^2$

[3] mg of toxicant consumed/day LD50 * wt. of a bird

| Table K-2 . Risk Presumptions for Aquatic Animals | | |
|---|---|---|
| **Risk Presumption** | **RQ** | **LOC** |
| Acute Risk | EEC[1]/LC50 or EC50 | 0.5 |
| Acute Restricted Use | EEC/LC50 or EC50 | 0.1 |
| Acute Endangered Species | EEC/LC50 or EC50 | 0.05 |
| Chronic Risk | EEC/MATC or NOAEC | 1 |

[1] EEC = (ppm or ppb) in water

| Table K-3. Risk presumptions for plants | | |
|---|---|---|
| **Risk Presumption** | **RQ** | **LOC** |
| Terrestrial and Semi-Aquatic Plants | | |
| Acute Risk | EEC[1]/EC25 | 1 |
| Acute Endangered Species | EEC/EC05 or NOAEC | 1 |
| Aquatic Plants | | |
| Acute High Risk | EEC[2]/EC50 | 1 |
| Acute Endangered Species | EEC/EC05 or NOAEC | 1 |

[1] EEC = lbs ai/A
[2] EEC = (ppb/ppm) in water

**Appendix L. Papers that were accepted for ECOTOX but that were not used in the risk assessment science chapter. These papers passed the screen for consideration in ecological risk assessment, but were ultimately not used because they did not add to or change the risk presumption.**

**Acceptable for ECOTOX and OPP**

Borzelleca, J. F., Depukat, K., and Hallagan, J. B. 1990. Lifetime Toxicity/Carcinogenicity Studies of FD-and-C Blue No. 1 (Brilliant Blue FCF) in Rats and Mice. *Food Chem.Toxicol.* 28: 221-234. EcoReference No.: 76018
Chemical of Concern: AQS; Habitat: T; Effect Codes: GRO,POCM,MOR,BEH,CEL.

Borzelleca, J. F. and Hallagan, J. B. 1988. A Chronic Toxicity/Carcinogenicity Study of FD & C Yellow No. 5 (Tartrazine) in Mice. *Food Chem.Toxicol.* 26: 189-194.EcoReference No.: 76019
Chemical of Concern: AQS; Habitat: T; Effect Codes: MOR,GRO,BEH,CEL.

Collins, T. F. X., Black, T. N., Brown, L. H., and Bulhack, P. 1990. Study of the Teratogenic Potential of FD and C Yellow No. 5 when Given by Gavage to Rats. *Food Chem.Toxicol.* 28: 821-827. EcoReference No.: 76021
Chemical of Concern: AQS; Habitat: T; Effect Codes: GRO,BEH,PHY,REP,MOR.

Collins, T. F. X., Black, T. N., O'Donnell, M. W. Jr., and Bulhack, P. 1992. Study of the Teratogenic Potential of FD & C Yellow No. 5 when Given in Drinking-Water. *Food Chem.Toxicol.* 30: 263-268.EcoReference No.: 76022
Chemical of Concern: AQS; Habitat: T; Effect Codes: GRO,BEH,PHY,REP,MOR.

Ershoff, B. H. 1977. Effects of Diet on Growth and Survival of Rats Fed Toxic Levels of Tartrazine (FD & C Yellow No. 5) and Sunset Yellow FCF (FD & C Yellow No. 6. *J.Nutr.* 107: 822-828. EcoReference No.: 76051
Chemical of Concern: AQS; Habitat: T; Effect Codes: GRO,MOR.

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