# NTP REPORT ON CARCINOGENS BACKGROUND DOCUMENT for DISPERSE BLUE 1

## FINAL
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Prepared by

Integrated Laboratory Systems
Post Office Box 13501
Research Triangle Park, North Carolina 27709
NIEHS Contract No. N01-ES-25346

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## NTP Report on Carcinogens Listing for Disperse Blue 1

### Carcinogenicity

Disperse Blue 1 is *reasonably anticipated to be a human carcinogen* based on evidence of malignant tumor formation in experimental animals to an unusual degree with regard to incidence, site and type of tumor (NTP 299, 1986), and because it is a member of a well defined, structurally related class of substances listed in a previous Annual Report on Carcinogens as either known to be human carcinogens, or reasonably anticipated to be human carcinogens (NTP, 1994).

Disperse Blue 1 was a carcinogen in both sexes of F344/N rats; the findings in $B6C3F_1$ mice were equivocal. Clear evidence of carcinogenicity in the urinary bladder of F344/N rats was evidenced by increased incidences of transitional cell neoplasms, leiomyomas or leiomyosarcomas, and squamous cell neoplasms (NTP 299, 1986). Transitional cell and squamous cell tumors of the urinary bladder of male F344 rats receiving Disperse Blue 1 were also noted in a small study reported by Burnett and Squire (1986).

### Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

Neoplasms in the urinary bladder of rats were associated with dose-dependent incidences of calculi that were thought to induce chronic inflammation and cell proliferation. Calculi and resulting inflammatory and proliferative lesions also occurred in the urinary bladders of both sexes of $B6C3F_1$ mice in the absence of significantly increased incidences of neoplastic lesions (NTP 299, 1986).

Data on the genotoxicity of Disperse Blue 1 indicate that it induced a weak positive response in *Salmonella* (Brown and Brown, 1976; cited by IARC V.48, 1990), DNA damage (SCE) and chromosomal aberrations in CHO cells (Anderson et al., 1990), *tk* gene mutations in mouse lymphoma L5178Y cells (Myhr et al., 1990; cited by Cosmetic Ingredient Review Board, 1995), and morphological transformation in Balb/c 3T3 mouse cells (Matthews et al., 1993). NTP studies of structurally related compounds, nitro and aminoanthraquinones, have demonstrated that each compound tested has some activity as a mutagen. Most compounds of this class that have been the subjects of two-year studies have also been found to be carcinogenic in one or more species. Sites of tumor development include the urinary bladder in rats and the liver of both rats and mice as well as other sites. Data are available to suggest that transitional cell and squamous cell tumors induced by Disperse Blue 1 in the urinary bladder would not occur in humans exposed to amounts of Disperse Blue 1 insufficient to also cause bladder calculi. However, compelling data which demonstrate a causal relationship between urinary bladder calculi and leiomyomas and leiomyosarcomas have not been sufficiently developed to establish that Disperse Blue 1 would not be *reasonably anticipated to be a human carcinogen.*

**Listing Criteria from the Report on Carcinogens, Eighth Edition**

*Known To Be A Human Carcinogen:*

There is sufficient evidence of carcinogenicity from studies in humans, which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

*Reasonably Anticipated To Be A Human Carcinogen:*

There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible but that alternative explanations, such as chance, bias, or confounding factors, could not adequately be excluded; or

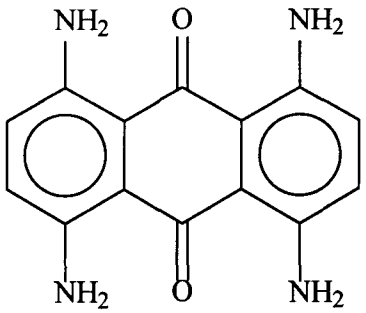
There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor, or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals; however, the agent, substance or mixture belongs to a well-defined, structurally related class of substances whose members are listed in a previous Report on Carcinogens as either a known to be human carcinogen or reasonably anticipated to be a human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgement, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

# 1.0 INTRODUCTION

Disperse Blue 1
[2475-45-8]




## 1.1 Chemical Identification

Disperse Blue 1 ($C_{14}H_{12}N_4O_2$, mol. wt. = 268.28) is also called:

9,10-Anthracenedione, 1,4,5,8-tetraamino- (9CI)
Anthraquinone, 1,4,5,8-tetraamino- (8CI)
Acetate Blue G
Acetoquinone Blue L
Acetoquinone Blue R
Acetylon Fast Blue G
Amacel Blue GG
Amacel Pure Blue B
Anthraquinone, 1,4,5,8-tetramino-
Artisil Blue Sap
Artisil Blue Sap Conc.
Brasilazet Blue GR
Celanthrene Pure Blue BRS
Celliton Blue BB-CF
Celliton Blue Extra
Celliton Blue G
Celliton Blue GA-CF
C.I. 64500
Cibacete Sapphire Blue G
Cibacet Sapphire Blue G
C.I. Disperse Blue 1
C.I. Disperse Blue No. 1
Cilla Blue Extra
C.I. Solvent Blue 18
Diacelliton Fast Blue R
Disperse Blue No. 1
Duranol Brilliant Blue CB
Fenacet Blue G
Grasol Blue 2GS
Kayalon Fast Blue BR
Microsetile Blue EB
Miketon Fast Blue
Miketon Fast Blue B
Nacelan Blue G
NCI-C54900
Neosetile Blue EB
Nyloquinone blue 2j
Nyloquinone Blue 2J
Oracet Sapphire Blue G
Perliton Blue B
Serinyl blue 2g
Serinyl Blue 2G
Serinyl blue 3g
Serinyl Blue 3G
Setacyl Blue 3GN
Setacyl Blue 2GS
Setacyl Blue 2GS II
Solvent Blue 18
Supracet Brilliant Blue 2GN
Supracet Deep Blue R
1,4,5,8-Tetraamino-9,10-anthracenedione
1,4,5,8-Tetraaminoanthraquinone
1,4,5,8-Tetraminoanthraquinone

## 1.2 Physical-Chemical Properties

Disperse Blue 1 contains approximately 50% 1,4,5,8-tetraaminoanthraquinone, 30% other compounds structurally related to 1,4,5,8-tetraaminoanthraquinone, and 20% water. When heated to decompositions, Disperse Blue 1 emits toxic fumes of nitrogen oxides ($NO_x$).

| Property | Information | Reference |
|---|---|---|
| Color | Blue-black | NTP (299, 1986) |
| Physical State | Microcrystalline powder | NTP (199, 1986) |
| Melting Point, °C | 332 | NTP (299, 1986) |
| Solubility: | | |
| Water at 25 °C | Very slightly soluble (30 µg/L) | Kuroiwa and Ogasawara (1973; cited by IARC, 1990) |
| Organic Solvents | Soluble in: acetone, ethanol, and Cellosolve<br>Slightly Soluble in: benzene and linseed oil | Enviro Control (1977; cited by IARC, 1990) |
| Partition Coefficients: | | |
| Log octanol/water (log P) | -0.96 | Baughman and Perenich (1988; cited by IARC, 1990) |
| Vapor Pressure, torr | $1.37 \times 10^{-5}$ (calculated by the Working Group) | Nishida et al. (1977; cited by IARC, 1990) |

## 1.3 Identification of Structural Analogues and Metabolites

Structural analogues and metabolites discussed in this report include the following:

2-Aminoanthraquinone ($C_{14}H_{10}NO_2$, CASRN 82-45-1, mol. wt. = 224.24)
1-Amino-2-methylanthraquinone ($C_{15}H_{11}NO_2$, mol. wt. = 237.26)
2-Methyl-1-nitroanthraquinone ($C_{15}H_9NO_4$, mol. wt. = 267.24).

Physical-chemical properties were found for 2-aminoanthraquinone.

### 2-Aminoanthraquinone

| Property | Information | Reference(s) |
|---|---|---|
| Solubility: | | |
| Water | Practically insoluble in water | Budavari (1996) |
| Organic Solvents | Soluble in ethanol, acetone, benzene, chloroform, glacial acetic acid, and hydrochloric acid | Weast and Astle (1980); Budavari (1996) |

## 1.4 Report Organization

The rest of this report is organized into six additional sections (2.0 Human Exposure, 3.0 Human Studies, 4.0 Mammalian Carcinogenicity, 5.0 Genotoxicity, 6.0 Other Relevant Studies, and 7.0 References) and two appendixes. Appendix A describes the literature search in online databases, and Appendix B provides explanatory information for Figure 5-1.

## 2.0 HUMAN EXPOSURE

### 2.1 Use

Disperse Blue 1 is not known to occur as a natural product but is produced and used as a mixture of chemicals. It is an aminoanthraquinone-based dye used in semi-permanent hair color formulations (nonoxidative hair dyes, colors and rinses [Cosmetic Ingredient Review Board, 1995]) and in coloring fabrics and plastics. Commercial preparations of Disperse Blue 1 contain approximately equal amounts of dye and lignosulfonate dispersants. In the mid 1980s, it was reported that over 3 million people in the United States ca. 1984 were using semi-permanent hair color preparations containing Disperse Blue 1 at concentrations of less than 1% (NTP 299, 1986). Disperse Blue 1 has been used as a fabric dye for nylon, cellulose acetate and triacetate, polyester, and acrylic fibers. It has also been used for surface dyeing of thermoplastics and as a solvent dye in cellulose acetate plastics (NTP 299, 1986; IARC, 1990). Disperse Blue 1 is also used to dye fur, solvents, resins, and lacquers (Green, 1990; cited by Haws et al., 1994).

### 2.2 Production

Disperse Blue 1 is no longer produced in the United States (NTP 299, 1986). Production of Disperse Blue 1 in the United States was reported to be 159 tons in 1972. Separate figures were not reported after 1972, but production of all Disperse Blue dyes was approximately 6030, 9940, and 5740 metric tons in 1975, 1980, and 1986, respectively (IARC, 1990). The United States imported approximately 7700 pounds of Disperse Blue 1 in 1980, 8800 pounds in 1981, 8085 pounds in 1982, and 13756 pounds in 1983 (USITCa, 1981-1984). Chem Sources (1996) identified three U.S. suppliers of Disperse Blue 1 in 1996.

### 2.3 Environmental Exposure

The primary routes of potential human exposure to Disperse Blue 1 are inhalation and dermal contact. Since Disperse Blue 1 is used in hair dyes, potential exposure by these routes exists for personnel producing and applying such products (IARC, 1990), as well as for those subjected to their administration. The National Occupational Hazard Survey, conducted by NIOSH from 1972 to 1974, estimated that 482 workers were potentially exposed to Disperse Blue 1 in the workplace (NIOSH, 1976). The National Occupational Exposure Survey (1981-1983) indicated that 43,522 workers, including 32,059 women, were potentially exposed to Disperse Blue 1 (NIOSH, 1990).

### 2.4 Regulations

OSHA regulates Disperse Blue 1 under the Hazard Communication Standard and as a chemical hazard in laboratories. There is no OSHA permissible exposure limit (PEL) and no ACGIH or NIOSH workroom air exposure criteria. Disperse Blue 1 was listed on the EPA TSCA Chemical Inventory List (1992) (CHEMTOX, 1996). An October 1996 search of the Federal Register (1988-1996) full text and Federal Register Abstracts (1977-1993) found no mention of Disperse Blue 1 except for the 1986 announcement of the availability of the NTP bioassay report (NTP 299, 1986).

REGULATIONS[a]

| | Regulatory Action | Effect of Regulation/Other Comments |
|---|---|---|
| OSHA | 29 CFR 1910.1200. Promulgated 2/15/89. OSH Act: Hazard Communication Standard. | Requires chemical manufacturers and importers and all employers to assess chemical hazards and to provide information to employees. Hazard Communication program to include labels, material safety data sheets, and worker training. |
| | 29 CFR 1910.1450. Promulgated 1/31/90. Amended 55 FR 12111, 3/30/90. OSH Act: Final rule for occupational exposure to hazardous chemicals in laboratories. | As a select carcinogen (IARC Group 2B), Disperse Blue 1 is included as a chemical hazard in laboratories. Employers are required to provide employee information and training and a Chemical Hygiene Plan. |

[a] The regulations in this table have been updated through the 1996 Code of Federal Regulations: 40 CFR, July 1, 1996; 21 CFR, April 1, 1996; 29 CFR, July 1, 1996.

## 3.0 HUMAN STUDIES

No human studies of Disperse Blue 1 were found. However, IARC (1993) noted that hairdressers may be exposed to permanent and semipermanent hair colorants, including aromatic amines, nitro-substituted aromatic amines and aminophenols, aminoanthraquinones [such as Disperse Blue 1], and azo dyes. In addition, they may be exposed to nitrosamines and numerous other chemicals including volatile solvents, propellants, and formaldehyde. Barbers traditionally have not used as wide a variety of products. IARC (1993) stated that "There is consistent evidence from five (all from Europe) of the six large cohort studies of an excess risk for cancer of the urinary bladder in male hairdressers and barbers." The overall risk relative to that in the general population amounted to approximately 1.6 in these studies, with a significant increase in urinary bladder carcinogenesis observed in 3 of 6 studies.

IARC (1993), following review of epidemiologic studies that have investigated the association between cancer and occupation as a hairdresser or barber, or between cancer and personal use of hair dyes, made the following conclusions:

> "There is *limited evidence* that occupation as a hairdresser or barber entails exposures that are carcinogenic" and "Occupation as a hairdresser or barber entails exposures that *are probably carcinogenic* (Group 2A)."
>
> "There is *inadequate evidence* that personal use of hair colourants entails exposures that are carcinogenic" and "Personal use of hair colourants *cannot be evaluated as to its carcinogenicity (Group 3)*."

The Cosmetic Ingredient Review Board (1995) concluded that "the relevance of the occupational data and conclusion for individuals using hair dyes is unclear."

## 4.0 MAMMALIAN CARCINOGENICITY

Experimental details discussed in this section are presented in Table 4-1.

**Summary:** There is "sufficient evidence" for the carcinogenicity of Disperse Blue 1 in experimental animals (IARC, 1990). NTP (299, 1986) concluded that for $B6C3F_1$ mice exposed to Disperse Blue 1 in the diet for 2 years, there was no evidence of carcinogenicity in female mice and equivocal evidence in males. There, however, was clear evidence of carcinogenicity for Disperse Blue 1 administered in the diet for 2 years in male and female F344/N rats based on the increased occurrence of transitional cell papillomas and carcinomas, of leiomyomas and leiomyosarcomas, and of squamous cell carcinomas and papillomas of the urinary bladder. In the groups of rats in which urinary bladder neoplasms were increased, a positive association existed between the presence of calculi and transitional cell neoplasms in male and female rats, leiomyomas or leiomyosarcomas (combined) in female rats, and squamous cell neoplasms in male rats administered Disperse Blue 1 in the diet for 2 years.

### 4.1 Mice

NTP (299, 1986) concluded that the evidence for the carcinogenicity of Disperse Blue 1 in male $B6C3F_1$ mice (7 weeks of age) administered Disperse Blue 1 in the diet (600, 1200, or 2500 ppm) for 2 years was equivocal: a marginally increased incidence of alveolar/broncheolar adenomas or carcinomas (combined) occurred in high dose males and a marginally increased incidence of hepatocellular adenomas or carcinomas (combined) occurred in dosed male mice. No evidence for the carcinogenicity of Disperse Blue 1 was found in female $B6C3F_1$ mice.

### 4.2 Rats

The NTP (299, 1986) concluded from a 2-year bioassay on Disperse Blue 1 administered in the diet (1250, 2500, or 5000 ppm) to F344/N rats beginning at age 7 weeks that there was clear evidence for carcinogenicity in male and female F344/N rats. This conclusion was based on the increased occurrence of transitional cell papillomas and carcinomas, of leiomyomas and leiomyosarcomas, and of squamous cell carcinomas and papillomas of the urinary bladder. In the groups of rats in which urinary bladder neoplasms were increased, a positive association existed between the presence of calculi and transitional cell neoplasms in male and female rats, leiomyomas or leiomyosarcomas (combined) in female rats, and squamous cell neoplasms in male rats administered Disperse Blue 1 in the diet for 2 years. In male rats, the occurrence of pancreatic islet cell adenomas or carcinomas (combined) was significantly increased in high-dose males.

Burnett and Squire (1986) fed Disperse Blue 1 to groups of 20, 20, and 60 F344/N rats/sex at dietary levels of 0.01, and 0.1% (100 and 1000 ppm) for 19 months and 1% (10,000 ppm) for 6 months, respectively. At six months, 12 rats/sex in the control group and 10 rats/sex in the high dose group were killed. Following necropsies at 6 months, the remaining rats in the high dose group were placed on control diet for the remainder of the study and urinary bladder calculi were removed from 15 rats/sex. "The object of the surgical removal of calculi after 6 months was to determine, whether, without further exposure to the test compound, the persisting

foreign material influences carcinogenesis." Urinary bladder lesions (squamous cell papillomas and transitional cell carcinoma) were seen only at the 1% level. At 6 months, 1/10 males and 1/10 females showed squamous cell papillomas and 1/10 males had a transitional cell carcinoma. The results at termination of the study (19 months) showed that tumors occurred in twice as many males and females that had surgical removal of calculi than those that were continued on control diet without surgical removal of calculi at six months. The authors stated that these findings "...emphasize the role of irritation or foreign matter in bladder carcinogenesis." In contrast to the NTP studies, sarcomas of the bladder wall as well as epithelial tumors were not observed by Burnett and Squire (1986). "We believe it is likely that the sarcoma response in the NTP study resulted from physical contact with the dye rather than from systemic exposure" (Burnett and Squire, 1986).

## Table 4-1. Mammalian Carcinogenicity of Disperse Blue 1

| Age, Strain, Species | No./Sex Exposed and Controls | Chemical Form and Purity | Dose Route | Duration of Exposure | Results/Comments | Reference |
|---|---|---|---|---|---|---|
| **Mice** | | | | | | |
| 7-wk-old B6C3F$_1$ mice | 50M, 50F<br>Controls: 50M;50F | Disperse Blue 1 (com. grade)[a] | Fed diets containing 600, 1200, or 2500 ppm | 104 wk | **Equivocal evidence for tumors listed. Survival:** A significant trend ($p = 0.028$) toward lower survival in M observed when early deaths excluded. Trend due to the decreased survival of the HD group (20/50) relative to that of the LD (30/50) and MD groups (35/50). No significant difference observed between any groups of F.<br><br>**Liver: Adenomas or carcinomas (combined)** in M (21/50 LD; 20/50 MD; 16/50 HD vs. 9/50 C).<br><br>**Bronchus and lung: Alveolar/broncheolar adenomas or carcinomas** in M (9/49 LD; 5/50 MD; 11/50 HD vs. 4/50 C).<br><br>**Urinary bladder: Gross calculi** in M (0/49 LD; 16/50 MD; 39/50 HD vs. 0/50 C) and F (0/50 LD; 0/50 MD; 30/50 HD, vs. 0/49 C). Significant increase in MD and HD M and HD F ($p<0.001$) vs. controls.<br><br>NTP summarized that there was equivocal evidence of carcinogenicity in male mice based on the marginally increased incidences of combined liver tumors in dosed males and on the marginally increased incidence of combined alveolar/broncheolar tumors in HD male mice. NTP found no evidence of carcinogenicity in female mice. | NTP (299, 1986) |
| **Rats** | | | | | | |
| 7-wk-old Fischer 344/N rats | 50M, 50F<br>Controls: 50M; 50F | Disperse Blue 1 (com. grade)[a] | Fed diets containing 1250, 2500, or 5000 ppm | 103 wk | **Positive for tumors listed.**<br>**Survival:** M (4/50) and F (15/50) HD groups were significantly reduced compared to that of controls (M, 29/50; F, 36/50), while survival of MD M (20/50) was marginally reduced after 100 wk.<br>**Urinary bladder (all lesions had significantly positive trends): Squamous-cell papilloma or carcinoma (combined)** in M (0/50LD; 2/50 MD; 4/49 HD[b] vs. 0/49 C) and F (0/50 LD; 1/50 MD; 11/48 HD[c] vs. 0/48 C). | NTP (299, 1986) |

## Table 4-1. Mammalian Carcinogenicity of Disperse Blue 1 (Continued)

| Age, Strain, Species | No./Sex Exposed and Controls | Chemical Form and Purity | Dose Route | Duration of Exposure | Results/Comments | Reference |
|---|---|---|---|---|---|---|
| 7-wk-old Fischer 344/N rats (contd.) | 50M, 50F<br>Controls:<br>50M; 50F | Disperse Blue 1 (com. grade)[a] | Fed diets containing 1250, 2500, or 5000 ppm | 103 wk | **Urinary bladder (contd.): Transitional cell papilloma or carcinoma** in M (0/50 LD; 10/50 MD[c]; 11/49 HD[c] vs. 0/49 C) and F (0/50 LD; 15/50 MD[c]; 21/48 HD[c] vs. 0/48 C).<br>**Leiomyoma or leiomyosarcoma** in M (0/50 LD; 7/50 MD[c]; 41/49 HD[c] vs. 0/49 C) and F (0/50 LD; 3/50 MD; 26/48 HD[c] vs. 0/48 C).<br>**Gross calculi** in M (0/50 LD; 16/50 MD[c]; 21/49 HD[c] vs. 0/49 C) and F (0/50 LD; 12/50 MD[c]; 37/48 HD[c] vs.0/48 C).<br>**Pancreatic islet cell adenoma or carcinoma (combined):** M (2/50 LD; 5/50 MD; 3/50 HD vs. 1/49 C). Significant in HD M by survival-adjusted analyses.<br>**Adenomas or carcinomas (combined)** in M (2/50 LD; 5/50 MD; 3/50 HD vs. 1/49 C). Significant in HD M by survival-adjusted analyses.<br>NTP concluded that there was clear evidence of carcinogenicity for M and F F344/N rats based on the urinary bladder tumors listed: Transitional cell neoplasms in M and F rats, leiomyomas, or leiomyosarcomas (combined) in F rats, and squamous cell neoplasms in M rats. | NTP (299, 1986) (contd.) |

## Table 4-1. Mammalian Carcinogenicity of Disperse Blue 1 (Continued)

| Age, Strain, Species | No./Sex Exposed and Controls | Chemical Form and Purity | Dose Route | Duration of Exposure | Results/Comments | Reference |
|---|---|---|---|---|---|---|
| Weanling Fischer 344 rats | 20M, 20F (LD)<br>20M, 20F (MD)<br>60M, 60F (HD)<br>Controls:<br>40M; 40F | Disperse Blue 1 (containing ~50% lignosulfonate dispersants) | 0.01, 0.1, and 1.0% in diet (100, 1,000 and 10,000 ppm) | At 6 mo, 10 rats/sex in high-dose group and 12 rats/sex in control group killed for necropsy. The remaining HD rats were placed on control diet for the remainder of the study.<br>Bladder stones were surgically removed from 15 rats/sex in HD group at 6 mo and placed on control diet for the remainder of the study (13 mo).<br>LD and MD fed Disperse Blue 1 until end of study (termination at 19 mo) | **Bladder lesions and calculi (observed only in HD): 6 mo**<br>**Calculi** in M (7/10 HD) and F (fewer than male, incidence not given).<br>**Squamous cell papilloma** in M (1/10) and F (1/10).<br>**Transitional cell carcinoma** in F (1/10).<br>**EFFECTS OF SURGERY**<br>**Without surgery:**<br>**Calculi** in M (7/14) and F (4/22).<br>**Transitional cell papillomas** in M (1/14) and F (3/22).<br>**With surgery:**<br>**Calculi** in M (2/15) and F (8/15).<br>**Transitional cell papilloma** in M (1/15) and F (3/15).<br>**Transitional cell carcinoma** in M (1/15) and F (2/15).<br>**Squamous cell carcinoma** in M (1/15).<br>**Conclusions:** Although statistical significance was not reported, Burnett and Squire found that transitional cell or squamous cell neoplasms of the urinary bladder were associated with dye deposits or calculi formation in F rats. The authors stated that "surgery apparently stimulated the development of even more calculi" than did the test compound in F. In addition, at termination of the study (19 mo), tumors occurred in twice as many M and F rats that had had the surgery than in those that had not. The authors stated that these findings "...emphasize the role of irritation or foreign matter in bladder carcinogenesis." | Burnett and Squire (1986) |

Abbreviations: C = control(s); com. = commercial; F = female(s); HD = high dose; LD = low dose; M = male(s); MD = mid dose

[a] Disperse Blue 1 (com. Grade) without lignosulfonate dispersants; 1,4,5,8-tetraaminoanthraquinone, 19.5% water, 30% impurities (primarily an isomer of tetraaminoanthraquinone and a nitroanthraquinone isomer).

[b] Significant by life-tables test only.

[c] Significant vs. Control at $p<0.05$.

## 5.0 GENOTOXICITY

Studies of the genotoxic effects of Disperse Blue 1 are summarized in Table 5-1.

**Summary:** Disperse Blue 1 has not been extensively tested for genotoxicity [see Genetic Activity Profile, Figure 5-1 (data limited to IARC, 1990)]. However, it was found to be mutagenic in *Salmonella typhimurium* strains TA1537 (+/- S9), TA97 (- S9), TA98 (+/- S9) and in TA 1535 (one +S9, another -S9), and in mouse lymphoma cells in the absence of S9; to induce sister chromatid exchanges (SCE) and chromosome aberrations in Chinese hamster ovary (CHO) cells with and without S9; and to induce cell transformations in BALB/c-3T3 cells in the absence of S9. It did not induce heritable translocations in Sprague-Dawley rats. Unless otherwise specified, rat liver S9 was the source of metabolic activation *in vitro.*

Information for studies reviewed by IARC (1990) was often limited to qualitative data. Pertinent information on study design, doses tested, chemical purity, etc., was generally not provided.

### 5.1 Noneukaryotic Systems

Brown and Brown (1976; cited by IARC, 1990) found that Disperse Blue 1 tested at doses from 100 to 2000 μg/plate (0.38 to 7.46 μmol/plate) induced a weak positive response in *S. typhimurium* strain TA1537 both with and without metabolic activation [LED = 100 μg/plate (0.38 μmol/plate)] and a positive response in strain TA1535 without metabolic activation only [LED = 2000 μg/plate (7.46 μmol/plate)]. Strains TA98, TA100, and TA1538 were all negative both with and without S9 activation [HID = 2000 μg/plate (7.46 μmol/plate)]. The NTP (299, 1986) reported that, using the pre-incubation assay, Disperse Blue 1 at 0.1 to 2000 μg/plate (0.0004 to 7.46 μmol/plate) induced a mutagenic response in *S. typhimurium* strains TA98 with and without S9, TA1535 with S9, and TA97 without S9 [LED = 10 μg/plate (0.04 μmol/plate)].

### 5.2 Mammalian Systems *In Vitro*

#### 5.2.1 DNA Damage

Anderson et al. (1990) reported that Disperse Blue 1 from 0.33 to 10.0 μg/mL (1.2 to 37.3 μM) induced a positive increase in SCE in CHO cells in both the presence (2-hour exposure) and absence (26-hour exposure) of exogenous metabolic activation [LED = 3.3 μg/mL (12 μM)].

#### 5.2.2 Gene Mutations

Myhr et al. (1990; cited by the Cosmetic Ingredient Review Board, 1995) found that a 4-hour exposure to Disperse Blue 1 at 2.5 to 160 μg/mL (9.3 to 596 μM) induced mutations at the *tk* locus in mouse lymphoma L5178Y cells, only in the absence of metabolic activation (LED not provided). The dye was tested in two trials.

#### 5.2.3 Chromosomal Damage

Anderson et al. (1990) reported that Disperse Blue 1 tested at 5.0 to 10.0 μg/mL (19 to 37.3 μM) for 2 hours in the presence of S9 activation and 8 hours in the absence of S9 activation induced a positive increase in chromosomal aberrations in CHO cells both with and without S9 [LED = 7.5 μg/mL (28 μM)].

5.2.4 Cell Transformation

Matthews et al. (1993) found that exposure for 48 hours to Disperse Blue 1 at doses from 117 to 3700 $\mu$M induced morphological transformations in BALB/c-3T3 cells (clone A-31-1-13) in the absence of metabolic activation [LED = 117 $\mu$M]. Significant levels of type I and type II foci, but not type III, were consistently induced in two trials.

**5.3 Mammalian Systems *In Vivo***

Burnett et al. (1981; cited by the Cosmetic Ingredient Review Board, 1995) reported that Disperse Blue 1 did not induce heritable translocations in male Sprague-Dawley rats administered 0.12% (1200 $\mu$g/L, 4500 $\mu$M) dye topically (dose per animal not provided) twice a week for 10 weeks. No effects were observed following three mating cycles.

## Table 5-1. Summary of Disperse Blue 1 Genotoxicity Studies

| Test System | Biological Endpoint | S9 Activation | Purity | Doses Used | Endpoint Response | Comments | Reference |
|---|---|---|---|---|---|---|---|
| **5.1 Noneukaryote Systems** | | | | | | | |
| *Salmonella typhimurium* strains TA100, TA98, TA1535, TA1537, and TA1538 | *his* gene mutations | +/- | n.p. | 100 to 2000 μg/plate (0.38 to 7.46 μmol/plate) | positive/ positive | Weakly mutagenic in strain TA1537 in the presence and absence of metabolic activation [LED = 100 μg/plate (0.38 μmol/plate)] and TA1535 without S9 [LED= 2000 μg/mL (7.46 μmol/plate)]. For all other strains - HID = 2000 μg/plate (7.46 μmol/plate). | Brown and Brown (1976; cited by IARC, 1990) |
| *S. typhimurium* strains TA100, TA97, TA98, and TA1535 | *his* gene mutations (pre-incubation assay) | +/- | 100% | 0.1 to 2000 μg/plate (0.0004 to 7.46 μmol/ plate) +/- rat or hamster S9 | positive/ positive | Positive in the pre-incubation assay in strains TA98 (+/-S9), TA1535 (+S9), and TA97 (-S9). [LED = 10 μg/plate (0.04 μmol/plate)]. For all other strains - HID = 2000 μg/plate (7.46 μmol/plate). | NTP (299, 1986) |
| **5.2 Mammalian Systems *In Vitro*** | | | | | | | |
| *5.2.1 DNA Damage* | | | | | | | |
| Chinese hamster ovary (CHO) cells | sister chromatid exchanges (SCE) | +/- | 100% | 0.33 to 10.0 μg/mL (1.2 to 37.3 μM) for 26 h -S9 and 1.0 to 10.0 μg/mL (3.7 to 37.3 μM) for 2 h +S9 | positive/ positive | LED = 3.3 μg/mL (12 μM) | Anderson et al. (1990) |
| *5.2.2 Gene Mutations* | | | | | | | |
| mouse lymphoma L5178Y cells | *tk* gene mutations | - | 100% | 2.5 to 160 μg/mL (9.3 to 596 μM) for 4 h | positive | Two positive trials were conducted. The LED was not provided. | Myhr et al. (1990; cited by Cosmetic Ingredient Review Board, 1995) |
| *5.2.3 Chromosomal Damage* | | | | | | | |
| CHO cells | chromosome aberrations | +/- | 100% | 5.0 to 10.0 μg/mL (19 to 37.3 μM) for 8 h -S9 and for 2 h +S9 | positive/ positive | LED = 7.5 μg/mL (28.0 μM) | Anderson et al. (1990) |

## Table 5-1. Summary of Disperse Blue 1 Genotoxicity Studies (Continued)

| Test System | Biological Endpoint | S9 Activation | Purity | Doses Used | Endpoint Response | Comments | Reference |
|---|---|---|---|---|---|---|---|
| *5.2.4 Cell Transformation* | | | | | | | |
| Balb/c-3T3 cells clone A-31-1-13 | morphological transformation | NA | n.p. | 117 to 3700 $\mu$M for 48 h | positive | Significant levels of type I and type II foci, but not type III, were consistently induced over two trials (LED = 117 $\mu$M). | Matthews et al. (1993) |
| **5.3 Mammalian Systems *In Vivo*** | | | | | | | |
| male Sprague-Dawley rats | heritable translocation | NA | n.p. | 0.12% (1200 $\mu$g/mL; 4500 $\mu$M) topically applied twice a wk for 10 wk | negative | No effects were observed following three mating cycles. The dose per animal was not provided. | Burnett et al. (1981; cited by Cosmetic Ingredient Review Board, 1995) |

Abbreviations: HID = highest ineffective dose; LED = lowest effective dose; NA = not applicable; n.p. = not provided

**Figure 5-1. Genetic Activity Profile of Disperse Blue 1 (Data limited to IARC, 1990)**

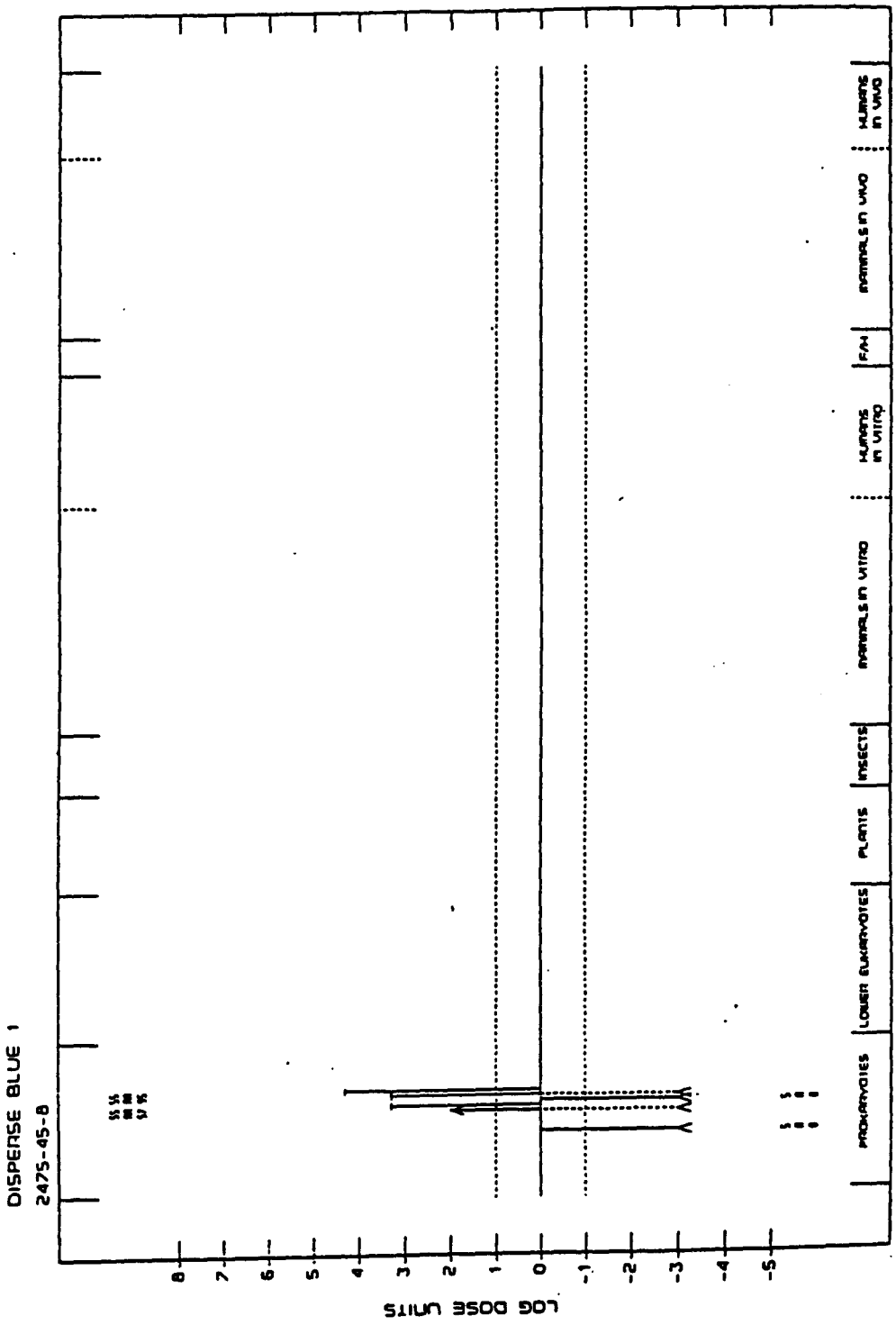
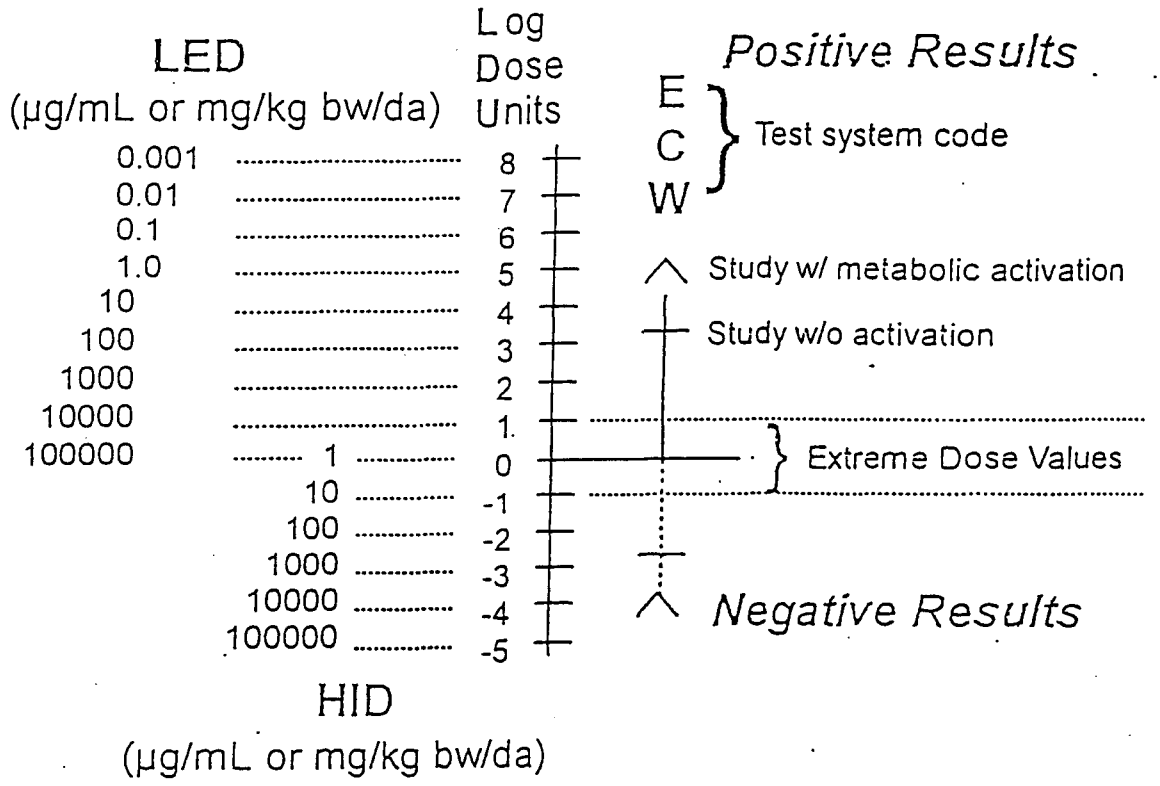


**Figure 5-2. Schematic View of a Genetic Activity Profile (GAP)**




A schematic view of a Genetic Activity Profile (GAP) representing four studies (two positive and two negative) for an example short-term test, ECW. Either the lowest effective dose (LED) or the highest ineffective dose (HID) is recorded from each study, and a simple mathematical transformation (as illustrated above) is used to convert LED or HID values into the logarithmic dose unit (LDU) values plotted in a GAP. For each test, the average of the LDUs of the majority call is plotted using a solid vertical bar drawn from the origin. A dashed vertical bar indicates studies that conflict with the majority call for the test. Note in cases where there are an equal number of positive and negative studies, as shown here, the overall call is determined positive. The GAP methodology and database have been reported previously (Garrett et al., 1984; Waters et al., 1988, 1991).

Garrett, N.E., H.F. Stack, M.R. Gross, and M.D. Waters. 1984. An analysis of the spectra of genetic activity produced by known or suspected human carcinogens. Mutat. Res. 143:89-111.

Waters, M.D., H.F. Stack, A.L. Brady, P.H.M. Lohman, L. Haroun, and H. Vainio. 1988. Use of computerized data listings and activity profiles of genetic and related effects in the review of 195 compounds. Mutat. Res. 205:295-312.

Waters, M.D., H.F. Stack, N.E. Garrett, and M.A. Jackson. 1991. The genetic activity profile database. Environ. Health Perspect. 96:41-45.

# 6.0 OTHER RELEVANT DATA

## 6.1 Absorption, Distribution, Metabolism and Excretion

No data were found.

## 6.2 Pharmacokinetics

No data were found.

## 6.3 Modes of Action

The potential mechanism of Disperse Blue 1-induced urinary bladder carcinogenesis found in male and female rats has been reviewed by Haws et al. (1994) and the Cosmetic Ingredient Review Board (1995) and is discussed below.

**Summary:** In the presence and/or absence of metabolic activation, Disperse Blue 1 has been positive for the induction of gene mutations in *Salmonella* and DNA damage, chromosomal aberrations, gene mutations, and transformation in mammalian cells. In rodents, Disperse Blue 1 or Disperse Blue 1 containing approximately 50% lignosulfonate dispersant may have operated via a secondary carcinogenic mechanism of action that involved the formation of urinary calculi to induce urinary bladder tumors in male and female rats. Although the occurrence of urinary bladder calculi and other nonneoplastic changes occurred in $B6C3F_1$ mice fed Disperse Blue 1 for 2 years, there was no evidence of significantly increased incidences of neoplastic lesions of the urinary bladder or kidney in mice of either sex. Disperse Blue 1 has been listed as 1 of 31 chemicals that induce a highly restricted pattern of tumor induction (in a specific site in a single sex in a single species). Chemicals in this category of rodent carcinogens may have a lower probability for potential human carcinogenicity as opposed to trans-species rodent carcinogens.

### 6.3.1 Genotoxicity

Although not adequately characterized for *in vivo* genotoxicity, Disperse Blue 1 has been positive for the induction of gene mutations in *Salmonella* and DNA damage, chromosomal aberrations, gene mutations, and transformation in mammalian cells, with and/or without metabolic activation. (See Section 5.0.)

### 6.3.2 Differences in Rodent Bladder Tumorigenesis

The NTP (299, 1986) and Burnett and Squire (1986) conducted rodent bioassays on Disperse Blue 1 and Disperse Blue 1 containing approximately 50% lignosulfonate dispersant, respectively (see Section 4.0 for details of the studies). Following review of the data, Haws et al. (1994) proposed that Disperse Blue 1 operated via a secondary carcinogenic mechanism of action that involved the formation of urinary calculi to induce urinary bladder tumors in male and female rats. "Such bladder calculi, while commonly seen in rats, do not appear to form in humans" (Cosmetic Ingredient Review Board, 1995). Although the occurrence of urinary bladder calculi and other nonneoplastic changes occurred in $B6C3F_1$ mice fed Disperse Blue 1 for 2 years (NTP 299, 1986), there was no evidence of significantly increased incidences of neoplastic lesions of the urinary bladder or kidney in mice of either sex. This observation is consistent with the findings of an NTP study of melamine (NTP 245, 1983; Melnick et al., 1984). Chronic administration of melamine resulted in increased incidences of calculi in the urinary bladders of both rats and mice, but produced an increased incidence of tumors only in rats.

Tennant (1993) proposed a ranking scheme for extrapolating rodent bioassays to human carcinogen hazards that was based on the premise that "inbred rodents are enriched or depleted for specific alleles of genes that have important functions in determining responses to chemicals." The author listed 31 of 159 chemicals, including Disperse Blue 1, that induce a highly restricted pattern of tumor induction, that is, in a specific site in a single sex of a single species (SS). It is thought that chemicals in this category of rodent carcinogens have a lower probability for potential human carcinogenicity as opposed to trans-species rodent carcinogens.

Eighty one percent of the 31 chemicals identified as SS rat carcinogens induced tumors at one of five sites, with the urinary bladder (Disperse Blue 1 included) and kidney the most prevalent. Tennant (1993) stated that these findings support the premise described above and due to the highly restricted site of distribution of SS neoplasia, these findings suggest the involvement of highly specific genes, especially when the diversity of the chemical structures among the carcinogens is considered (Ashby and Tennant, 1991; cited by Tennant, 1993). He pointed out a potential weakness of this scheme in that specific mouse or rat strains may, by chance, possess a susceptibility similar to that of humans. Tennant (1993), however, stated that the evidence supporting this stratification scheme can be found in the characteristics of known human carcinogens.

## 6.4 Structure-Activity Relationships

This subsection summarizes the carcinogenic activity of structurally related compounds.

The NTP (299, 1986) identified three structurally related compounds that were also found to be carcinogenic to rodents: 2-aminoanthraquinone, 1-amino-2-methylanthraquinone, and 2-methyl-1-nitroanthraquinone. However, only 2-methyl-1-nitroanthraquinone produced an increased incidence of papillomas and transitional cell papillomas or sarcomas (combined) of the urinary bladder in female rats (NCI, 1978c).

### 6.4.1 2-Aminoanthraquinone

2-Aminoanthraquinone, an intermediate in the manufacture of anthraquinone, was administered in feed to male and female F344/N rats and male and female B6C3F$_1$ mice for 78 weeks (80 weeks for high dose mice). In male rats, 2-aminoanthraquinone was found to be carcinogenic, causing neoplastic nodules or hepatocellular carcinomas (combined) of the liver. In male and female mice, 2-aminoanthraquinone caused hepatocellular carcinomas, and malignant lymphomas in female mice only (NCI, 1978a).

### 6.4.2 1-Amino-2-methylanthraquinone; CI Disperse Orange II

1-Amino-2-methylanthraquinone was administered in feed to male and female F344 rats and male and female B6C3F$_1$ mice for 78 and 73 weeks, respectively. An increased occurrence of hepatocellular carcinomas in male and female F344/N rats was observed, while in female B6C3F$_1$ mice, hepatocellular carcinomas or neoplastic liver nodules (combined) were found. In male rats, 1-amino-2-methylanthraquinone produced a significant increase in kidney neoplasms (NCI, 1978b).

### 6.4.3 2-Methyl-1-nitroanthraquinone

Administered in feed for 78 weeks, 2-methyl-1-nitroanthraquinone, an intermediate in the manufacture of CI Vat Red 39, caused hepatocellular carcinomas and neoplastic nodules in male

F344/N rats. In male and female rats, increased incidences of subcutaneous fibromas were observed in each sex. In female rats, an increased incidence of papillomas and transitional cell papillomas or sarcomas (combined) of the urinary bladder were observed (control, 0/46; low dose, 600 ppm in feed, 3/43, 7%; high dose, 1200 ppm, 4/44, 9%). In male and female $B6C3F_1$ mice, 2-methyl-1-nitroanthraquinone also produced hemangiosarcomas at dietary concentrations of 300 and 600 ppm (NCI, 1978c).

## 6.5 Cell Proliferation

Experimental details discussed in this section are presented in Table 6-1.

**Summary:** In mice, there was an increase in the incidences of inflammation and hyperplasia of the urinary bladder with administration of Disperse Blue 1 in the diet for 91 days or 104 weeks (NTP 299, 1986). In rats, hyperplasia and/or inflammation of the urinary bladder was detected in rats administered Disperse Blue 1 for 4 days, 91 days, or 103 weeks (NTP 299, 1986; Burnett and Squire, 1986). "Extensive labeling of transitional epithelial cell nuclei [was detected] in [the urinary bladders of] most rats" administered Disperse Blue 1 in the diet for 5, 9, or 17 weeks. The extent of labeling correlated with the degree of simple or papillary hyperplasia. Hyperplasia was detected in the renal epithelium of rats after 2 to 3 days of treatment with Disperse Blue 1 in the diet or by gavage and after 103 weeks of treatment with Disperse Blue 1 in the diet (Burnett and Squire, 1986).

### 6.5.1 Mice

There was an increase in the incidences of chronic inflammation of the urinary bladder and hyperplasia of the transitional epithelium of the urinary bladder in male and female B6C3F1 mice that were administered 2500, 5000, or 10,000 ppm Disperse Blue 1 (50% Disperse Blue 1, 19.5% water, 30% other compounds) in the diet for 91 days beginning at 5 to 6 weeks of age. There was no increase in either incidence, however, when lower doses (600 or 1200 ppm) were similarly administered (NTP 299, 1986).

In male and female B6C3F1 mice administered 600, 1200, or 2500 ppm Disperse Blue 1 (50% Disperse Blue 1, 19.5% water, 30% other compounds) in the diet for 104 weeks beginning at 7 weeks of age, the incidence of inflammation of the urinary bladder was significantly increased in mid-dose and high-dose males and females and the incidences of epithelial hyperplasia and fibrosis of the urinary bladder were significantly increased in mid-dose and high-dose males and in high-dose females. In the stomach, the incidence of cysts was increased in all dosed mice, but statistical analysis was not performed (NTP 299, 1986).

### 6.5.2 Rats

In male and female F344 rats administered Disperse Blue 1 (50% Disperse Blue 1 and isomers, 50% lignosulfonate dispersants) by gavage (1000 mg/kg/day [3727 μmol/kg/day] or 500 mg/kg [1860 μmol/kg] twice/day for 1, 2, or 3 consecutive days) or in the diet (1% [10,000 ppm] for 4 days), Disperse Blue 1 "accumulated in the kidney tubules and resulted in hyperplasia of the renal pelvis epithelium, in some cases within 2 days. All animals of either sex were so affected after 3 days." Incidences were not given and there were no controls. Urinary bladders were described as "normal" (Burnett and Squire, 1986).

In male and female F344 rats administered 5000 or 10,000 ppm Disperse Blue 1 with dispersants (50% Disperse Blue 1 and isomers, 50% lignosulfonate dispersants) or 2500 or 5000 ppm Disperse Blue 1 without dispersants in the diet for 4 days beginning at 10 weeks of age, low-grade hyperplasia of the urinary bladder urothelium was detected in all rats after treatment for 4 days with 10,000 ppm Disperse Blue 1. Also, after 4 days of treatment with the 10,000-ppm dose, "there was no accumulation in the kidneys." With the 5000-ppm dose, "the corresponding level of dye [Disperse Blue 1] without dispersants had a similar effect, providing no evidence that the dispersants had a major effect on absorption or elimination of the dye." The "effect" of the dye, however, was not described. No details were given about the effects of the 2500-ppm dose and there were no controls (Burnett and Squire, 1986).

In male and female F344/N rats administered 1200, 2500, 5000, 10,000, or 20,000 ppm Disperse Blue 1 (50% Disperse Blue 1, 19.5% water, 30% other compounds) in the diet for 91 days beginning at 5 to 6 weeks of age, there was an increase in the incidences of urinary bladder chronic inflammation and urinary bladder transitional epithelium hyperplasia in all Disperse Blue 1-treated rats except males and females that received the 1200-ppm dose (NTP 299, 1986).

In male and female F344 rats (age not specified) administered 100, 1000, or 10,000 ppm Disperse Blue 1 (50% Disperse Blue 1 and isomers, 50% lignosulfonate dispersants) in the diet and injected i.p. with [*Me*-$^3$H]thymidine after 5, 9, or 17 weeks of treatment, "extensive labeling of transitional epithelial cell nuclei [was detected] in [the urinary bladders of] most rats fed the 1% [10,000 ppm] level. The extent of labeling correlated with the degree of simple or papillary hyperplasia. No increase in nuclear labeling over background occurred in animals fed the 0.1 or 0.01% [1000 or 100 ppm] level. No labeling was present in subepithelial mesenchymal cells at any exposure level. Histopathological evaluation of bladder lesions showed that hyperplasia was slight to moderate by week 5 at the 1% [10,000 ppm] level and by weeks 9 and 17 squamous metaplasia was also present." Incidences were not given. At 6 months, epithelial hyperplasia was detected in all high-dose males and females. The incidence in controls was not mentioned. At 19 months, hyperplasia was detected in 8/14 high-dose males and in 8/22 high-dose females (basal-diet controls were not mentioned). Also, "compound-related effects in the kidneys were apparent at the high-dose level and their severity correlated with the amount of dye present in the renal tissues and the length of treatment. The primary lesion involved tubular degeneration and regeneration with interstitial fibrosis and inflammation, similar to chronic nephropathy in aging rats...Hyperplasia of pelvis epithelium [detected in all high-dose rats] also correlated with the amount of dye in the renal parenchyma...Kidneys of animals exposed to 0.01 or 0.1% [100 or 1000 ppm Disperse Blue 1] for up to 9 weeks were indistinguishable from those of controls, and no dye was present." The labeling index in kidneys was not mentioned (Burnett and Squire, 1986).

In male and female F344/N rats administered 1250, 2500, or 5000 ppm Disperse Blue 1 (50% Disperse Blue 1, 19.5% water, 30% other compounds) in the diet for 103 weeks beginning at 7 weeks of age, the incidences of epithelial hyperplasia of the urinary bladder and kidneys were significantly increased in mid-dose and high-dose rats and the incidence of hyperplasia of the parathyroid was increased in mid-dose and high-dose males and in high-dose females. Epithelial hyperplasia of the prostate was detected in 1/50 low-dose, 12/50 mid-dose, and 5/50 high-dose males (vs. 3/49 controls). Statistical analysis of these incidences, however, was not performed (NTP 299, 1986).

## Table 6-1. Cell Proliferation Induced by Disperse Blue 1

| Age, Strain, Species | No./Sex Exposed and Control | Chemical Form and Purity | Dose | Duration of Exposure | Results/Comments | Reference |
|---|---|---|---|---|---|---|
| **Mice - *Oral Administration*** | | | | | | |
| 5- to 6-wk-old B6C3F1 mice | 10M; 10F per dose<br>Controls: 10M; 10F (basal diet alone) | Disperse Blue 1 (50% Disperse Blue 1, 19.5% water, 30% other compounds) | 600, 1200, 2500, 5000, or 10,000 ppm in diet | 91 days | Survivors were killed at the end of the treatment period. Necropsy was performed on all mice. Skin, mandibular and mesenteric lymph nodes, mammary glands, salivary glands, thigh muscle, femur (including marrow), thymus, trachea, lungs and bronchi, heart, thyroid gland, parathyroids, esophagus, stomach, small intestine, colon, liver, pancreas, brain, spleen, kidneys, adrenal glands, urinary bladder, vesicular gland/prostate/testes or ovaries/uterus, and pituitary gland were examined histologically in all high dose mice, mice that died before the end of the study, and all controls.<br>**Urinary Bladder:**<br>Positive (for proliferative activity, as indicated by presence of chronic inflammation and transitional epithelium hyperplasia with 3 highest doses)<br>There was an increase in the incidence of chronic inflammation of the urinary bladder in all Disperse Blue 1-treated mice except in males and females that received 600 or 1200 ppm (males [in order of increasing dose, starting at 1200 ppm]: 1/10, 3/10, 10/10, and 6/8 vs. 0/10 controls; females [in order of increasing dose, starting at 1200 ppm]: 0/10, 10/10, 10/10, 7/8 vs. 0/10 controls).<br>There was an increase in the incidence of hyperplasia of the transitional epithelium of the urinary bladder in all Disperse Blue 1-treated mice except males and females that received 600 or 1200 ppm (males [in order of increasing dose, starting at 1200 ppm]: 0/10, 3/10, 4/10, 4/8 vs. 0/10 controls; females [in order of increasing dose, starting at 1200 ppm]: 0/10, 6/10, 6/10, 2/8 vs. 0/10 controls).<br>Incidences were not given for the 600-ppm groups and there was no mention of statistical analysis. | NTP (299, 1986) |

## Table 6-1. Cell Proliferation Induced by Disperse Blue 1 (Continued)

| Age, Strain, Species | No./Sex Exposed and Control | Chemical Form and Purity | Dose | Duration of Exposure | Results/Comments | Reference |
|---|---|---|---|---|---|---|
| 7-wk-old B6C3F1 mice | 50M, 50F per dose<br>Controls: 50M; 50F (basal diet alone) | Disperse Blue 1 (50% Disperse Blue 1, 19.5% water, 30% other compounds) | 600, 1200, or 2500 ppm in diet | 104 wk | Survivors were killed at the end of the treatment period. Necropsy and histologic examination were performed on all mice, including those found dead unless they were excessively autolyzed or cannibalized. Examined tissues were the same as those for the NTP 91-day study (see above), with the addition of sciatic nerve, costochondral junction, larynx, duodenum, jejunum, ileum, cecum, rectum, nasal cavity, spinal cord (examined selectively), and eyes (examined selectively). Incidences were analyzed using 1 of 3 statistical tests: life-table analysis (Mantel-Haenszel method), incidental analysis, or unadjusted analysis. All p-values were one-sided.<br>**Urinary Bladder:**<br>Positive (for proliferative activity, as indicated by presence of inflammation (MD and HD), epithelial hyperplasia (MD and HD [males], HD [females]), and fibrosis (MD and HD [males], HD [females])<br>The incidence of inflammation was significantly increased in MD and HD mice (males: 11/50 MD [$p < 0.01$], 36/50 HD [$p < 0.001$] vs. 2/50 controls; females: 8/50 MD [$p < 0.01$], 37/50 HD [$p < 0.001$] vs. 0/49 controls).<br>The incidence of epithelial hyperplasia was significantly increased in MD and HD males and in HD females (males: 11/50 MD [$p < 0.001$], 42/50 HD [$p < 0.001$] vs. 0/50 controls; females: 26/50 HD [$p < 0.001$] vs. 0/49 controls).<br>The incidence of fibrosis was significantly increased in MD and HD males and in HD females (males: 7/50 MD [$p < 0.01$], 22/50 HD [$p < 0.001$]; females: 23/50 HD [$p < 0.001$]).<br>**Stomach:**<br>Positive (for proliferative activity, as indicated by presence of cysts)<br>The incidence of cysts was increased in Disperse Blue 1-treated mice (males: 7/49 LD, 12/50 MD, 6/50 HD vs. 3/50 controls; females: 10/50 LD, 8/50 MD, 9/49 HD vs. 3/49 controls; statistical analysis not performed). | NTP (299, 1986) |
| **Rats - *Oral Administration*** | | | | | | |
| 10-wk-old F344 rats | 15M, 15F per treatment regimen<br>Controls: 0 | Disperse Blue 1 (50% Disperse Blue 1 and isomers, 50% lignosulfonate dispersants) | 1000 mg/kg/day (3727 µmol/kg/day) or 500 mg/kg (1860 µmol/kg) twice/day by gavage or 1.0% in diet for 4 days | 1, 2, or 3 consecutive days or 4 days | Rats were killed 1 day after the end of treatment. Urinary bladder and kidneys were examined.<br>**Kidneys:**<br>Disperse Blue 1 "accumulated in the kidney tubules and resulted in hyperplasia of the renal pelvis epithelium, in some cases within 2 days. All animals of either sex were so affected after 3 days." Incidences were not given and there were no controls.<br>**Urinary Bladder:**<br>Negative<br>Urinary bladders were "normal". | Burnett and Squire (1986) |

## Table 6-1. Cell Proliferation Induced by Disperse Blue 1 (Continued)

| Age, Strain, Species | No./Sex Exposed and Control | Chemical Form and Purity | Dose | Duration of Exposure | Results/Comments | Reference |
|---|---|---|---|---|---|---|
| 10-wk-old F344 rats | 15M, 15F<br>Controls: 0 | Disperse Blue 1 (50% Disperse Blue 1 and isomers, 50% lignosulfonate dispersants) | 5000 or 10,000 ppm (Disperse Blue 1 with dispersants) or 2500 or 5000 ppm (Disperse Blue 1 without dispersants [see NTP study for purity]) in diet | 4 days | Rats were killed 1 day after the end of treatment. Urinary bladder and kidneys were examined.<br>**Kidneys:**<br>After 4 days of treatment with the 10,000-ppm dose, "there was no accumulation in the kidneys." No other details were given.<br>**Urinary Bladder:**<br>After 4 days of treatment with 10,000 ppm, low-grade hyperplasia of the urothelium was detected in all rats.<br>With the 5000-ppm dose, "the corresponding level of dye [Disperse Blue 1] without dispersants had a similar effect, providing no evidence that the dispersants had a major effect on absorption or elimination of the dye." The "effect" of the dye, however, was not described. No details were given about the effects of the 2500-ppm dose and there were no controls. | Burnett and Squire (1986) |
| 5- to 6-wk-old F344/N rats | 10M, 10F per dose<br>Controls: 10M; 10F (basal diet alone) | Disperse Blue 1 (50% Disperse Blue 1, 19.5% water, 30% other compounds) | 1200, 2500, 5000, 10,000, or 20,000 ppm in diet | 91 days | Survivors were killed at the end of the treatment period. Necropsy was performed on all rats. All high-dose rats, rats that died before the end of the study, and all controls were examined histologically. The following tissues were examined: skin, mandibular and mesenteric lymph nodes, mammary glands, salivary glands, thigh muscle, femur (including marrow), thymus, trachea, lungs and bronchi, heart, thyroid gland, parathyroids, esophagus, stomach, small intestine, colon, liver, pancreas, brain, spleen, kidneys, adrenal glands, urinary bladder, vesicular gland/prostate/testes or ovaries/uterus, and pituitary gland.<br>**Urinary Bladder:**<br>Positive (for proliferative activity, as indicated by presence of chronic inflammation and transitional epithelium hyperplasia with 4 highest doses)<br>There was an increase in the incidence of urinary bladder chronic inflammation in all Disperse Blue 1-treated rats except males and females that received 1200 ppm (males [in order of increasing dose]: 0/10, 3/9, 4/10,10/10, and 10/10 vs. 0/10 controls; females [in order of increasing dose]: 0/10, 4/10, 2/10, 9/10, and 7/10 vs. 0/10 controls).<br>There was an increase in the incidence of urinary bladder transitional epithelium hyperplasia in all Disperse Blue 1-treated rats except males and females that received 1200 ppm (males [in order of increasing dose]: 0/10, 9/9, 10/10, 10/10, 10/10 vs. 0/10 controls; females [in order of increasing dose]: 0/10, 2/10, 8/10, 10/10, 9/10 vs. 0/10 controls).<br>There was no mention of statistical analysis. | NTP (299, 1986) |

## Table 6-1. Cell Proliferation Induced by Disperse Blue 1 (Continued)

| Age, Strain, Species | No./Sex Exposed and Control | Chemical Form and Purity | Dose | Duration of Exposure | Results/Comments | Reference |
|---|---|---|---|---|---|---|
| F344 rats | 20M, 20F (LD)<br>20M, 20F (MD)<br>60M, 60F (HD)<br>Controls: 40M; 40F (basal diet alone) | Disperse Blue 1 (50% Disperse Blue 1 and isomers, 50% lignosulfonate dispersants) | 100, 1000, or 10,000 ppm in diet | 5 wk, 9 wk, 17 wk, 6 mo (control and HD only), or 19 mo | After 5, 9, and 17 wk of treatment, between 3 and 5 rats of each sex from each group were injected i.p. with [*Me*-$^3$H]thymidine for autoradiographic studies and were killed 1 hour later. Kidneys (from the 5-wk and 9-wk groups only) and bladders (from the 5-, 9-, and 17-wk groups) were examined. After 6 months of treatment, 10 rats of each sex from the HD group and 12 rats/sex from the control group were killed and "an extensive set of tissues, including kidneys and bladders" was preserved. Bladders and kidneys were examined microscopically. After 19 months, rats that died or were killed when moribund, or were killed at study termination were necropsied and 30 tissues were preserved.<br>**Urinary Bladder:**<br>Positive (for proliferative activity with HD, as indicated by labeling index and presence of hyperplasia)<br>"Extensive labeling of transitional epithelial cell nuclei [was detected] in most rats fed the 1% [10,000 ppm] level. The extent of labeling correlated with the degree of simple or papillary hyperplasia. No increase in nuclear labeling over background occurred in animals fed the 0.1 or 0.01% [1000 or 100 ppm] level. No labeling was present in subepithelial mesenchymal cells at any exposure level. Histopathological evaluation of bladder lesions showed that hyperplasia was slight to moderate by wk 5 at the 1% [10,000 ppm] level and by weeks 9 and 17 squamous metaplasia was also present." Incidences were not given. At 6 months, epithelial hyperplasia was detected in all HD males and females. The incidence in controls was not mentioned. At 19 months, hyperplasia was detected in 8/14 HD males and in 8/22 HD females (basal-diet controls were not mentioned).<br>**Kidneys:**<br>Positive (for proliferative activity with HD, as indicated by presence of hyperplasia)<br>"Compound-related effects in the kidneys were apparent at the HD level and their severity correlated with the amount of dye present in the renal tissues and the length of treatment. The primary lesion involved tubular degeneration and regeneration with interstitial fibrosis and inflammation, similar to chronic nephropathy in aging rats...Hyperplasia of pelvis epithelium [detected in all HD rats] also correlated with the amount of dye in the renal parenchyma...Kidneys of animals exposed to 0.01 or 0.1% [100 or 1000 ppm Disperse Blue 1] for up to 9 wk were indistinguishable from those of controls, and no dye was present." The labeling index in kidneys was not mentioned. | Burnett and Squire (1986) |

## Table 6-1. Cell Proliferation Induced by Disperse Blue 1 (Continued)

| Age, Strain, Species | No./Sex Exposed and Control | Chemical Form and Purity | Dose | Duration of Exposure | Results/Comments | Reference |
|---|---|---|---|---|---|---|
| 7-wk-old F344/N rats | 50M, 50F per dose<br>Controls: 50M; 50F (basal diet alone) | Disperse Blue 1 (50% Disperse Blue 1, 19.5% water, 30% other compounds) | 1250, 2500, or 5000 ppm in diet | 103 wk | Survivors were killed at the end of the treatment period. Necropsy and histologic examination were performed on all rats, including those found dead unless they were excessively autolyzed or cannibalized. Examined tissues were the same as those for the NTP 91-day study (see above), with the addition of sciatic nerve, costochrondral junction, larynx, duodenum, jejunum, ileum, cecum, rectum, nasal cavity, spinal cord (examined selectively), and eyes (examined selectively). Incidences were analyzed using 1 of 3 statistical tests: life-table analysis (Mantel-Haenszel method), incidental analysis, or unadjusted analysis. All p-values were one-sided.<br>**Urinary Bladder:**<br>Positive (for proliferative activity, as indicated by presence of epithelial hyperplasia with MD and HD)<br>The incidence of epithelial hyperplasia was significantly increased in MD and HD rats (males: 28/50 MD [$p < 0.01$], 42/49 HD [$p < 0.01$] vs. 0/49 controls; females: 42/50 MD [$p < 0.01$], 40/48 HD [$p < 0.01$] vs. 0/48 controls).<br>**Kidneys:**<br>Positive (for proliferative activity, as indicated by presence of epithelial hyperplasia with MD and HD)<br>The incidence of epithelial hyperplasia was significantly increased in MD and HD rats (males: 8/50 MD [$p < 0.01$], 11/50 HD [$p < 0.01$] vs. 0/49 controls; females: 12/50 MD [$p < 0.01$], 15/50 HD [$p < 0.01$] vs. 0/49 controls).<br>**Prostate:**<br>Positive (for proliferative activity, as indicated by presence of epithelial hyperplasia; MD males)<br>Epithelial hyperplasia was detected in 1/50 LD, 12/50 MD, and 5/50 HD males (vs. 3/49 controls; statistical analysis not performed).<br>**Parathyroid:**<br>Positive (for proliferative activity, as indicated by presence of hyperplasia with MD [males] and HD [males and females])<br>The incidence of hyperplasia was increased in MD and HD males and in HD females (males: 7/49 MD, 7/49 HD vs. 0/46 controls; females: 4/48 HD vs. 0/47 controls; statistical analysis not performed). | NTP (299, 1986) |

Abbreviations: HD = high dose; LD = low dose; MD = mid dose

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# APPENDIX A

# DESCRIPTION OF ONLINE LITERATURE SEARCHES FOR DISPERSE BLUE 1

## DESCRIPTION OF ONLINE LITERATURE SEARCHES FOR DISPERSE BLUE 1 (IARC Monograph in Vol. 48, 1990)

The searches described below were conducted between March and October 1996. An exhaustive search of all pertinent databases was not attempted, but the ones chosen were expected to provide citations for most of the relevant recently published literature. No attempt was made in the search strategy to find toxicity information for metabolites and other structural analogues.

Generally, if an IARC monograph or another authoritative review had been published, literature searches were generally restricted from the year before publication to the current year.

Older literature that needed to be examined was identified from the reviews and original articles as they were acquired. Current awareness was maintained by conducting weekly searches of Current Contents on Diskette® Life Sciences 1200 [journals] edition.

TOXLINE (on STN International): Fifty-one records in the entire database (1940s to date) were indexed by the Chemical Abstracts Service Registry Number (CASRN) (47) and/or name (17). Many of the 13 references of interest had already been identified in the EMIC and EMICBACK searches.

CANCERLIT: There were 5 records in the entire database (1963 to June 1996) indexed by name and 4, by its CASRN. Only 3 had been published since 1988, and they were all identified by the MEDLINE search as well.

EMBASE: Only 2 records resulted from a search strategy like that used for TOXLIT. Both described highly useful recent reviews.

EMIC/EMICBACK: Six records were indexed by the CASRN in EMIC, and 10 records were indexed by CASRN in EMICBACK. Two of the EMIC publications were selected for acquisition.

IRIS: The database did not include Disperse Blue 1 (searched by CASRN) in March 1996.

MEDLINE: Four records were indexed by compound name or CASRN and published after 1988. None of the records was unique to this database.

TOXLIT: All of the records (64) in the database (1965 to 1996) on Disperse Blue 1 were indexed by CASRN; only 6 of them were indexed by name. Forty of the 64 records had been published since 1988. The set of 40 was reduced to 22 by combination with the truncated (use of ? with the word stem) keywords in the statement "carcinogen? or mechanism? or toxicokinetic? or pharmacokinetic? or metaboli? or neoplas? or hyperplas? or metaplas? or foci? or tumor? or tumour?" Nineteen publications were selected for acquisition.

In September 1996, the contractor performed searches for updating sections 1 and 2, which had been last updated in 1994 with regulatory information from print sources and REGMAT (May 1993 version). REGMAT had broad coverage of EPA regulations, but it is no longer available. Databases searched in 1996 included CSCHEM and CSCORP for U.S. suppliers (databases produced by Chem Sources); HSDB; the Chemical Information System's databases SANSS (the Structure and Nomenclature Search System) and ISHOW (for physical-chemical properties); Chemical Abstracts Service's (CAS) File CHEMLIST for TSCA and SARA updates in 1996; and CAS's CA File sections 59 (Air Pollution and Industrial Hygiene), 60 (Waste Disposal and Treatment), and 61 (Water) for environmental exposure information.

# APPENDIX B

# LISTING OF GAP TEST CODES IN ALPHABETICAL ORDER

# LISTING OF GAP TEST CODES IN ALPHABETICAL ORDER

| Test Code | Definition |
|---|---|
| ACC | Allium cepa, chromosomal aberrations |
| AIA | Aneuploidy, animal cells in vitro |
| AIH | Aneuploidy, human cells in vitro |
| ANF | Aspergillus nidulans, forward mutation |
| ANG | Aspergillus nidulans, genetic crossing-over |
| ANN | Aspergillus nidulans, aneuploidy |
| ANR | Aspergillus nidulans, reverse mutation |
| ASM | Arabidopsis species, mutation |
| AVA | Aneuploidy, animal cells in vivo |
| AVH | Aneuploidy, human cells in vivo |
| BFA | Body fluids from animals, microbial mutagenicity |
| BFH | Body fluids from humans, microbial mutagenicity |
| BHD | Binding (covalent) to DNA, human cells in vivo |
| BHP | Binding (covalent) to RNA or protein, human cells in vivo |
| BID | Binding (covalent) to DNA in vitro |
| BIP | Binding (covalent) to RNA or protein in vitro |
| BPF | Bacteriophage, forward mutation |
| BPR | Bacteriophage, reverse mutation |
| BRD | Other DNA repair-deficient bacteria, differential toxicity |
| BSD | Bacillus subtilis rec strains, differential toxicity |
| BSM | Bacillus subtilis multi-gene test |
| BVD | Binding (covalent) to DNA, animal cells in vivo |
| BVP | Binding (covalent) to RNA or protein, animal cells in vivo |
| CBA | Chromosomal aberrations, animal bone-marrow cells in vivo |
| CBH | Chromosomal aberrations, human bone-marrow cells in vivo |
| CCC | Chromosomal aberrations, spermatocytes treated in vivo and cytes obs. |
| CGC | Chromosomal aberrations, spermatogonia treated in vivo and cytes obs. |
| CGG | Chromosomal aberrations, spermatogonia treated in vivo and gonia obs. |
| CHF | Chromosomal aberrations, human fibroblasts in vitro |
| CHL | Chromosomal aberrations, human lymphocyte in vitro |
| CHT | Chromosomal aberrations, transformed human cells in vitro |
| CIA | Chromosomal aberrations, other animal cells in vitro |
| CIC | Chromosomal aberrations, Chinese hamster cells in vitro |
| CIH | Chromosomal aberrations, other human cells in vitro |
| CIM | Chromosomal aberrations, mouse cells in vitro |
| CIR | Chromosomal aberrations, rat cells in vitro |
| CIS | Chromosomal aberrations, Syrian hamster cells in vitro |
| CIT | Chromosomal aberrations, transformed animal cells in vitro |
| CLA | Chromosomal aberrations, animal leukocytes in vivo |
| CLH | Chromosomal aberrations, human lymphocytes in vivo |

| Test Code | Definition |
|---|---|
| COE | Chromosomal aberrations, oocytes or embryos treated in vivo |
| CVA | Chromosomal aberrations, other animal cells in vivo |
| CVH | Chromosomal aberrations, other human cells in vivo |
| DIA | DNA strand breaks, cross-links or rel. damage, animal cells in vitro |
| DIH | DNA strand breaks, cross-links or rel. damage, human cells in vitro |
| DLM | Dominant lethal test, mice |
| DLR | Dominant lethal test, rats |
| DMC | Drosophila melanogaster, chromosomal aberrations |
| DMG | Drosophila melanogaster, genetic crossing-over or recombination |
| DMH | Drosophila melanogaster, heritable translocation test |
| DML | Drosophila melanogaster, dominant lethal test |
| DMM | Drosophila melanogaster, somatic mutation (and recombination) |
| DMN | Drosophila melanogaster, aneuploidy |
| DMX | Drosophila melanogaster, sex-linked recessive lethal mutation |
| DVA | DNA strand breaks, cross-links or rel. damage, animal cells in vivo |
| DVH | DNA strand breaks, cross-links or rel. damage, human cells in vivo |
| ECB | Escherichia coli (or E. coli DNA), strand breaks, cross-links or repair |
| ECD | Escherichia coli pol A/W3110-P3478, diff. toxicity (spot test) |
| ECF | Escherichia coli (excluding strain K12), forward mutation |
| ECK | Escherichia coli K12, forward or reverse mutation |
| ECL | Escherichia coli pol A/W3110-P3478, diff. toxicity (liquid susp. test) |
| ECR | Escherichia coli, miscellaneous strains, reverse mutation |
| ECW | Escherichia coli WP2 uvrA, reverse mutation |
| EC2 | Escherichia coli WP2, reverse mutation |
| ERD | Escherichia coli rec strains, differential toxicity |
| FSC | Fish, chromosomal aberrations |
| FSI | Fish, micronuclei |
| FSM | Fish, mutation |
| FSS | Fish, sister chromatid exchange |
| FSU | Fish, unscheduled DNA synthesis |
| GCL | Gene mutation, Chinese hamster lung cells exclusive of V79 in vitro |
| GCO | Gene mutation, Chinese hamster ovary cells in vitro |
| GHT | Gene mutation, transformed human cells in vivo |
| GIA | Gene mutation, other animal cells in vitro |
| GIH | Gene mutation, human cells in vitro |
| GML | Gene mutation, mouse lymphoma cells exclusive of L5178Y in vitro |
| GVA | Gene mutation, animal cells in vivo |
| G5T | Gene mutation, mouse lymphoma L5178Y cells in vitro, TK locus |
| G51 | Gene mutation, mouse lymphoma L5178Y cells in vitro, all other loci |
| G9H | Gene mutation, Chinese hamster lung V-79 cells in vitro, HPRT locus |
| G9O | Gene mutation, Chinese hamster lung V-79 cells in vitro, ouabain resistance |
| HIM | Haemophilus influenzae, mutation |
| HMA | Host mediated assay, animal cells in animal hosts |

| Test Code | Definition |
|---|---|
| HMH | Host mediated assay, human cells in animal hosts |
| HMM | Host mediated assay, microbial cells in animal hosts |
| HSC | Hordeum species, chromosomal aberrations |
| HSM | Hordeum species, mutation |
| ICH | Inhibition of intercellular communication, human cells in vitro |
| ICR | Inhibition of intercellular communication, rodent cells in vitro |
| KPF | Klebsiella pneumonia, forward mutation |
| MAF | Micrococcus aureus, forward mutation |
| MHT | Mouse heritable translocation test |
| MIA | Micronucleus test, animal cells in vitro |
| MIH | Micronucleus test, human cells in vitro |
| MST | Mouse spot test |
| MVA | Micronucleus test, other animals in vivo |
| MVC | Micronucleus test, hamsters in vivo |
| MVH | Micronucleus test, human cells in vivo |
| MVM | Micronucleus test, mice in vivo |
| MVR | Micronucleus test, rats in vivo |
| NCF | Neurospora crassa, forward mutation |
| NCN | Neurospora crassa, aneuploidy |
| NCR | Neurospora crassa, reverse mutation |
| PLC | Plants (other), chromosomal aberrations |
| PLI | Plants (other), micronuclei |
| PLM | Plants (other), mutation |
| PLS | Plants (other), sister chromatid exchanges |
| PLU | Plants, unscheduled DNA synthesis |
| PRB | Prophage, induction, SOS repair, DNA strand breaks, or cross-links |
| PSC | Paramecium species, chromosomal aberrations |
| PSM | Paramecium species, mutation |
| RIA | DNA repair exclusive of UDS, animal cells in vitro |
| RIH | DNA repair exclusive of UDS, human cells in vitro |
| RVA | DNA repair exclusive of UDS, animal cells in vivo |
| SAD | Salmonella typhimurium, DNA repair-deficient strains, differential toxicity |
| SAF | Salmonella typhimurium, forward mutation |
| SAL | Salmonella typhimurium, all strains, reverse mutation |
| SAS | Salmonella typhimurium (other misc. strains), reverse mutation |
| SA0 | Salmonella typhimurium TA100, reverse mutation |
| SA1 | Salmonella typhimurium TA97, reverse mutation |
| SA2 | Salmonella typhimurium TA102, reverse mutation |
| SA3 | Salmonella typhimurium TA1530, reverse mutation |
| SA4 | Salmonella typhimurium TA104, reverse mutation |
| SA5 | Salmonella typhimurium TA1535, reverse mutation |
| SA7 | Salmonella typhimurium TA1537, reverse mutation |
| SA8 | Salmonella typhimurium TA1538, reverse mutation |

| Test Code | Definition |
|---|---|
| SA9 | Salmonella typhimurium TA98, reverse mutation |
| SCF | Saccharomyces cerevisiae, forward mutation |
| SCG | Saccharomyces cerevisiae, gene conversion |
| SCH | Saccharomyces cerevisiae, homozygosis by recombination or gene conversion |
| SCN | Saccharomyces cerevisiae, aneuploidy |
| SCR | Saccharomyces cerevisiae, reverse mutation |
| SGR | Streptomyces griseoflavus, reverse mutation |
| SHF | Sister chromatid exchange, human fibroblasts in vitro |
| SHL | Sister chromatid exchange, human lymphocytes in vitro |
| SHT | Sister chromatid exchange, transformed human cells in vitro |
| SIA | Sister chromatid exchange, other animal cells in vitro |
| SIC | Sister chromatid exchange, Chinese hamster cells in vitro |
| SIH | Sister chromatid exchange, other human cells in vitro |
| SIM | Sister chromatid exchange, mouse cells in vitro |
| SIR | Sister chromatid exchange, rat cells in vitro |
| SIS | Sister chromatid exchange, Syrian hamster cells in vitro |
| SIT | Sister chromatid exchange, transformed cells in vitro |
| SLH | Sister chromatid exchange, human lymphocytes in vivo |
| SLO | Mouse specific locus test, other stages |
| SLP | Mouse specific locus test, postspermatogonia |
| SPF | Sperm morphology, F1 mouse |
| SPH | Sperm morphology, human |
| SPM | Sperm morphology, mouse |
| SPR | Sperm morphology, rat |
| SPS | Sperm morphology, sheep |
| SSB | Saccharomyces species, DNA breaks, cross-links or related damage |
| SSD | Saccharomyces cerevisiae, DNA repair-deficient strains, diff. toxicity |
| STF | Streptomyces coelicolor, forward mutation |
| STR | Streptomyces coelicolor, reverse mutation |
| SVA | Sister chromatid exchange, animal cells in vivo |
| SVH | Sister chromatid exchange, other human cells in vivo |
| SZD | Schizosaccharomyces pombe, DNA repair-deficient strains, diff. toxicity |
| SZF | Schizosaccharomyces pombe, forward mutation |
| SZG | Schizosaccharomyces pombe, gene conversion |
| SZR | Schizosaccharomyces pombe, reverse mutation |
| T7R | Cell transformation, SA7/rat cells |
| T7S | Cell transformation, SA7/Syrian hamster embryo cells |
| TBM | Cell transformation, BALB/C3T3 mouse cells |
| TCL | Cell transformation, other established cell lines |
| TCM | Cell transformation, C3H10T1/2 mouse cells |
| TCS | Cell transformation, Syrian hamster embryo cells, clonal assay |
| TEV | Cell transformation, other viral enhancement systems |
| TFS | Cell transformation, Syrian hamster embryo cells, focus assay |

| Test Code | Definition |
|---|---|
| TIH | Cell transformation, human cells in vitro |
| TPM | Cell transformation, mouse prostate cells |
| TRR | Cell transformation, RLV/Fischer rat embryo cells |
| TSC | Tradescantia species, chromosomal aberrations |
| TSI | Tradescantia species, micronuclei |
| TSM | Tradescantia species, mutation |
| TVI | Cell transformation, treated in vivo, scored in vitro |
| UBH | Unscheduled DNA synthesis, human bone-marrow cells in vivo |
| UHF | Unscheduled DNA synthesis, human fibroblasts in vitro |
| UHL | Unscheduled DNA synthesis, human lymphocytes in vitro |
| UHT | Unscheduled DNA synthesis, transformed human cells in vitro |
| UIA | Unscheduled DNA synthesis, other animal cells in vitro |
| UIH | Unscheduled DNA synthesis, other human cells in vitro |
| UPR | Unscheduled DNA synthesis, rat hepatocytes in vivo |
| URP | Unscheduled DNA synthesis, rat primary hepatocytes |
| UVA | Unscheduled DNA synthesis, other animal cells in vivo |
| UVC | Unscheduled DNA synthesis, hamster cells in vivo |
| UVH | Unscheduled DNA synthesis, other human cells in vivo |
| UVM | Unscheduled DNA synthesis, mouse cells in vivo |
| UVR | Unscheduled DNA synthesis, rat cells (other than hepatocytes) in vivo |
| VFC | Vicia faba, chromosomal aberrations |
| VFS | Vicia faba, sister chromatid exchange |