Crystal violet and related dyes: Human health tier II assessment

13 February 2015

Chemicals in this assessment

Chemical Name in the Inventory	CAS Number
Methanaminium, N-[4-[bis[4-(dimethylamino)phenyl]methylene]-2,5-cyclohexadien-1-ylidene]-N-methyl-, chloride	548-62-9
Benzenemethanaminium, N-[4-[[4-(dimethylamino)phenyl][4-[ethyl[(3-sulfophenyl)methyl]amino]phenyl]methylene]-2,5-cyclohexadien-1-ylidene]-N-ethyl-3-sulfo-, hydroxide, inner salt, sodium salt	1694-09-3
Ethanaminium, N-[4-[bis[4-(diethylamino)phenyl]methylene]-2,5-cyclohexadien-1-ylidene]-N-ethyl-, chloride	2390-59-2
Ethanaminium, N-[4-[[4-(diethylamino)phenyl][4-(ethylamino)-1-naphthalenyl]methylene]-2,5-cyclohexadien-1-ylidene]-N-ethyl-, chloride	2390-60-5
Methanaminium, N-[4-[[4-(dimethylamino)phenyl][4-(phenylamino)-1-naphthalenyl]methylene]-2,5-cyclohexadien-1-ylidene]-N-methyl-, chloride	2580-56-5
Methylium, [4-(dimethylamino)phenyl]bis[4-(ethylamino)-3-methylphenyl]-, acetate	72102-55-7

Preface

This assessment was carried out by staff of the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) using the Inventory Multi-tiered Assessment and Prioritisation (IMAP) framework.

The IMAP framework addresses the human health and environmental impacts of previously unassessed industrial chemicals listed on the Australian Inventory of Chemical Substances (the Inventory).

The framework was developed with significant input from stakeholders and provides a more rapid, flexible and transparent approach for the assessment of chemicals listed on the Inventory.

Stage One of the implementation of this framework, which lasted four years from 1 July 2012, examined 3000 chemicals meeting characteristics identified by stakeholders as needing priority assessment. This included chemicals for which NICNAS already held exposure information, chemicals identified as a concern or for which regulatory action had been taken overseas, and chemicals detected in international studies analysing chemicals present in babies' umbilical cord blood.

Stage Two of IMAP began in July 2016. We are continuing to assess chemicals on the Inventory, including chemicals identified as a concern for which action has been taken overseas and chemicals that can be rapidly identified and assessed by using Stage One information. We are also continuing to publish information for chemicals on the Inventory that pose a low risk to human health or the environment or both. This work provides efficiencies and enables us to identify higher risk chemicals requiring assessment.

The IMAP framework is a science and risk-based model designed to align the assessment effort with the human health and environmental impacts of chemicals. It has three tiers of assessment, with the assessment effort increasing with each tier. The Tier I assessment is a high throughput approach using tabulated electronic data. The Tier II assessment is an evaluation of risk on a substance-by-substance or chemical category-by-category basis. Tier III assessments are conducted to address specific concerns that could not be resolved during the Tier II assessment.

These assessments are carried out by staff employed by the Australian Government Department of Health and the Australian Government Department of the Environment and Energy. The human health and environment risk assessments are conducted and published separately, using information available at the time, and may be undertaken at different tiers.

This chemical or group of chemicals are being assessed at Tier II because the Tier I assessment indicated that it needed further investigation.

For more detail on this program please visit:**www.nicnas.gov.au**

Disclaimer

ACRONYMS & ABBREVIATIONS

Grouping Rationale

These chemicals are synthetic organic compounds, used extensively as colourants in various applications. They are chemically similar in that they all possess a triarylmethane backbone with similar chemical chromophores without any clear toxicological differences. There are reports of these chemicals being used as cosmetics in hair dye products.

Import, Manufacture and Use

Australian

The following Australian industrial use was reported under previous mandatory and/or voluntary calls for information.

The chemicals, basic blue 7 (CAS No. 2390-60-5) and C.I. basic blue 26 (CAS No. 2580-56-5) have reported cosmetic use in hair colourant formulations.

International

The following international uses have been identified through:

- the European Union (EU) Registration, Evaluation and Authorisation of Chemicals (REACH) dossiers;
- Galleria Chemica;
- the Substances and Preparations in the Nordic countries (SPIN) database;
- the European Commission Cosmetic Ingredients and Substances (CosIng) database;
- the United States (US) Personal Care Product Council International Nomenclature of Cosmetic Ingredients (INCI) Dictionary;
- the US Environmental Protection Agency Aggregated Computer Toxicology Resource (ACToR);
- the US National Library of Medicine Hazardous Substances Data Bank (HSDB); and
- several journal articles (Littlefield et al., 1985; Docampo & Moreno, 1990)

The chemicals have reported cosmetic use as hair colourants (CAS No. 548-62-9; CAS No. 2390-59-2; CAS No. 2580-56-5; CAS No. 2390-60-5).

The chemicals have reported domestic use including as dyes and colourants (CAS No. 548-62-9; CAS No. 1694-09-3; CAS No. 72102-55-7; CAS No. 2580-56-5).

The chemicals have reported commercial use including as colourants for oils, solvents, plastics, petrol and waxes (CAS No. 2580-56-5; CAS No. 2390-60-5).

The chemicals have reported site-limited uses including as:

- fixative agents (CAS No. 548-62-9; CAS No. 2580-56-5); and
- reprographic agents (CAS No. 2580-56-5).

The chemicals have reported non-industrial uses including in topical antibacterial preparations (CAS No. 548-62-9).

Restrictions

Australian

The chemical crystal violet (CAS No. 548-62-9) is listed in the *Poisons Standard*—the *Standard for the Uniform Scheduling of Medicines and Poisons* (SUSMP) in Schedule 4 (SUSMP, 2014) as follows:

'CRYSTAL VIOLET for human use except when used as a dermal marker.'

Schedule 4 chemicals are described as 'Substances, the use or supply of which should be by or on the order of persons permitted under the Act to prescribe and should be available from a pharmacist on prescription.' (SUSMP, 2014).

No other chemicals in this group are listed in the *Poisons Schedule*.

International

The chemicals are listed on the following (Galleria Chemica):

- EU Cosmetics Regulation 1223/2009 Annex II—List of substances prohibited in cosmetic products (CAS No. 548-62-9; CAS No. 1694-09-3; CAS No. 2390-59-2; CAS No. 2390-60-5; CAS No. 2580-56-5)
- New Zealand Cosmetic Products Group Standard—Schedule 4: Components cosmetic products must not contain (CAS No. 548-62-9; CAS No. 1694-09-3; CAS No. 2580-56-5);
- Health Canada List of prohibited and restricted cosmetic ingredients (The Cosmetic Ingredient 'Hotlist') (CAS No. 548-62-9; CAS No. 1694-09-3; CAS No. 2390-59-2; CAS No. 2390-60-5; CAS No. 2580-56-5); and
- The Association of Southeast Asian Nations (ASEAN) Cosmetic Directive Annex II Part 1: List of substances which must not form part of the composition of cosmetic products (CAS No. 548-62-9; CAS No. 1694-09-3; CAS No. 2580-56-5).

The chemicals (CAS No. 548-62-9; CAS No. 2580-56-5) are listed on the candidate list of substances of very high concern (SVHC) for eventual inclusion in Annex XIV (ECHA, 2014). In the EU, companies could have legal obligations if the chemical that they produce, supply or use is included on the candidate list whether on its own, in mixtures, or present in articles.

Existing Worker Health and Safety Controls

Hazard Classification

The chemical crystal violet (CAS No. 548-62-9) is classified as hazardous, with the following risk phrases for human health in the Hazardous Substances Information System (HSIS) (Safe Work Australia):

- Xn; R22 (acute toxicity)
- Xi; R41 (irritation)
- R40 Carc. Cat 3 (carcinogenicity)

The chemical benzyl violet (CAS No. 1694-09-3) is classified as hazardous, with the following risk phrases for human health in the HSIS (Safe Work Australia):

- R40 Carc. Cat 3 (carcinogenicity)

Exposure Standards

Australian

No specific exposure standards are available.

International

No specific exposure standards are available.

Health Hazard Information

In the past, the term gentian violet was used to refer to a mixture of crystal violet and other methyl violets, a group that includes crystal violet as well as analogues where the nitrogen atoms are not completely methyl substituted. More recently, the term is used synonymously with crystal violet (CAS No. 548-62-9). In this report, the term is used in one instance to refer to a non-specific mixture of methyl violets.

Toxicokinetics

Absorption

Very little information is available on the absorption of these chemicals. Crystal violet has been demonstrated to be absorbed in the gastrointestinal tract; however, few experimental details have been provided (WHO, 2014).

The toxicokinetics of benzyl violet (CAS No. 1694-09-3) were investigated in Wistar and Sprague Dawley (SD) rats. In brief, absorption of the chemical when administered orally was minimal. Only 0.89 % of the dose was recovered from the bile after 24 hours, while in contrast, the chemical, when administered intravenously, was found to be rapidly excreted via the bile (Minegishi et al., 1977).

As the chemicals in this group contain large quarternary ammonium ions, the rate of penetration across the epidermis is expected to be slow (Diamante et al., 2009).

Metabolism

The chemical is metabolised by cytochrome P-450 in the liver to give rise to a carbon-centred radical metabolite (Diamante et al., 2009). The chemical has been shown to hinder adenosine triphosphate (ATP) synthesis and enhance ATPase activity. It has also been shown to be actively demethylated by NADPH-supplemented liver microsomes to pentamethylpararosaniline chloride, N,N,N',N'-tetramethylpararosaniline chloride and N,N,N',N?-tetramethylpararosaniline chloride (WHO, 2014). Furthermore, evidence suggests crystal violet could be a substrate for metabolism by prostaglandin synthase, potentially giving rise to carcinogenic degradants (Docampo & Moreno, 1990).

Distribution

Studies have assessed the distribution of crystal violet in male and female B6C3F1 mice. Multiple doses of ^{14}C-labelled crystal violet (CAS No. 548-62-9) (5.6 mg/kg body weight (bw)) were administered to animals by gavage at 12-hour intervals for seven days. The test material was found to be distributed preferentially to the liver and adipose tissue. Similar patterns of distribution have been observed in Fischer 344 (F344) rats (WHO, 2014).

Excretion

Crystal violet (CAS No. 548-62-9) is excreted in the faeces of rats following oral dosing (Decampo & Moreno, 1990). Another study, performed on B5C3F1 mice, demonstrated that orally administered crystal violet is excreted via the faeces (65.9 % and 67.4 % of total dose for males and females, respectively) and urine (5.9 % and 8.1 % of total dose for males and females, respectively) (WHO, 2014).

Acute Toxicity

Oral

Crystal violet (CAS No. 548-62-9) is classified as hazardous with the risk phrase ‘Harmful if swallowed’ (Xn; R22) in the HSIS (Safe Work Australia).

Crystal violet was found to have moderate to high acute toxicity based on results from animal tests following oral exposure. The median lethal dose (LD50) in rats ranges from 90 mg/kg bw to 650 mg/kg bw for chemicals in this group. Data on ethyl violet indicate similar toxicity. As the chemicals in this group are close analogues of crystal violet or ethyl violet, the classification should apply to all the members of this group (see **Recommendation** section).

The acute toxicity of crystal violet (CAS No. 548-69-2) was studied in male ICR mice and SD rats. The chemical was prepared as a suspension in polyethylene glycol, and animals were administered the chemical by oral gavage. Oral LD50 values of 800 and 180 mg/kg bw for mice and rats, respectively were determined in these studies (Hodge et al., 1972).

In another study, crystal violet was administered via gavage to 44 young and 24 adult rats (strain and sex not stated). Animals received the chemical in solution at concentrations of 1 % or 2 %. The oral LD50 was approximately 90 mg/kg bw for young rats and approximately 650 mg/kg bw for adult rats. No further experimental details were provided (Diamante et al., 2009).

In a non-guideline study, crystal violet (CAS No. 548-62-9) was administered to adult ChR-CD male rats by intragastric injection at 30-11,000 mg/kg bw. An approximate minimum lethal dose (the lowest dose at which mortality occurred) of 670 mg/kg bw was determined from this study. Sublethal effects included lethargy on the second day after dosing, stained perineal area, aesthenia and weight loss. No LD50 was reported (REACH).

In a non-guideline study, ethyl violet (CAS No. 2390-59-2) was assessed for acute toxicity in SD rats (five/group). Males received the chemical at doses of 0, 250, 500, 595, 707 or 1000 mg/kg bw, whereas females were dosed at 0, 62.5, 125, 250, 500, or 1000 mg/kg bw (both by gavage). The investigators reported that the chemical had an LD50 of 549 mg/kg bw in male rats and 308 mg/kg bw in females. Signs of acute toxicity included sluggishness, unsteady gait, facial erythema, tremors, diarrhoea, emaciation and a moribund appearance (Diamante et al., 2009).

No data are available for the other chemicals in this group.

Dermal

No data are available.

Inhalation

No data are available.

Corrosion / Irritation

Skin Irritation

The chemicals in this group could cause mild skin irritation.

Crystal violet (CAS No. 548-62-9) was assessed as a skin irritant in a 1977 study. The chemical (at an unreported concentration) was applied to human skin and was reported to cause mild irritation (REACH). Very few experimental details were provided and, as a result, the study is considered to be unreliable .

No data are available for the other chemicals in this group.

Eye Irritation

The chemical, crystal violet (CAS No. 548-62-9) is classified as hazardous with the risk phrase 'Risk of serious damage to eyes' (Xi; R41) in the HSIS (Safe Work Australia). The available data support this classification and the extension of this classification to other chemicals in this group, due to the chemical similarity of the cationic species.

In eye irritation studies, crystal violet caused serious eye damage and the effects were not reversible within the observation period.

The potential for crystal violet (CAS No. 548-62-9) to cause ocular irritation has been assessed in several studies. In one study, described as a standard rabbit eye irritation test, the chemical was administered at 20 mg/mL to the eyes of rabbits (strain not stated). Application resulted in rapid severe and persistent blepharitis (inflammation of the eyelid) with hyperaemia, oedema and

necrosis of the conjunctivae and nictitating membrane. Three weeks after application, there was gross opacification, deformity and vascularisation of the cornea (Diamante et al., 2009).

In a separate investigation, a 1 % crystal violet solution was instilled in the conjunctival sac of two rabbits, three times a day (number of days was not reported). Both rabbits developed conjunctival vascular congestion and discharge the following day. Three days after administration, some necrosis of the conjunctivae was evident. Histological assessment of conjunctival biopsies revealed epithelial thinning, goblet cell loss and capillary congestion with neutrophilic infiltration (Diamante et al., 2009).

No data are available for the other chemicals in this assessment.

Sensitisation

Skin Sensitisation

The chemicals in this group do not contain a structural alert for skin sensitisation (OECD Toolbox). Data summarised below from a close structural analogue, malachite green oxalate (CAS No. 2437-29-8), support the conclusion that the chemicals in this group are not skin sensitisers.

A guinea pig maximisation test with malachite green oxalate (CAS No. 2437-29-8) was reported (Clemmensen et al., 1984). Animals were intradermally induced at 0.2 % followed by topical induction at 20 %. Animals were then challenged seven days later by topical application of 0.05, 0.1 and 1 % solutions. No responses indicative of skin sensitisation were observed.

No data are available for the other chemicals in this group.

Repeated Dose Toxicity

Oral

Relevant data for repeated dose toxicity are from a two-year carcinogenicity and two developmental toxicity studies in rats. In the rat study, the lowest observed adverse effect level (LOAEL), based on mortality rates, is 10 mg/kg bw/day.

In a life-span dosing study to assess the carcinogenicity of gentian violet (an unspecified mixture of methyl violets), male and female R6C3F1 mice (720 mice/sex) were dosed with the chemical at 0, 100, 300 or 600 ppm in the diet. Conversions to mg/kg bw were not provided in the study. However, based on conversion factors this is roughly equivalent to 0, 5, 15 or 30 mg/kg bw/day (Nielsen et al., 2008). Selected animals were euthanised after 12, 18 and 24 months of continuous dosing. No effect on feed consumption or body weights was observed. Mortality was statistically significantly increased at all treatment doses (100, 300 or 600 ppm) in female animals at 28, 27 and 64 %, respectively compared with 13 % for controls. Mortality in males was 14, 20 and 23 %, respectively compared with 13 % for controls. Other effects included erythropoiesis in the spleen and atrophy of the ovaries after 18 months of treatment. These results are difficult to interpret in the context of repeated dose toxicity, given that mortality was reported to be very low up until after approximately 450 days. This suggests that the dose-dependent increase in deaths was a result of neoplasm-related mortality, rather than repeated dose toxicity-related mortality (Littlefield et al., 1985).

In another two-year study, male and female weanling rats were dosed with crystal violet in the diet at 0, 100, 300 or 600 mg/kg for 80 days (WHO, 2014). During dosing, females and males at the same dose level were mated. Two males and two females were selected from each litter at random (F1a generation). The F1a animals continued on the same dose levels as their parents, with a total of 570 male and 570 female F1a rats treated (doses were approximately 0, 30, 80 and 160 mg/kg bw/day for males and 0, 40, 100 and 200 mg/kg bw/day for females, respectively). Mortality rates at the age of 24 months for the females were 38, 60 and 66 % for the low-dose, mid-dose and high-dose groups, respectively compared with 33 % for the control. For males, the same respective dose groups had mortality rates after 104 weeks of 33, 48 and 39 % compared with 33 % for the controls (refer **Carcinogenicity** for other details).

In a developmental toxicity study, New Zealand White rabbits were dosed by gavage on gestation days (GD) 6–19 with the crystal violet at 0, 0.5, 1.0 or 2.0 mg/kg bw/day.

Maternal mortality was also reported as 7.4 % in the 0.5 mg/kg bw/day group, 15.4 % in the 1.0 mg/kg bw/day group and 22.6 % in the 2.0 mg/kg bw/day group, compared with 0.0 % in the control group. (See **Reproductive and developmental toxicity** for further details.)

In another developmental toxicity study, female rats (n = 153) were mated with breeder males (n = 127) and dosed daily via gavage with crystal violet at doses of 0.0, 2.5, 5.0 or 10.0 mg/kg bw/day on GD 6–15. The maternal mortality rate was 9.4 % in the 10 mg/kg bw/day group. All other dams from all other doses survived until the conclusion of the experiment. (See **Reproductive and developmental toxicity** for experimental details.)

No data are available for the other chemicals in this assessment.

Dermal

No data are available.

Inhalation

No data are available.

Genotoxicity

Positive results were reported for crystal violet (CAS No. 548-62-9) in two in vitro point mutation assays (Ames test) in *Salmonella typhimurium* strains TA 98, TA 1535 and TA 1538, with or without metabolic activation. Positive results were also observed in several other in vitro assays conducted with the chemical. While there is a concern for mutagenicity, in the absence of clear positive in vivo data, the available data do not meet the criteria for classification.

In vitro

In a bacterial reverse mutation assay, crystal violet (CAS No. 548-62-9) was incubated with *S. typhimurium* TA98, TA100, TA1535, TA1537, and TA1538 at concentrations ranging up to 12 µg/plate both with and without metabolic activation. The compound was mutagenic in the TA98 and TA1538 strains. In the TA1538 strain, there was a nine-fold increase in the frequency of mutations compared with controls (REACH).

In another bacterial reverse mutation assay, crystal violet (CAS No. 548-62-9) was assessed for mutagenicity in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 at up to 3.2 µg/mL. The test material was found only to be mutagenic in *S. typhimurium* strain TA1535 in the absence of S9 metabolic activation. No further experimental details were provided (Diamante et al., 2009).

Chinese hamster ovary (CHO) cells were treated with gentian violet, a formulation containing no less than 96 % crystal violet (CAS No. 548-62-9), at 1 and 10 µg/mL for two, five and eight hours in an in vitro genotoxicity study. The increased frequency of chromosomal abnormalities (chromatin bridges, lagging chromosomes, chromosome fragments and sticky chromosomes) was dose- and time-dependent. Three other mammalian cell types (HeLa lymphocytes and two fibroblast-like cells) also showed similar genotoxic responses to the chemical (Au et al., 1978).

No data are available for the other chemicals in this assessment.

In vivo

Crystal violet (CAS No. 548-62-9) was applied to the inner shell membrane of 74-hour Cornell K-strain chicken embryos at doses of up to 2000 µg per embryo. Embryos were then grown in the presence of crystal violet (0.5–10 µg). At doses up to 10 µg, no sister chromatid exchange was seen. One surviving embryo at 100 µg exhibited significant sister chromatid exchange (Diamente et al., 2009).

In another study, Swiss albino mice were administered crystal violet at 20 or 40 µg/mL in drinking water (calculated to be approximately 4 µg/mL and 8 mg/kg/day respectively). Animals were selected weekly at random for four weeks, euthanised and

bone marrow cells harvested for chromosome analysis. The chemical failed to induce statistically significant chromosome damage at either concentration in any of the animals.

Carcinogenicity

The chemicals, crystal violet (CAS No. 548-62-9) and benzyl violet (CAS No. 1694-09-3) are classified as hazardous—Category 3 carcinogenic substance—with the risk phrase 'Limited evidence of carcinogenic effect' (Xn; R40) in the HSIS (Safe Work Australia). The available data support this classification.

Crystal violet (CAS No. 548-62-9) was assessed for carcinogenicity in B6C3F1 mice in a life-time dosing study. Male and female mice (150 mice/sex/dose) were dosed with the chemical by dietary administration at 0, 100, 300 or 600 ppm. Conversions to mg/kg bw were not provided in the study. However, based on conversion factors this is roughly equivalent to 0, 5, 15 or 30 mg/kg bw/day (Nielsen et al., 2008) (refer **Repeated dose toxicity** for other details). Hepatocellular adenomas and carcinomas were the most common lesions, with significant dose-related increases found at 24 months in males and at both 18 and 24 months in females. The females also showed statistically significant dose-related increases in adenoma of the Harderian gland and in type A reticulum cell sarcoma in the urinary bladder, uterus, ovaries and vagina. Under these test conditions, the chemical was found to be carcinogenic in mice at several different organs. Female mice appeared to be affected more than males (Littlefield et al., 1985; WHO, 2014).

Benzyl violet (CAS No. 1694-09-3) produced mammary carcinomas and squamous cell carcinomas of the skin of female rats (strain not stated) following oral administration. Subcutaneous injection of the test material resulted in local fibrosarcomas. No further experimental details were provided (Cheremisinoff, 1994).

In a carcinogenicity study conducted with good laboratory practice (GLP), male and female weanling rats were dosed with crystal violet (CAS No. 548-62-9) at 0, 100, 300 or 600 mg/kg in diet for 80 days. During dosing, females and males from the same dose level were mated. Two males and two females were selected from each litter at random (F1a generation). The F1a animals continued on the same dose levels as their respective parents. In total, 570 male and 570 female F1a rats were fed crystal violet 0, 100, 300 or 600 mg/kg diet (equal to approximately 0, 30, 80 and 160 mg/kg bw/day for males and 0, 40, 100 and 200 mg/kg bw/day for females, respectively) for 12, 18 and 24 months. Food consumption, body weights and clinical signs were recorded weekly. Complete necropsy, histopathological examination and clinical chemistry analysis were performed on selected animals at (12, 18 and 24 months) (WHO, 2014).

No dose-related pathology was observed in any animals euthanised after 12 months of dosing. Male and female rats fed 600 mg/kg in diet for 24 months showed a decrease in body weight (refer **Repeated dose toxicity** for other details). The majority of neoplastic lesions were observed only at the 24-month necropsy. Increases in the incidence of follicular cell adenocarcinomas of the thyroid were statisticaly significnat in males in the highest dose group, and in females in the two highest dose groups. Hepatocellular adenomas were significantly increased at 24 months in males in the 300 mg/kg and 600 mg/kg feed groups (WHO, 2014).

No data are available for the other chemicals in this assessment.

Reproductive and Developmental Toxicity

Skeletal variations observed in rat offspring were only observed at maternally toxic doses.

Crystal violet (CAS No. 548-62-9) was assessed for developmental toxicity following maternal exposure in a non-guideline study. New Zealand White rabbits were dosed by gavage on GD 6–19 with the chemical at 0, 0.5, 1.0 or 2.0 mg/kg bw/day. Females were euthanised on GD 30 and were evaluated for body weight, liver weight, gravid uterine weight and the status of uterine implantation sites. Live foetuses were removed from the uterus and assessed for body weight, sex ratios and morphological abnormalities. Maternal mortality was also reported: 0.0 % in the control group, 7.4% in the 0.5 mg/kg bw/day group, 15.4% in the 1.0 mg/kg bw/day group and 22.6 % in the 2.0 mg/kg bw/day group. All groups exposed to the test material had significantly lower body weight gain than the control groups for both the treatment and gestation periods. Clinical signs including wheezing, diarrhoea, congestion, wet nose, dyspnoea, lacrimation, anorexia and cyanosis were elevated in a dose-dependent manner. There were also reductions in the average foetal body weights per litter in all dosed groups versus control groups.

There were no significant dose-related effects on the incidence of gross, visceral or skeletal malformations per litter, nor in the number of foetuses, malformed foetuses per litter, nor in the number of litters with malformed foetuses. In conclusion, no evidence of teratogenicity of crystal violet was seen when it was administered by gavage to pregnant New Zealand White rabbits during organogenesis at doses that produced evidence of maternal mortality and toxicity (NTP, 1983).

In a similar study, crystal violet (CAS No. 548-62-9) was assessed for developmental toxicity (Diamante et al., 2009). Female rats (n = 153) were mated with breeder males (n = 127) and dosed daily via gavage with crystal violet at doses of 0.0, 2.5, 5.0 or 10.0 mg/kg bw/day on GD 6–15. The maternal mortality rate was 9.4 % in the 10 mg/kg bw group. All other dams in the other dose groups survived until the conclusion of the experiment (WHO, 2014).

No dose-dependent significant differences were observed in the following parameters: number of implantation sites per litter; number or percent of resorptions, foetal deaths; or dead pups per litter. However, there was a significant trend toward an increased number and percentage of pups affected (dead or malformed) per litter with increasing doses. The number of litters with affected foetuses was significantly increased in the 10.0 mg/kg bw/day group versus controls. The number and percentage of malformed foetuses, both males and females, per litter was also significantly increased in the highest dose group versus controls. Because there was no significant incidence of malformations in the lower dose groups, the investigators suggested that foetal response to the chemical could be secondary to maternal toxicity (WHO, 2014).

In a three-generation reproductive toxicity study, crystal violet (CAS No. 548-62-9) was administered to F344 rats at doses of 0, 100, 300 or 600 mg/kg (equivalent to 0, 5, 15 and 30 mg/kg bw per day, respectively) in their feed. Males and females of the same dose group were mated. Offspring were selected and mated with animals of the same dose group for three generations in total. A dose-dependent effect on body weight was noted in the highest dose group. The test material had no effect on the number of pups per litter. The number of stillborn animals compared across the generations or across doses did not show a consistent pattern. The number of animals not surviving to weanling age and the sex ratio did not show significant dose or generation effects. No dose-related effects on the incidence of gross deformities were noted in examinations of pups of each generation. The only significant histopathological changes noted in the third (F3a) generation were a dose-related trend for focal dilatation of the renal cortex and tubules, a statistically significant dose-related trend for necrosis of the thymus and a statistically significant inverse dose–response relationship for red pulp haematopoietic cell proliferation of the spleen (WHO, 2014).

The no observed adverse effect level (NOAEL) for parental toxicity was 15 mg/kg bw/day, based on reductions in body weight at 30 mg/kg bw/day. A NOAEL for offspring toxicity could not be determined, as effects in the F3 generation were present in all dose groups. The NOAEL for reproductive toxicity was 30 mg/kg bw/day, the highest dose tested (WHO, 2014).

No data are available for the other chemicals in this group.

Risk Characterisation

Critical Health Effects

The critical health effects for risk characterisation include a systemic long-term effect (carcinogenicity) and systemic acute effect (acute toxicity from oral exposure). The chemicals can also cause serious eye damage and harmful effects following repeated oral exposure.

Public Risk Characterisation

The chemicals, basic blue 7 (CAS No. 2390-60-5) and C.I. basic blue 26 (CAS No. 2580-56-5) have reported cosmetic use in hair colourant formulations in Australia, based on a survey undertaken by industry (NICNAS).

Overseas, several of the chemicals (CAS No. 548-62-9; CAS No. 2390-59-2; CAS No. 2580-56-5; CAS No. 2390-60-5) are being, or have been, used as hair colourants. In addition, several of the chemicals in the group (CAS No. 548-62-9; CAS No. 1694-09-3; CAS No. 72102-55-7; and CAS No. 2580-56-5) have dye and colourant use in domestic products overseas.

The EU and ASEAN as well as Canada and New Zealand have prohibited the use of these chemicals in cosmetics. While it appears that crystal violet (CAS No. 548-62-9) is prohibited for cosmetic use, given the Schedule 4 listing for human use, except

when used as a dermal marker (SUSMP, 2014), the other chemicals in this group are not scheduled or risk managed for cosmetic or domestic use.

Considering the high acute toxicity, carcinogenicity, repeated dose toxicity and the serious eye damage that could be caused by these chemicals, there is a concern for the use of these chemicals in cosmetic and domestic products without any risk management measures. Although dermal absorption is thought to be poor through human skin (Diamante et al., 2009) there are no toxicokinetic or dermal acute/chronic data to verify this.

Occupational Risk Characterisation

During product formulation, oral, dermal and ocular exposure might occur, particularly where manual or open processes are used. These could include transfer and blending activities, quality control analysis, and cleaning and maintaining equipment. Worker exposure to the chemicals at lower concentrations could also occur while using formulated products containing the chemicals. The level and route of exposure will vary depending on the method of application and work practices employed.

Given the critical systemic long-term, systemic acute and local health effects, the chemicals could pose an unreasonable risk to workers unless adequate control measures to minimise dermal and ocular exposure are implemented. The chemicals should be appropriately classified and labelled to ensure that a person conducting a business or undertaking (PCBU) at a workplace (such as an employer) has adequate information to determine the appropriate controls.

The data available support an amendment to the hazard classification in the HSIS (Safe Work Australia) (refer to **Recommendation** section.

NICNAS Recommendation

Further risk management is required. Sufficient information is available to recommend that risks to public health and safety from the potential use of the chemicals in cosmetics and/or domestic products be managed through changes to the Poisons Standard (SUSMP), and risks for workplace health and safety be managed through changes to classification and labelling.

Assessment of the chemicals is considered to be sufficient provided that risk management recommendations are implemented and all requirements are met under workplace health and safety and poisons legislation as adopted by the relevant state or territory.

Regulatory Control

Public Health

It is recommended that the chemical be listed in Schedule 4 of the SUSMP to preclude the use of this chemical in hair dye preparations.

Work Health and Safety

The chemicals are recommended for classification and labelling under the current approved criteria and adopted GHS as below. This assessment does not consider classification of physical and environmental hazards.

Hazard	Approved Criteria (HSIS)[a]	GHS Classification (HCIS)[b]
Acute Toxicity	Toxic if swallowed (T; R25)	Toxic if swallowed - Cat. 3 (H301)

Hazard	Approved Criteria (HSIS)[a]	GHS Classification (HCIS)[b]
Irritation / Corrosivity	Risk of serious eye damage (Xi; R41)	Causes serious eye damage - Cat. 1 (H318)
Carcinogenicity	Carc. Cat 3 - Limited evidence of a carcinogenic effect (Xn; R40)	Suspected of causing cancer - Cat. 2 (H351)

[a] Approved Criteria for Classifying Hazardous Substances [NOHSC:1008(2004)].

[b] Globally Harmonized System of Classification and Labelling of Chemicals (GHS) United Nations, 2009. Third Edition.

[*] Existing Hazard Classification. No change recommended to this classification

Advice for consumers

Products containing the chemicals should be used according to the instructions on the label.

Advice for industry

Control measures

Control measures to minimise the risk from oral/dermal/ocular exposure to the chemicals should be implemented in accordance with the hierarchy of controls. Approaches to minimise risk include substitution, isolation and engineering controls. Measures required to eliminate, or minimise risk arising from storing, handling and using a hazardous chemical depend on the physical form and the manner in which the chemicals are used. Examples of control measures which could minimise the risk include, but are not limited to:

- using closed systems or isolating operations;
- health monitoring for any worker who is at risk of exposure to the chemicals, if valid techniques are available to monitor the effect on the worker's health;
- minimising manual processes and work tasks through automating processes;
- work procedures that minimise splashes and spills;
- regularly cleaning equipment and work areas; and
- using protective equipment that is designed, constructed, and operated to ensure that the worker does not come into contact with the chemicals.

Guidance on managing risks from hazardous chemicals are provided in the *Managing risks of hazardous chemicals in the workplace—Code of practice* available on the Safe Work Australia website.

Personal protective equipment should not solely be relied upon to control risk and should only be used when all other reasonably practicable control measures do not eliminate or sufficiently minimise risk. Guidance in selecting personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

Obligations under workplace health and safety legislation

Information in this report should be taken into account to help meet obligations under workplace health and safety legislation as adopted by the relevant state or territory. This includes, but is not limited to:

- ensuring that hazardous chemicals are correctly classified and labelled;

- ensuring that (material) safety data sheets ((M)SDS) containing accurate information about the hazards (relating to both health hazards and physicochemical (physical) hazards) of the chemicals are prepared; and
- managing risks arising from storing, handling and using a hazardous chemical.

Your work health and safety regulator should be contacted for information on the work health and safety laws in your jurisdiction.

Information on how to prepare an (M)SDS and how to label containers of hazardous chemicals are provided in relevant codes of practice such as the *Preparation of safety data sheets for hazardous chemicals—Code of practice* and *Labelling of workplace hazardous chemicals—Code of practice*, respectively. These codes of practice are available from the Safe Work Australia website.

A review of the physical hazards of these chemicals has not been undertaken as part of this assessment.

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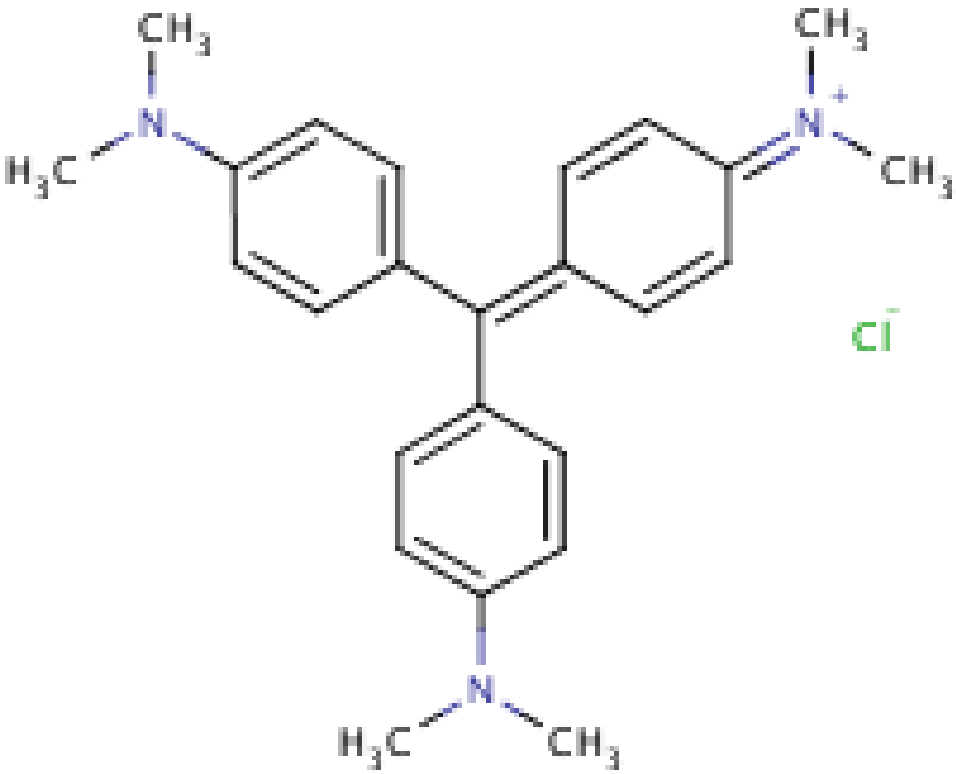
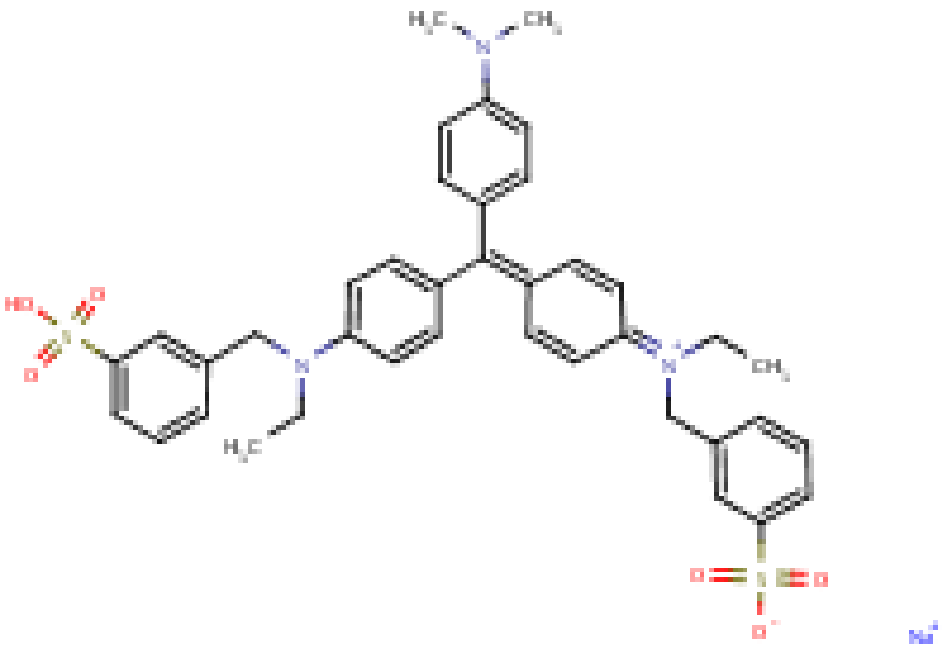
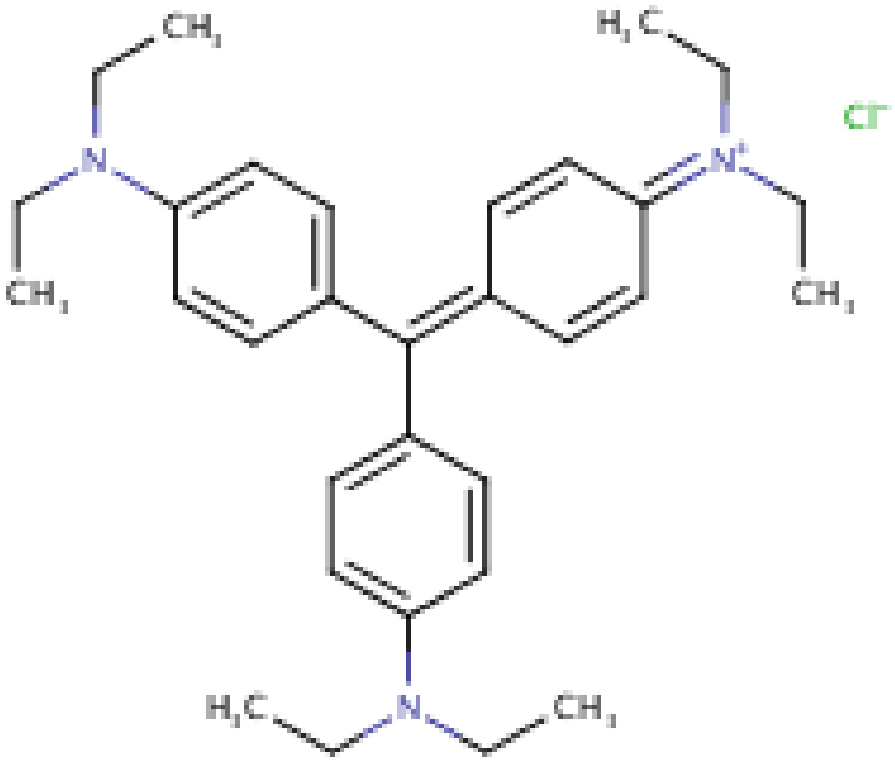
Last Update 13 February 2015

Chemical Identities

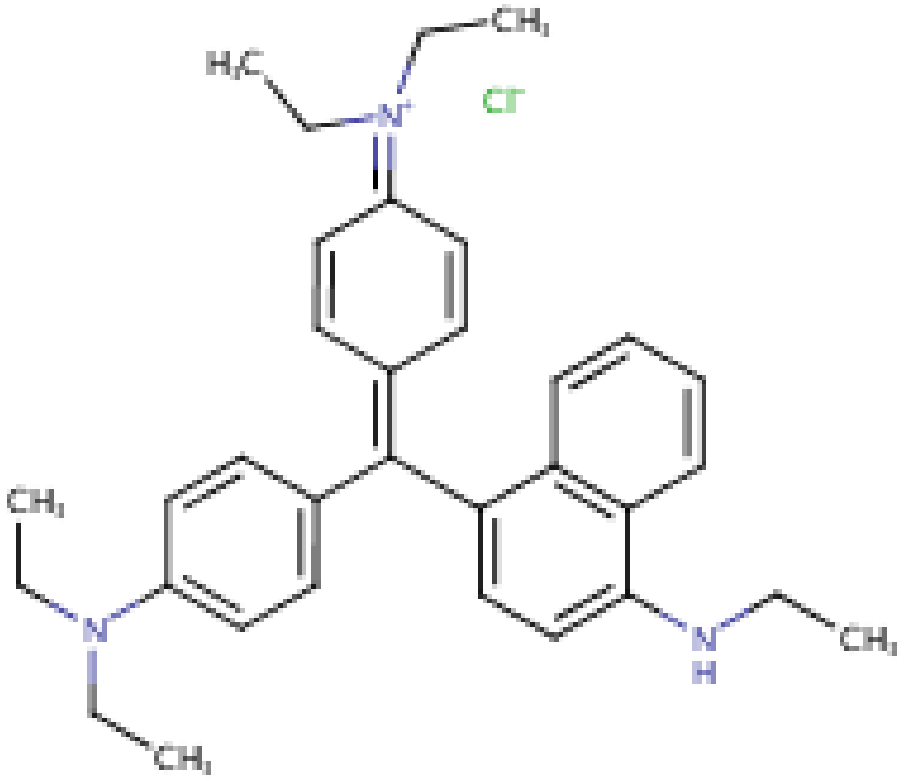
Chemical Name in the Inventory and Synonyms	**Methanaminium, N-[4-[bis[4-(dimethylamino)phenyl]methylene]-2,5-cyclohexadien-1-ylidene]-N-methyl-, chloride** C.I. basic violet 3 crystal violet (4-(Bis(para-(dimethylamino)phenyl)methylene)-2,5-cyclohexadien-1-ylidene)dimethylammonium chloride hexamethyl para-rosaniline hydrochloride methanaminium, N-(4-(bis(4-(dimethylamino)phenyl)methylene)-2,5-cyclohexadien-1-ylidene)-N-methyl-, chloride (1:1)
CAS Number	548-62-9
Structural Formula	
Molecular Formula	C25H30N3.Cl

Molecular Weight	407.99

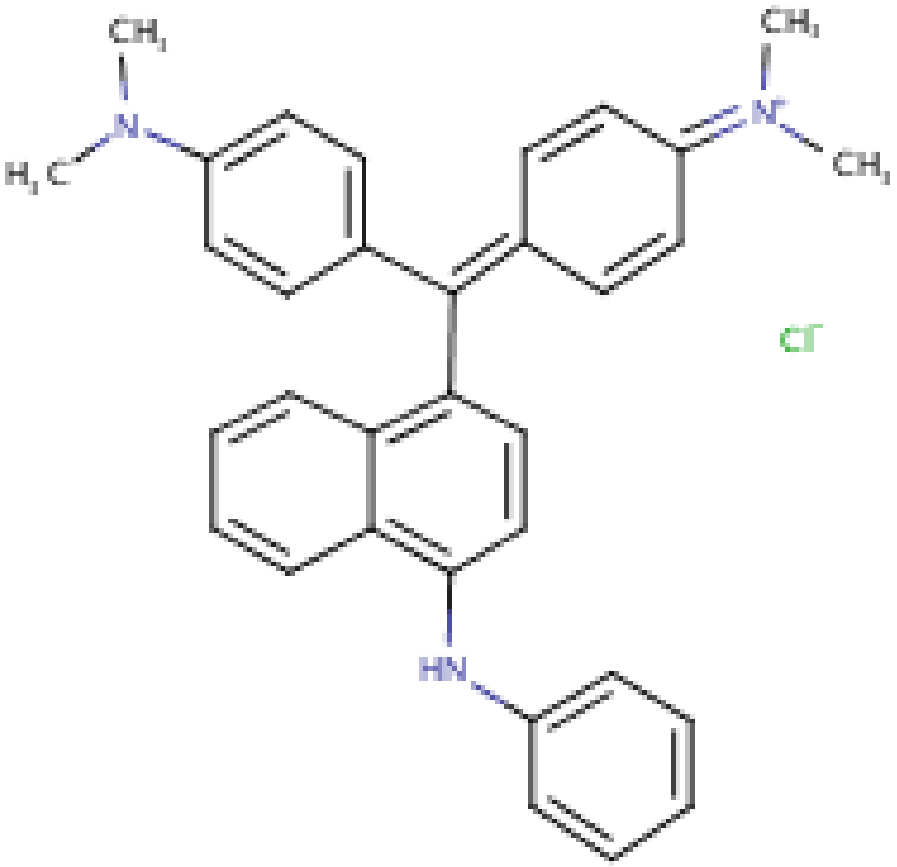
Chemical Name in the Inventory and Synonyms	**Benzenemethanaminium, N-[4-[[4-(dimethylamino)phenyl][4-[ethyl[(3-sulfophenyl)methyl]amino]phenyl]methylene]-2,5-cyclohexadien-1-ylidene]-N-ethyl-3-sulfo-, hydroxide, inner salt, sodium salt** FD & C violet acid violet 6B benzyl violet benzenemethanaminium, N-(4-((4-(dimethylamino)phenyl)(4-(ethyl((3-sulfophenyl)methyl)amino)phenyl)methylene)-2,5-cyclohexadien-1-ylidene)-N-ethyl-3-sulfo-, inner salt, sodium salt (4-((4-(dimethylamino)phenyl)(4-(ethyl(3-sulphonatobenzyl)amino)phenyl)methylene)cyclohexa-2,5-dien-1-ylidene)(ethyl)(3-sulphonatobenzyl)ammonium, sodium salt
CAS Number	1694-09-3
Structural Formula	
Molecular Formula	C39H41N3O6S2.Na
Molecular Weight	734.89

Chemical Name in the Inventory and Synonyms	**Ethanaminium, N-[4-[bis[4-(diethylamino)phenyl]methylene]-2,5-cyclohexadien-1-ylidene]-N-ethyl-, chloride** ethyl violet C.I basic violet 4 (4-(bis(4-(diethylamino)phenyl)methylene)-2,5-cyclohexadien-1-ylidene)diethylammonium chloride ethanaminium, N-(4-(bis(4-(diethylamino)phenyl)methylene)-2,5-cyclohexadien-1-ylidene)-N-ethyl-, chloride C.I. 42600
CAS Number	2390-59-2
Structural Formula	
Molecular Formula	C31H42N3.Cl
Molecular Weight	492.147

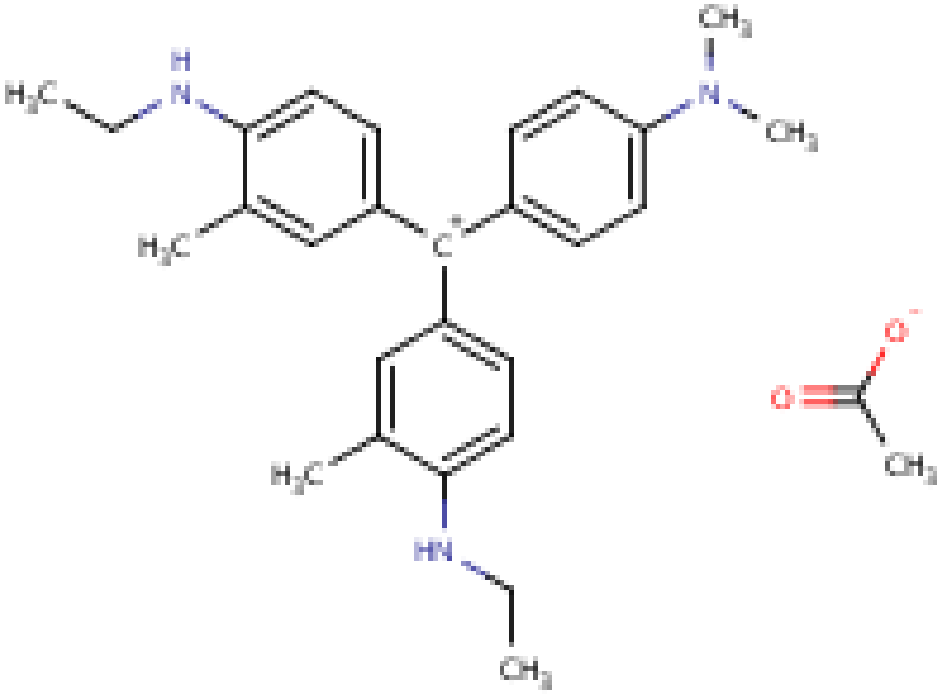
Chemical Name in the Inventory and Synonyms	**Ethanaminium, N-[4-[[4-(diethylamino)phenyl][4-(ethylamino)-1-naphthalenyl]methylene]-2,5-cyclohexadien-1-ylidene]-N-ethyl-, chloride** C.I. basic blue 7 victoria pure blue ethanaminium, N-(4-((4-(diethylamino)phenyl)(4-(ethylamino)-1-naphthalenyl)meth- ylene)-2,5-cyclohexadien-1-ylidene)-N-ethyl-, chloride (4-(4-(diethylamino)-alpha-(4-(ethylamino)-1-naphthyl)benzylidene)cyclohexa-2,5-dien-1-ylidene)diethylammonium chloride

CAS Number	2390-60-5
Structural Formula	
Molecular Formula	C33H40N3.Cl
Molecular Weight	514.153

Chemical Name in the Inventory and Synonyms	**Methanaminium, N-[4-[[4-(dimethylamino)phenyl][4-(phenylamino)-1-naphthalenyl]methylene]-2,5-cyclohexadien-1-ylidene]-N-methyl-, chloride** C.I. basic blue 26 2-methanaminium, N-(4-((4-(dimethylamino)phenyl)(4-(phenylamino)-1-naphthalenyl)me- thylene)-2,5-cyclohexadien-1-ylidene)-N-methyl-, chloride (4-((4-anilino-1-naphthyl)(4-(dimethylamino)phenyl)methylene)cyclohexa-2,5-dien-1-ylidene)dimethylammonium chloride
CAS Number	2580-56-5
Structural Formula	

	
Molecular Formula	C33H32N3.Cl
Molecular Weight	506.09

Chemical Name in the Inventory and Synonyms	**Methylium, [4-(dimethylamino)phenyl]bis[4-(ethylamino)-3-methylphenyl]-, acetate** [4-(dimethylamino)phenyl)bis(4-(ethylamino)-3-methylphenyl)methylium acetate methylium, (4-(dimethylamino)phenyl)bis(4-(ethylamino)-3-methylphenyl)-, acetate
CAS Number	72102-55-7
Structural Formula	

	
Molecular Formula	C27H34N3.C2H3O2
Molecular Weight	459.6303

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