Malachite green and related chemicals: Human health tier II assessment

10 March 2017

Chemicals in this assessment

Chemical Name in the Inventory	CAS Number
Methanaminium, N-[4-[[4-(dimethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]-N-methyl-, chloride	569-64-2
Ethanaminium, N-[4-[[4-(diethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]-N-ethyl-, sulfate (1:1)	633-03-4
Methanaminium, N-[4-[[4-(dimethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]-N-methyl-, ethanedioate, ethanedioate (2:2:1)	2437-29-8
Methanaminium, N-[4-[[4-(dimethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]-N-methyl-, chloride, compound with 3-methyl-1-butanol (1:3)	7278-09-3
Methanaminium, N-[4-[[4-(dimethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]-N-methyl-, ethanedioate (1:1)	13425-25-7

Chemical Name in the Inventory	CAS Number
Methanaminium, N-[4-[[4-(dimethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]-N-methyl-, hydrogen sulfate	16044-24-9
Methanaminium, N-[4-[[4-(dimethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]-N-methyl-, ethanedioate	18015-76-4
Methanaminium, N-[4-[[4-(dimethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]-N-methyl-, molybdate tungstate phosphate	61725-50-6
Ethanaminium, N-[4-[[4-(diethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]-N-ethyl-, molybdate phosphate	68814-00-6
Methanaminium, N-[4-[[4-(dimethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]-N-methyl-, sulfate (2:1)	85188-05-2
Methanaminium, N-[4-[[4-(dimethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]-N-methyl-, methyl sulfate	85204-56-4

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 compounds are members of the malachite green family of dyes consisting of malachite green chloride, other salts of malachite green and chemically related substances. They are chemically similar as they all possess triarylmethane backbones. They have similar uses including in the dyeing industry and as stains in molecular biology.

Import, Manufacture and Use

Australian

The chemical malachite green chloride (CAS No. 569-64-2) has reported non-industrial use as an antimicrobial agent for external fungal and bacterial infections in aquarium fish (FSANZ).

International

The following international uses have been identified through: Galleria Chemica; the Substances and Preparations in Nordic countries (SPIN) database; the United States (US) Personal Care Product Council International Nomenclature of Cosmetic Ingredients (INCI) Dictionary; the US National Library of Medicine's Hazardous Substances Data Bank (HSDB); and various international assessments (ECHA, 2010 a; ECHA, 2010 b; NFI, 2007).

The chemicals have reported cosmetic use as hair colourants (malachite green chloride (CAS No. 569-64-2) and basic green 1 (CAS No. 633-03-4)).

The chemicals have reported domestic and commercial use including as dyes and colourants for fabrics and paper.

The chemicals have reported non-industrial uses including:

- as antimicrobials in fish farming (malachite green chloride (CAS No. 569-64-2) and malachite green oxalate (CAS No. 2437-29-8)); and

- as an intestinal antihelminthic pharmaceutical (malachite green chloride (CAS No. 569-64-2).

Restrictions

Australian

Malachite green is listed in the *Poisons standard—the Standard for the uniform scheduling of medicines and poisons* (SUSMP) in Schedules 5 and 7 (SUSMP, 2014).

Schedule 5:

'in preparations for veterinary use containing 10 per cent or less of malachite green.'

Schedule 7:

'For veterinary use **except** when included in Schedule 5'.

Schedule 5 chemicals are described as 'Substances with a low potential for causing harm, the extent of which can be reduced through the use of appropriate packaging with simple warnings and safety directions on the label.' Schedule 5 chemicals are labelled with 'Caution' (SUSMP, 2014).

Schedule 7 chemicals are described as 'Substances with a high potential for causing harm at low exposure and which require special precautions during manufacture, handling or use. These poisons should be available only to specialised or authorised users who have the skills necessary to handle them safely. Special regulations restricting their availability, possession, storage or use may apply' (SUSMP, 2014).

International

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

- Association of Southeast Asian Nations (ASEAN) Cosmetic Directive Annex II Part I: List of substances which must not form part of the composition of cosmetic products (CAS No. 569-64-2; CAS No. 18015-76-4);
- European Union (EU) Cosmetics Regulation 1223/2009 Annex II—List of substances prohibited in cosmetic products (CAS No. 569-64-2; CAS No. 18015-76-4; CAS No. 633-03-4);
- New Zealand Cosmetic Products Group Standard—Schedule 4: Components cosmetic products must not contain (CAS No. 569-64-2; CAS No. 633-03-4); and
- Health Canada List of prohibited and restricted cosmetic ingredients (The Cosmetic Ingredient 'Hotlist') (CAS No. 569-64-2).

Existing Worker Health and Safety Controls

Hazard Classification

The chemicals malachite green (CAS No. 569-64-2) and basic green 4 oxalate (CAS No. 18015-76-4) are classified as hazardous, with the following hazard categories and hazard statements for human health in the Hazardous Chemical Information System (HCIS) (Safe Work Australia):

- Acute toxicity – category 4; H302 (Harmful if swallowed)
- Eye damage – category 1; H318 (Causes serious eye damage)

- Reproductive toxicity – category 2; H361d (Suspected of damaging the unborn child)

Exposure Standards

Australian

No specific exposure standards are available.

International

No specific exposure standards are available.

Health Hazard Information

This group of chemicals consists of a range of salts of the malachite green cation, as well as several salts of the ethyl substituted analogue of malachite green. The toxicity of the chemicals is assumed to be due to the triarylmethane cation.

Malachite green is available in a number of forms, most commonly as the oxalate (CAS No. 2437-29-8) or chloride salt (CAS No. 569-64-2), but also as basic green 4 oxalate (CAS No. 18015-76-4) and malachite green phosphotungstomolybdate (CAS No. 61725-50-6).

These chemicals are used extensively in the textile industry as colourants. The group is of particular concern to human health as there is evidence to support a role for them in carcinogenesis and reproductive/developmental toxicity.

Toxicokinetics

The toxicokinetics of these chemicals have not been studied extensively in any conventional experiments.

Malachite green has been shown to be metabolised into its reduced form, leucomalachite green, after entering the body. A 1968 study showed that the metabolite was detected in the liver, kidneys, heart, lungs and muscles of rats, two hours after an intravenous injection of malachite green chloride. It has been demonstrated that a multitude of bacterial species present in the intestinal micro flora of mice, rats, rhesus monkeys and humans can reduce malachite green into leucomalachite green (NTP Technical Report, 2004; NFI, 2007).

In a study performed in Fischer 344 (F344) rats, N-demethylated and N-oxidised malachite green and leucomalachite green metabolites as well as primary arylamines were present in the animal livers following consumption of malachite green and leucomalachite green (Culp et al., 1999).

Acute Toxicity

Oral

The chemicals malachite green chloride (CAS No. 569-64-2) and basic green 4 oxalate (CAS No. 18015-76-4) are classified as hazardous with the hazard category 'Acute toxicity – category 4' and hazard statement 'Harmful if swallowed' (H302) in the HCIS (Safe Work Australia). The evidence below suggests this classification should be extended to the other chemicals in this group (see **Recommendation** section).

Very few studies have assessed the acute toxicity of these chemicals in mammals. In one study, male and female Wistar rats were administered (by gavage) a single dose of malachite green oxalate (CAS No. 2437-29-8) at various concentrations and

observed for 14 days. Sub-lethal effects included reduced motor activity, hyperaemia and atonia of the intestinal walls. The oral median lethal dose (LD50) of malachite green oxalate was determined to be 275 mg/kg bodyweight (bw). These investigators also reported an LD50 of 50 mg/kg bw for NMRI mice. However, experimental details were not provided (Clemmensen et al., 1984; ECHA, 2010 a).

The acute oral toxicity of malachite green chloride (CAS No. 569-64-2) has also been assessed in Sprague Dawley (SD) rats. Animals were observed for signs of toxicity following administration of the test material at 300, 450, 600 or 750 mg/kg bw. An oral LD50 of 520 mg/kg bw was determined under these conditions. Sublethal effects of administration included prostration, emaciation and coma (Meyer & Jorgenson, 1983).

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

Dermal

The chemicals in this group have low acute toxicity based on results from animal tests following dermal exposure.

Malachite green oxalate (CAS No. 2437-29-8) was assessed for acute toxicity from dermal exposure in a 1984 non-guideline study. The chemical was applied to the clipped skin of both male and female Wistar rats under occlusive patches at a concentration of 2000 mg/kg bw. Animals were observed and evaluated for evidence of toxicity at one, two, three and five hours after dosing, and for a further 14 days. No signs of systemic toxicity were observed. The LD50 was found to be >2000 mg/kg bw (Clemmensen et al., 1984).

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

Inhalation

No data are available.

Corrosion / Irritation

Skin Irritation

These chemicals have not been assessed for skin irritation in any conventional studies. However, one study reported that a 20 % suspension of malachite green oxalate (CAS No. 2437-29-8) caused no visible erythema or oedema when applied to the skin of Wistar rats or guinea pigs. No further experimental details were provided (Clemmensen et al., 1984).

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

Eye Irritation

The chemicals malachite green chloride (CAS No. 569-64-2) and basic green 4 oxalate (CAS No. 18015-76-4) are classified as hazardous with the hazard category ‘Eye damage – category 1’ and hazard statement ‘Causes serious eye damage’ (H318) in the HCIS (Safe Work Australia). The severity and persistence of the observed effects in the study below are sufficient to support the current classification for malachite green and basic green 4 oxalate. Due to the similarities of the cationic species in this group, it is recommended that the classification be extended to the other chemicals in the group (see **Recommendation** section).

Malachite green oxalate (CAS No. 2437-29-8) was assessed as an ocular irritant in a study conducted similarly to the Organisation for Economic Co-operation and Development (OECD) Test Guideline (TG) 405 (acute eye irritation/corrosion). A solution containing the chemical at a concentration of 76 mg/kg (in 100 µL), was deposited in the conjunctival sac of three Ssc:CPH albino rabbits of unspecified sex. The treatment resulted in marked oedema, ocular discharge and slight hyperaemia

of the conjunctivae. The effects resolved within 24 hours in 2/3 animals. No effect on the corneas or irises was observed (Clemmensen et al,. 1984; NFI, 2007).

A single rabbit was treated with fine crystals of malachite green oxalate (particle size 60–90 µm), which produced a totally opaque cornea and bright red and oedematous conjunctivae that remained for a fortnight (Clemmensen et al,. 1984). Although few details are available, these findings demonstrate that the chemical caused severe ocular irritation under these test conditions.

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

Observation in humans

Malachite green has been reported to be injurious to the human eye (NTP Technical Report, 2004).

Sensitisation

Skin Sensitisation

The negative result observed for the chemical malachite green oxalate in a skin sensitisation animal study (guinea pig maximisation test) supports a 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 conducted (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 a topical application of 0.05, 0.1 or 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

The results from the repeated dose toxicity studies summarised below indicate limited changes in liver weight and minor haematological changes. These do not meet the classification criteria for repeated dose toxicity.

Malachite green oxalate (CAS No. 2497-29-8) was assessed for toxicity following repeated dosing in a non-guideline study. Groups of eight Wistar rats/sex were fed diets containing 0, 10, 100, and 1000 ppm of the chemical (approximately 1, 10 and 100 mg/kg bw/day) for 28 days. Animals were evaluated throughout the study period with the following parameters assessed: clinical signs, body weights, feed consumption, haematology, serum biochemistry (alkaline phosphatase, aspartate aminotransferase, urea, creatinine glucose and methaemoglobin), organ weights, and macro- and microscopic evaluations of the liver, kidneys, adrenals, and testes. Adverse effects were only observed in the high-dose group (1000 ppm). These included hyperactivity, significant reductions in feed intake and weight gain. Among the females an increase in lymphocytes and a concomitant decrease in neutrophils were seen. From this study, a no observed adverse effect level (NOAEL) of 10 mg/kg bw/day was derived (Clemmensen et al., 1984).

A 28-day repeated dose toxicity study assessed the effect of malachite green chloride (CAS No. 569-64-2) on both F344 rats and B6C3F1 mice (ECHA, 2010 a). Eight rats per group were fed diets containing malachite green at concentrations of 0, 25, 100, 300, 600, or 1200 ppm (approximately 0, 2.5, 10, 30, 60, and 120 mg/kg bw/day) for 28 days. Significant reduction in body weights was observed among the female rats in the 1200 ppm group. Elevated liver weights were dose dependent among female rats in the 300, 600 and 1200 ppm groups. Females in the highest dose group had significantly reduced erythrocyte counts and haemoglobin, haematocrit, mean erythrocyte haemoglobin, and mean erythrocyte concentrations. In male rats, statistically significant reductions in erythrocyte levels were seen in the 300, 600, and 1200 ppm diet groups. The only

histopathological change reported was vacuolisation of hepatocytes in the 1200 ppm group. Under these test conditions, investigators reported a NOAEL of 10 mg/kg bw/day for rats.

A similar study was undertaken in B6C3F1 mice (eight/sex/group). Animals were fed diets containing malachite green at 0, 25, 100, 300, 600, or 1200 ppm (approximately 0, 3.75, 15, 45, 90, and 180 mg/kg bw/day) for 28 days. Multiple physiological, biochemical and haematological parameters were assessed. Significant reductions in body weight were seen among female mice in the 1200 ppm group. There was no significant change in the body weights of male mice. Under these test conditions, a NOAEL of 15 mg/kg bw was determined in mice, based on increased erythrocyte volume and reticulocyte levels following exposure to 45 mg/kg bw/day (Culp et al., 1999).

Dermal

No data are available.

Inhalation

No data are available.

Genotoxicity

The genotoxicity of malachite green has been investigated in a number of studies. The positive in vitro data, together with reports from in vivo studies that malachite green can bind to DNA covalently (ECHA, 2010 a), suggest that malachite green may cause genotoxicity and, therefore, warrants hazard classification (see **Recommendation** section).

In vitro

In a bacterial reverse mutation assay, malachite green oxalate (CAS No. 2437-29-8) was mutagenic in *Salmonella typhimurium* strain TA98 in the presence of S9 activation enzymes, but not mutagenic in TA100, TA1535, or TA1537 at up to 160 µg/plate, with or without metabolic activation (Clemmensen et al., 1984).

In a similar investigation, it was reported that malachite green oxalate did not induce mutations in *S. typhimurium* in TA97a, 98, TA100, and TA102 strains at concentrations up to 10 µg/plate in the absence or presence of metabolic activation.

A comet assay found no evidence of DNA damage in cultured Chinese hamster ovary (CHO) cells incubated for five hours with malachite green (CAS No. 569-64-2) at a concentration of 20 µM.

In another comet assay using CHO cells, malachite green oxalate was shown to induce DNA damage following exposure for one hour at doses ≥3 µg/mL in the absence of metabolic activation. In the presence of metabolic activation, significant increases in DNA damage were observed at 15 and 20 µg/mL, with only a moderate associated decrease in cell viability (ECHA, 2010 a).

An unspecified form of malachite green was incubated with Chinese hamster lung (V79) cells at a concentration of 4.0 mg/mL in the absence of a metabolic activation system. Under these conditions, the test material caused a significant increase (28 %) in the number of chromosomal aberrations in cells following incubation for 48 hours. No details were provided on the types of aberrations observed (ECHA, 2010 a).

In vivo

In a non-guideline study, no increase in micronucleated erythrocytes was seen in bone marrow cells harvested from mice administered a single dose of 37.5 mg/kg malachite green oxalate by oral gavage (Clemmensen et al., 1984).

In another study, male F344 rats (eight per group) and female B6C3F1 mice (eight per group) were fed diets containing malachite green at 0, 100 or 600 ppm for 28 days. At the end of the treatment period, DNA was isolated from the livers and adduct levels were measured using a ^{32}P-postlabeling assay. A single adduct (or co-eluting adducts) were observed in both species (levels were lower in mice). Significant increases in DNA adduct levels were dose-dependent in both species (ECHA, 2010 a).

A micronucleus test was conducted according to OECD TG 474 (mammalian erythrocyte micronucleus test). Mice (strain unspecified) (five/group) were administered a single oral gavage dose of malachite green oxalate (CAS No. 2437-29-8) at 37.5 mg/kg. Animals were euthanised 24, 42 or 66 hours after dosing. No significant increase in the number of micronuclei in polychromatic erythrocytes was observed in any of the treated groups.

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

Carcinogenicity

The available data do not indicate that malachite green is a carcinogen.

In a study where 48 F344 rats were fed diets containing malachite green at 0, 100, 300 or 600 ppm (equivalent to 7, 21 or 43 mg/kg bw/day) (females only) for 104 weeks, there was no significant toxicity at any dose and survival was similar across all dose groups. There were no statistically significant increases in tumour incidence; however, thyroid follicular cell adenomas/carcinomas (combined) were observed in the two highest dose groups (3/47 and 2/46 at 21 and 43 mg/kg bw/day, respectively). Hepatocellular adenomas were also observed in several animals (NTP Technical Report, 2004; Culp et al., 2006; ECHA, 2010 a).

In a similar study, 48 female B6C3F1 mice per group were fed diets containing malachite green at 0, 100, 225 or 450 ppm (equivalent to 15, 33 and 67 mg/kg bw/day) for 104 weeks. Feed consumption and body weights were recorded throughout the study, and complete necropsies and histopathological examinations of a number of tissues were performed on all animals, including those that died prematurely (NTP Technical Report, 2004; Culp et al., 2006; ECHA, 2010 a).

There was no significant toxicity at any dose and survival was similar in all dose groups. Body weight gain was reduced in the highest dose and relative kidney weights were lower in dosed mice compared with controls. No treatment-related increase in the incidence of neoplasms was observed under these test conditions.

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

Reproductive and Developmental Toxicity

The chemicals malachite green chloride (CAS No. 569-64-2) and basic green 4 oxalate (CAS No. 18015-76-4) are classified as hazardous with hazard category ‘Reproductive toxicity – category 2’ and hazard statement ‘Suspected of damaging fertility or the unborn child’ (H361) in the HCIS (Safe Work Australia). The available data from the rabbit study support this classification and, due to the similarities of the cationic species in this group, it is recommended that this classification be extended to the other chemicals in the group (see **Recommendation** section).

Malachite green oxalate (CAS No. 2437-29-8) was assessed in a non-guideline developmental toxicity study. New Zealand White rabbits were administered the chemical at 0, 5, 10 or 20 mg/kg bw/day by gavage on gestation days (GD) 6–8. Reductions in mean maternal body weight relative to control were observed at the two highest doses; however, this effect was only statistically significant at 10 mg/kg and not clearly related to dose. All three doses caused statistically significant increases in preimplantation loss, primarily as a result of the reabsorptions and decreases in the number of living foetuses. The body weights of the offspring were reduced across all groups. However, this change was only statistically significant in the 5 and 20 mg/kg groups. Developmental abnormalities, including enlargement of the liver, heart and abdominal cavity, were observed in all treated groups. Skeletal abnormalities were also evident and included incomplete ossification of the skull, malformed skull, twisted ankles, shortened tail and malformed scapula. The percentage of offspring with abnormalities were 18.5, 38.0, 33.9, and 47.0 % in the 0, 5, 10, and 20 mg/kg treatment groups, respectively (ECHA, 2010 a).

In another study, malachite green oxalate was administered by gavage to CD rats on GD 6–15 at 0, 10, 30 or 100 mg/kg bw/day (six animals per group). Decreased body weight gain and food consumption occurred in the dams in the highest dose group. No treatment-related macroscopic changes occurred in the dams or their pups. Litter size, in utero survival and mean foetal and placental weights were unaffected by treatment. No further details were provided (ECHA, 2010 a).

In a developmental toxicity study using malachite green oxalate (CAS No. 2437-29-8), CD rats (22 per group) were administered the test material by oral gavage at 0, 2, 10, 50 or 100 mg/kg bw/day on GD 6–15. Five animals in the highest dose group had to be euthanised. Decreased weight gain and food consumption were observed in dams in the highest dose group. No treatment-

related macroscopic changes occurred in the dams or pups. Litter size and in utero survival and placental weights were also unaffected by treatment with the test material (ECHA, 2010 a).

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

Risk Characterisation

Critical Health Effects

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

Public Risk Characterisation

While it is not known whether the chemicals in this group are used in cosmetic products in Australia, the chemicals are reported to be used overseas as hair colourants. However, the likelihood of availability of cosmetic and domestic products containing the chemicals in this group is remote considering that there is currently no documented use of the chemicals in cosmetic products in the United States (Personal Care Products Council, 2011) and no use in domestic products, other than for pet care, in the US Household Products Database (US HPD).

While there is a concern based on the critical health effects, particularly the high acute toxicity of the chemicals in this group (LD50 of 275–520 mg/kg bw), the likelihood of exposure to the Australian public is low. Therefore, the risk to public health is not considered to be unreasonable and further risk management is not considered necessary for public safety.

Occupational Risk Characterisation

During product formulation, oral, dermal, ocular and inhalation exposure may 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 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 HCIS (Safe Work Australia) (see **Recommendation** section).

NICNAS Recommendation

Assessment of these chemicals are considered to be sufficient, provided that the recommended amendment to the classification is adopted, and labelling and all other requirements are met under workplace health and safety and poisons legislation as adopted by the relevant state or territory.

Regulatory Control

Work Health and Safety

The chemicals are recommended for classification and labelling aligned with the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) as below. This does not consider classification of physical hazards and environmental hazards. The existing hazard classifications for malachite green (CAS No. 569-64-2) and basic green 4 oxalate (CAS No. 18015-76-4) should be extended to all chemicals in this group. The hazard classification for genotoxicity should be added to all chemicals in this group.

From 1 January 2017, under the model Work Health and Safety Regulations, chemicals are no longer to be classified under the Approved Criteria for Classifying Hazardous Substances system.

Hazard	Approved Criteria (HSIS)[a]	GHS Classification (HCIS)[b]
Acute Toxicity	Not Applicable	Harmful if swallowed - Cat. 4 (H302)
Irritation / Corrosivity	Not Applicable	Causes serious eye damage - Cat. 1 (H318)
Genotoxicity	Not Applicable	Suspected of causing genetic defects - Cat. 2 (H341)
Reproductive and Developmental Toxicity	Not Applicable	Suspected of damaging the unborn child - Cat. 2 (H361d)

[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 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.

References

Aquasonic Australia. MSDS for products containing malachite green (CAS No. 569-64-2). Accessed January 2015 at **http://www.aquasonic.com.au/wp-content/uploads/pdf/Formalin-Malachite-Soln.pdf**

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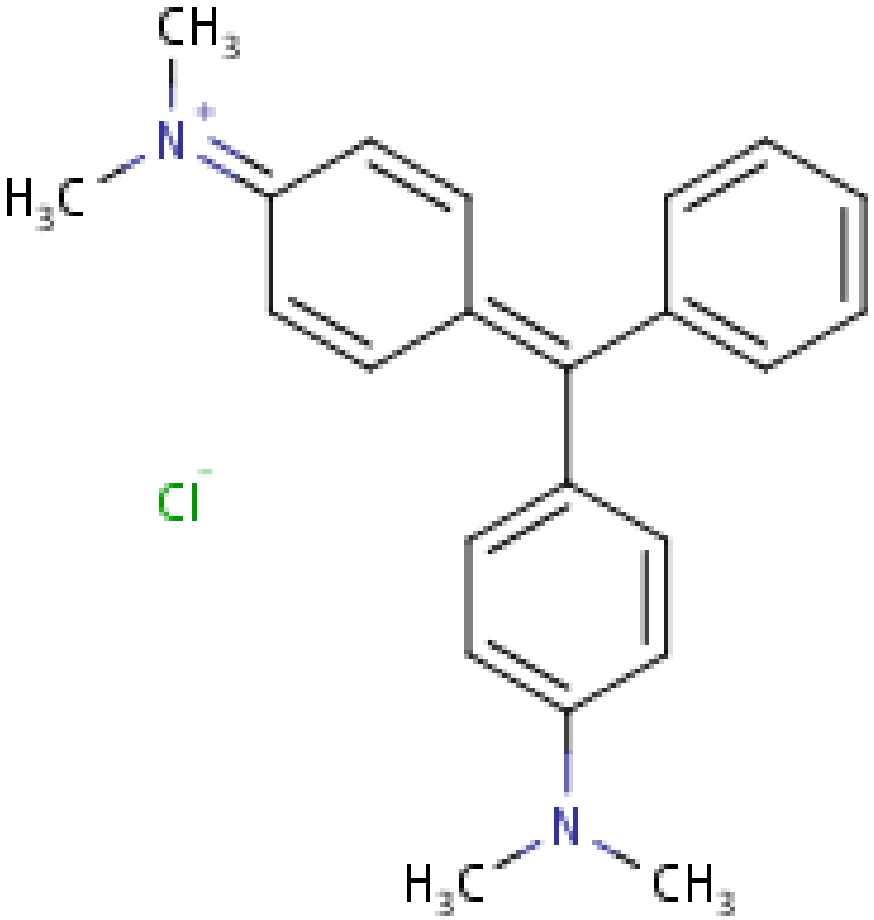
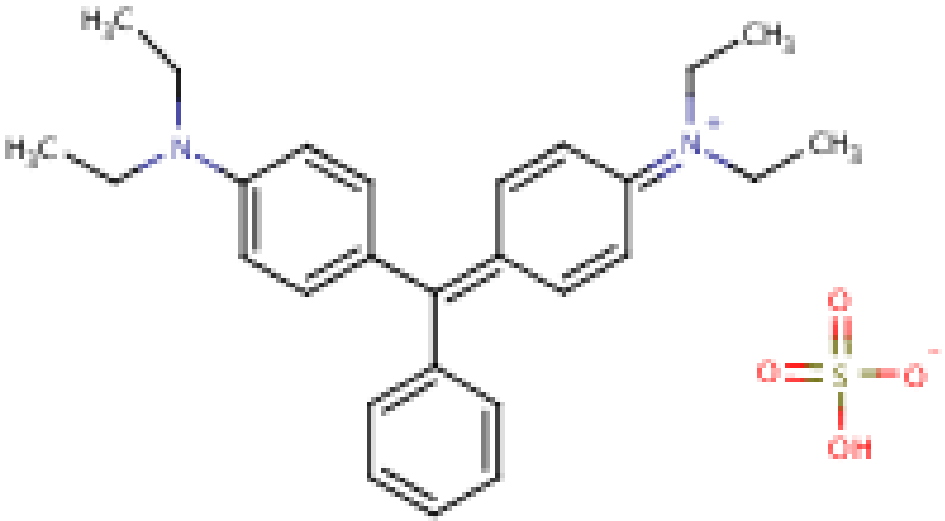
Last Update 10 March 2017

Chemical Identities

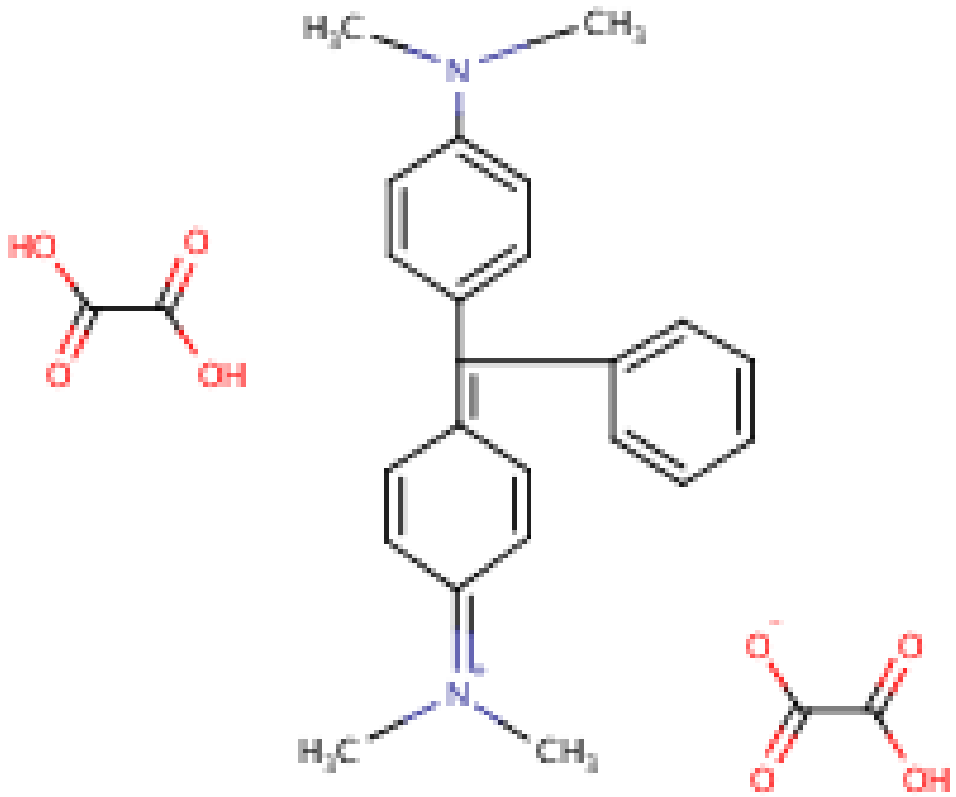
Chemical Name in the Inventory and Synonyms	**Methanaminium, N-[4-[[4-(dimethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]-N-methyl-, chloride** malachite green chloride C.I. basic green 4 4-(4-dimethylaminobenzhydriylidene)cyclohexa-2,5-dienylidene)dimethylammonium chloride light green N (biological stain)
CAS Number	569-64-2
Structural Formula	

	
Molecular Formula	C23H25N2.Cl
Molecular Weight	364.9

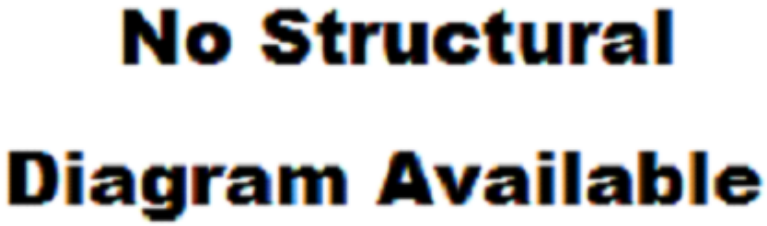
Chemical Name in the Inventory and Synonyms	**Ethanaminium, N-[4-[[4-(diethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]-N-ethyl-, sulfate (1:1)** C.I. Basic Green 1 N-(4-((4-(diethylamino)phenyl)phenylmethylene)-2,5-cyclohexadien-1-ylidene)-N-ethylethanaminium sulfate (1:1) (4-(4-(diethylamino)benzhydrylene)cyclohexa-2,5-dien-1-ylidene)diethylammonium hydrogen sulphate brilliant green crystals ammonium, (4-(p-(diethylamino)-alpha-phenylbenzylidene)-2,5-cyclohexadien-1-ylidene) diethyl-,sulfate (1:1)
CAS Number	633-03-4
Structural Formula	

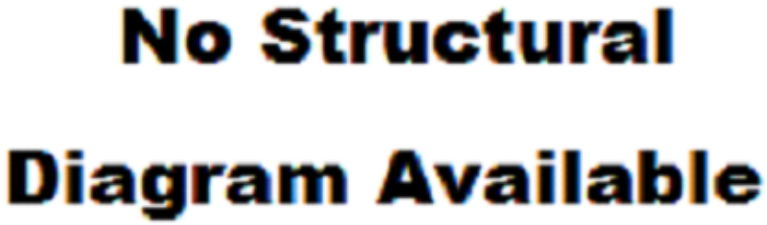
	
Molecular Formula	C27H33N2.HO4S
Molecular Weight	482.642

Chemical Name in the Inventory and Synonyms	**Methanaminium, N-[4-[[4-(dimethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]-N-methyl-, ethanedioate, ethanedioate (2:2:1)** malachite green oxalate ammonium, (4-(p-(dimethylamino)-alpha-phenylbenzylidene)-2,5-cyclohexadien-1-ylidene)-dimethyl-, oxalate (2:1), oxalate (1:1) bis((4-(4-(dimethylamino)benzhydrylidene)cyclohexa-2,5-dien-1-ylidene)dimethylammonium) oxalate, dioxalate
CAS Number	2437-29-8
Structural Formula	

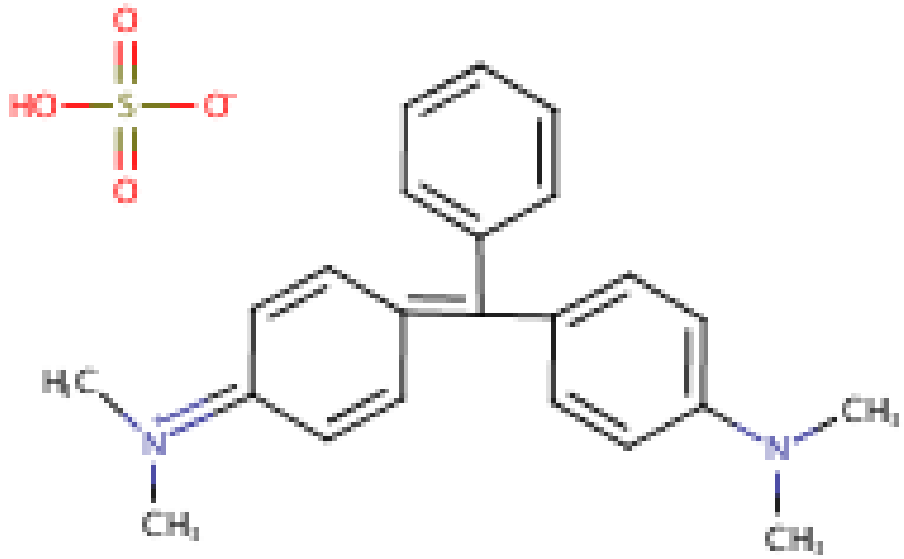
	
Molecular Formula	C23H25N2.1/2C2H2O4.C2HO4
Molecular Weight	508.5

Chemical Name in the Inventory and Synonyms	**Methanaminium, N-[4-[[4-(dimethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]-N-methyl-, chloride, compound with 3-methyl-1-butanol (1:3)** C.I. basic green 4, compound with isopentyl alcohol (1:3)
CAS Number	7278-09-3
Structural Formula	

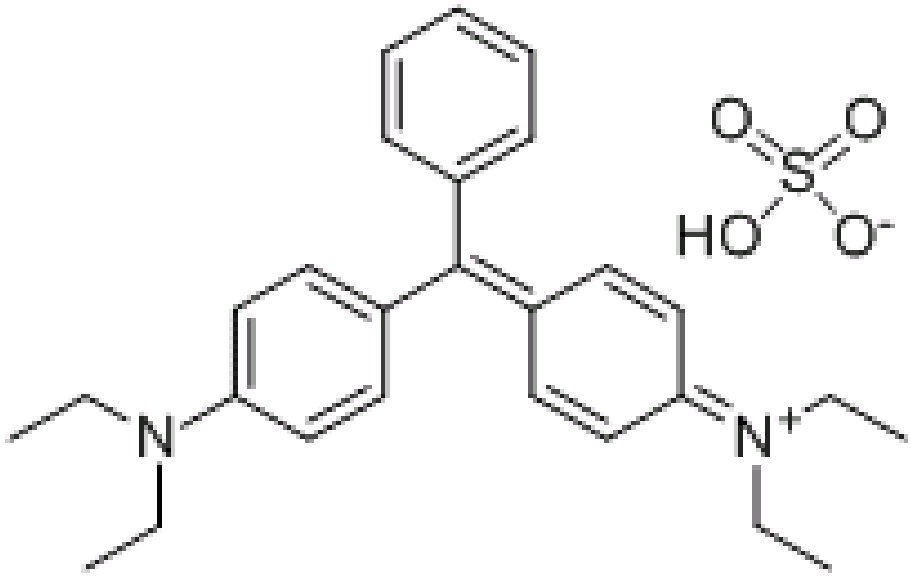
	
Molecular Formula	C23H25N2.3C5H12O.Cl
Molecular Weight	

Chemical Name in the Inventory and Synonyms	**Methanaminium, N-[4-[[4-(dimethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]-N-methyl-, ethanedioate (1:1)** C.I. basic green 4, oxalate (1:1)
CAS Number	13425-25-7
Structural Formula	
Molecular Formula	C23H25N2.C2HO4
Molecular Weight	

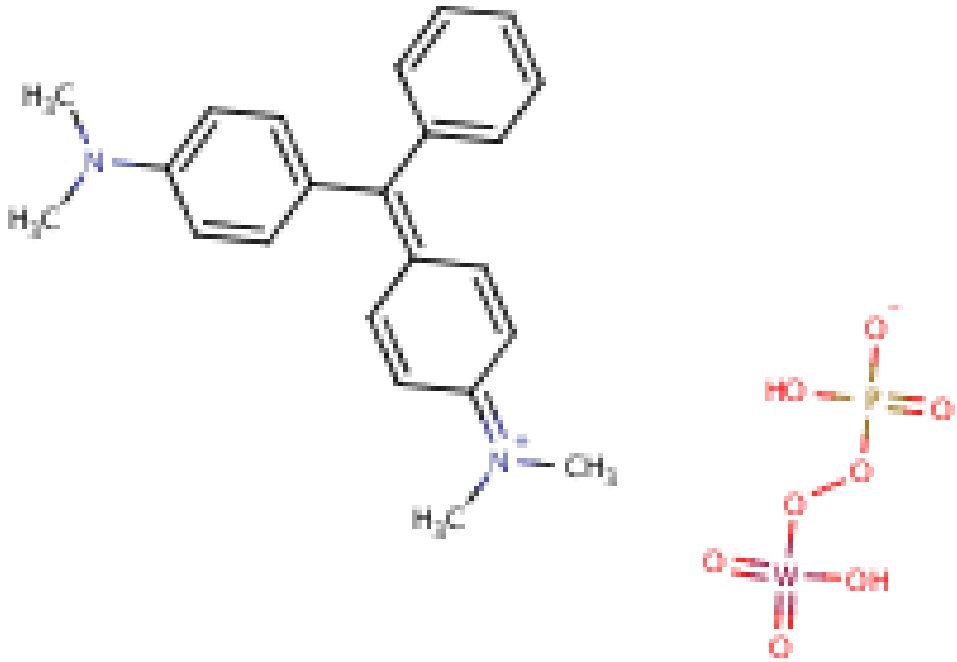
Chemical Name in the Inventory and Synonyms	**Methanaminium, N-[4-[[4-(dimethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]-N-methyl-, hydrogen sulfate** dimethyl(4-(4-(dimethylamino)-alpha-phenylbenzylidene)-2,5-cyclohexadien-1-ylidene)ammonium hydrogen sulphate

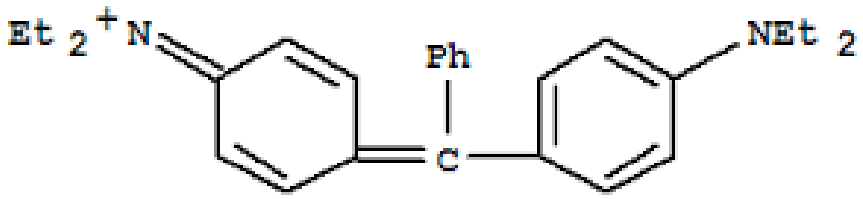
	C.I. basic green 4 (hydrogen sulfate)
CAS Number	16044-24-9
Structural Formula	
Molecular Formula	C23H25N2.HO4S
Molecular Weight	426.534

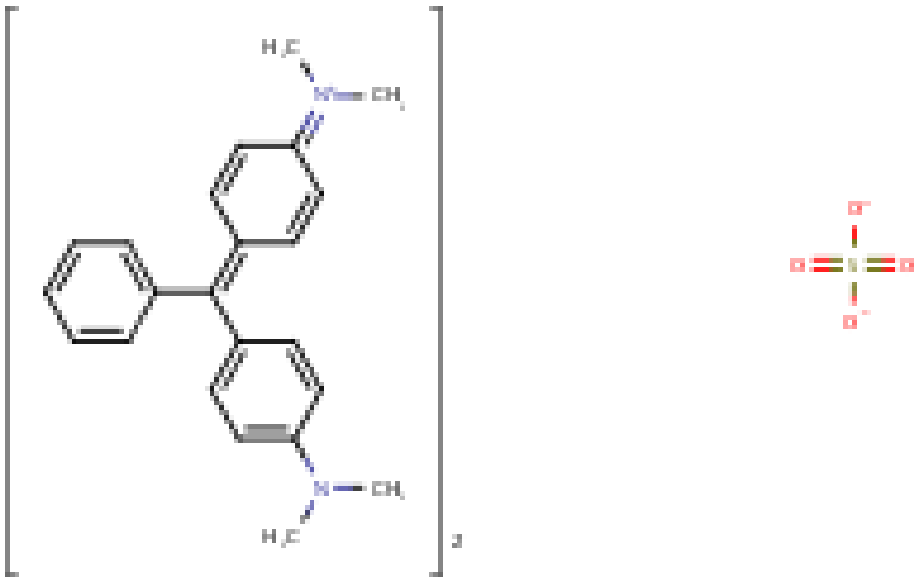
Chemical Name in the Inventory and Synonyms	**Methanaminium, N-[4-[[4-(dimethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]-N-methyl-, ethanedioate** methanaminium, N-(4-((4-(dimethylamino)phenyl)-4-phenylmethylene)-2,5-cyclohexadien-1-ylidene)-N-methyl-, oxalate basic green 4 oxalate
CAS Number	18015-76-4
Structural Formula	

	
Molecular Formula	C23H25N2.x
Molecular Weight	482.6

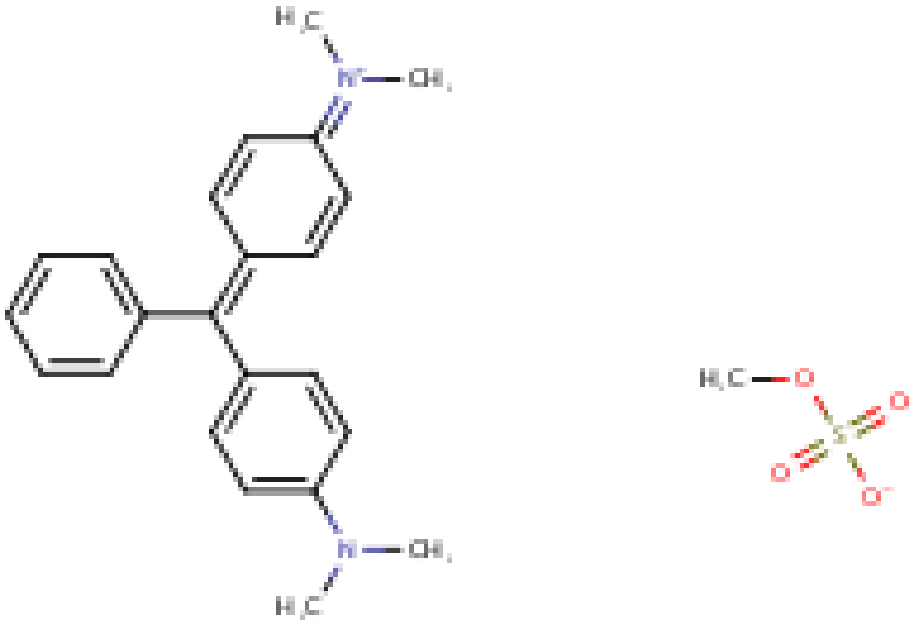
Chemical Name in the Inventory and Synonyms	**Methanaminium, N-[4-[[4-(dimethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]-N-methyl-, molybdate tungstate phosphate** C.I. basic green 4, phosphotungstomolybdic acid complex methanaminium, N-(4-((4-(dimethylamino)phenyl)phenylmethylene)-2,5-cyclohexadien-1-ylidene)-N-methyl-, molybdatetungstatephosphate malachite green phosphotungstomolybdate
CAS Number	61725-50-6
Structural Formula	

	
Molecular Formula	C23H25N2.x
Molecular Weight	674.3

Chemical Name in the Inventory and Synonyms	**Ethanaminium, N-[4-[[4-(diethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]-N-ethyl-, molybdate phosphate** C.I. basic green 1, phosphomolybdic acid complex
CAS Number	68814-00-6
Structural Formula	
Molecular Formula	C27H33N2.x
Molecular Weight	

Chemical Name in the Inventory and Synonyms	**Methanaminium, N-[4-[[4-(dimethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]-N-methyl-, sulfate (2:1)** dimethyl(4-((4-(dimethylamino)phenyl)benzylidene)-2,5-cyclohexadien-1-ylidene)ammonium sulphate (2:1)
CAS Number	85188-05-2
Structural Formula	
Molecular Formula	C23H25N2.1/2O4S
Molecular Weight	754.991

Chemical Name in the Inventory and Synonyms	**Methanaminium, N-[4-[[4-(dimethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]-N-methyl-, methyl sulfate** dimethyl(4-((4-(dimethylamino)phenyl)benzylidene)-2,5-cyclohexadien-1-ylidene)ammonium methyl sulphate

CAS Number	85204-56-4
Structural Formula	
Molecular Formula	C23H25N2.CH3O4S
Molecular Weight	440.5612

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