HC Blue No. 12 and its hydrochloride: Human health tier II assessment

24 April 2015

Chemicals in this assessment

Chemical Name in the Inventory	CAS Number
Ethanol, 2-[4-[ethyl[(2-hydroxyethyl)amino]-2-nitrophenyl]amino]-	104516-93-0
Ethanol, 2-[[4-[ethyl(2-hydroxyethyl)amino]-2-nitrophenyl]amino]-, hydrochloride (1:1)	132885-85-9

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

The chemicals in this group contain a free base (CAS No. 104516-93-0) and its hydrochloride salt (CAS No. 132885-85-9). The free base (CAS No. 104516-93-0) is also referred to as 'the parent chemical' in this report. Therefore, as the toxicokinetics and the toxicity of these chemicals are expected to be similar, they are grouped together for human health risk assessment, although the hydrochloride salt could have different properties with respect to local effects. The speciation of these chemicals in biological fluids will be dependent on pH, but is independent of the original form (SCCP, 2008).

It is also noted that a different CAS No. (104576-93-0) has been stated for the free base in the Scientific Committee on Consumer Products (SCCP) opinion on HC Blue no 12 (SCCP, 2008).

Import, Manufacture and Use

Australian

The chemicals are on the 'List of chemicals used as dyes in permanent and semi-permanent hair dyes in Australia' (NICNAS, 2007).

International

The following international uses have been identified through:

- Galleria Chemica;

- the European Commission Cosmetic Ingredients and Substances (CosIng) database;
- the United States (US) Personal Care Product Council International Nomenclature of Cosmetic Ingredients (INCI) Dictionary; and

the US Environmental Protection Agency's Aggregated Computer Toxicology Resource (ACToR).

The chemicals have reported cosmetic use in hair dye preparations.

Restrictions

Australian

The chemicals are not listed in the *Poisons Standard—the Standard for the Uniform Scheduling of Medicines and Poisons* (SUSMP, 2015). However, there is a group entry in Schedule 6 and Appendix C of the SUSMP that will include this chemical to be implemented on 1 June 2015. (TGA, 2014)

Schedule 6:

'PHENYLENEDIAMINES including alkylated, arylated and nitro derivatives not elsewhere specified in these Schedules:

a. in preparations packed and labelled for photographic purposes;

b. in preparations packed and labelled for testing water except tablets containing 10 mg or less of diethyl-para-phenylenediamine or dimethyl-para-phenylenediamine in opaque strip packaging provided the directions for use include the statement, "Do not discard testing solutions into the pool";

c. in hair dye preparations except when the immediate container and primary pack are labelled with the following statements: KEEP OUT OF REACH OF CHILDREN, and WARNING - This product contains ingredients which may cause skin irritation to certain individuals. A preliminary test according to the accompanying directions should be made before use. This product must not be used for dyeing eyelashes or eyebrows; to do so may be injurious to the eye. written in letters not less than 1.5 mm in height;

d. in eyelash and eyebrow tinting products when the immediate container and primary pack are labelled with the following statement: WARNING - This product contains ingredients which may cause skin irritation to certain individuals, and when used for eyelash and eyebrow tinting may cause injury to the eye. A preliminary test according to the accompanying directions should be made before use. written in letters not less than 1.5 mm in height.'

Schedule 6 chemicals are labelled with 'Poison'. These are substances with a moderate potential for causing harm, the extent of which can be reduced by using distinctive packaging with strong warnings and safety directions on the label.

Appendix C:

'PHENYLENEDIAMINES, including alkylated, arylated and nitro derivatives, in preparations for skin colouration, tattooing and dyeing of eyelashes or eyebrows except when included in Schedule 6.'

Appendix C chemicals are substances of such danger to health as to warrant prohibition of sale, supply and use.

International

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

European Union (EU) Cosmetics Regulation 1223/2009 Annex III, part 1—List of substances which cosmetic products must not contain except subject to the restrictions and conditions laid down. The restrictions include the following:

- "in combination with hydrogen peroxide, the maximum use concentration upon application is 0.75 % (as hydrochloride);

- do not use with nitrosating systems;
- maximum nitrosamine content: 50 µg/kg; and
- keep in nitrite-free containers"

New Zealand Cosmetic Products Group Standard (2006)—Schedule 5: Components cosmetic products must not contain except subject to the restrictions and conditions laid down. These restrictions and conditions are similar to those indicated above.

The Association of Southeast Asian Nations (ASEAN) Cosmetic Directive Annex III – Part 1 List of substances which cosmetic products must not contain except subject to restrictions and conditions laid down.

The parent base only is listed in:

ASEAN Cosmetic Directive Annex III—Part 2 List of substances provisionally allowed. The restrictions and conditions are similar to the restriction indicated above.

The Scientific Committee on Consumer Products (SCCP) was of the opinion that the use of the chemicals "as an ingredient in non-oxidative hair dye formulations with a maximum on-head concentration of 1.5 % and in oxidative hair dye formulations with a maximum on-head concentration of 0.75 % does not pose a risk to the health of the consumer, apart from skin sensitising potential" (moderate skin sensitiser). It was also noted that the chemicals should not be used in combination with nitrosating substances. The nitrosamine content should be < 50 ppb (SCCP, 2008).

Existing Worker Health and Safety Controls

Hazard Classification

The hydrochloride salt (CAS No. 132885-85-9) 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; R43 (sensitisation)

Exposure Standards

Australian

No specific exposure standards are available.

International

There are no specific exposure standards identified for these two chemicals (Galleria Chemica).

Health Hazard Information

The human health hazards of both the parent chemical and hydrochloride salt was assessed using the toxicological data available on the parent chemical. Where data are unavailable for the parent chemical, available data for the hydrochloride salt are considered relevant for the hazard assessment due to the structural similarity of the two chemicals. However, the hydrochloride salt could have different properties with respect to local effects.

Toxicokinetics

The bioavailability of the parent chemical across the intestinal barrier was investigated in vitro in human epithelial cells. The result indicated possible good absorption of the chemical after oral administration due to high permeability in this assay.

The dermal absorbtion of a formulation containing radioactively-labelled parent chemical was also observed in Long Evans rats. The chemical was applied in a hair dye formulation or a reference dose in dimethylsulfoxide (DMSO). The percutaneous absorption of the parent chemical in the commercial formulation was stated to be approximately 1.3 % in female rats (0.8 % urine, 0.3 % faeces, 0.1 % tissues and organs), under non-oxidative conditions (SCCP, 2008).

Acute Toxicity

Oral

The hydrochloride salt is classified as hazardous with the risk phrase ‘Harmful if swallowed’ (Xn; R22) in the Hazardous Substances Information System (HSIS).

Although the data for the hydrochloride salt are not available, the available data for the parent chemical (median lethal dose—LD50 of 1668 mg/kg bw in female rats) support this classification. The parent chemical (CAS No. 104516-93-0) is therefore also recommended for classification (refer to **Recommendation** section). Reported signs of toxicity included reduced animal activity, abdominal position, and blue discolouration of the extremities (SCCP, 2008).

Dermal

The chemicals in this group are likely to have low acute toxicity based on results from animal tests following dermal exposure. The parent chemical (CAS No. 104516-93-0) has reported low dermal acute toxicity in rabbits, with an LD50 of >2000 mg/kg bw.

There were no reported mortality and other observed sub-lethal effects. Skin alterations could not be evaluated because of the skin discolouration due to the applied chemical (SCCP, 2008).

Inhalation

No data are available.

Corrosion / Irritation

Skin Irritation

The chemical produced no skin irritation in studies that were performed in accordance with the Organisation for Economic Co-operation and Development (OECD) Test Guideline (TG) 404 in which the parent chemical (CAS No. 104516-93-0) (0.5 g) was applied to intact New Zealand White rabbit skin for four hours under occlusive conditions (SCCP, 2008).

Eye Irritation

The parent chemical (CAS No. 104516-93-0) is reported to be a slight eye irritant in animal studies. The effects were not sufficient to warrant hazard classification. The chemical 0.1 ml (30–50 mg) was applied into the conjunctival sac of the right eye

of three female New Zealand White rabbits. No effects on the cornea or the iris were noted at any reading. Mild conjunctival erythema was noted in two animals for up to 48 hours after instillation (SCCP, 2008).

Sensitisation

Skin Sensitisation

The hydrochloride salt is classified as hazardous with the risk phrase ‘May cause sensitisation by skin contact’ (R43) in the HSIS (Safe Work Australia).

Although data for the hydrochloride salt are not available, the available data for the parent chemical support this classification. The parent chemical is therefore also recommended for classification (refer to **Recommendation** section), based on skin sensitisation observed in an animal study (SCCP, 2008).

The skin sensitising potential of the parent chemical was investigated in CBA/J mice by local lymph node assay (LLNA). Stimulation indices of 1.4, 1.5, 3.0 and 2.7 were obtained for the four test concentrations of 0.5, 1.5, 5.0 and 10 %, respectively, for the chemical in DMSO. The estimated concentration needed to produce a three-fold increase in lymphocyte proliferation (EC3) value was estimated as 5.0 %. It was also noted that the chemical produced no skin sensitisation if a mixture of water/acetone (1:1) mixed with olive oil (4:1) was used as the vehicle. The SCCP classified the chemical as a moderate skin sensitising agent, 'according to the observed EC3 value of 5.0 % with the vehicle DMSO in the LLNA' (SCCP, 2008)

A structurally similar chemical, 1,4-benzenediamine, 2-nitro- (CAS No. 5307-14-2), has been characterised as having a sensitising potential (NICNASa) indicating that the chemicals in this group could have weak sensitisation potential.

Quantitative Structure-Activity Relationship (QSAR) modelling using OASIS–TIMES (Optimized Approach based on Structural Indices Set–Tissue Metabolism Simulator) resulted in a strong sensitisation result for the chemicals, although it should be noted that the result was out of the applicability domain of the models. If a prediction is out of the applicability domain of the model, it indicates that there is a greater uncertainty about the reliability of the models since the performance statistics from the training set might not be applicable to the chemical. Thus, QSAR model predictions for this chemical will not be included in the weight of evidence analysis of the skin sensitisation potential of the chemical.

Repeated Dose Toxicity

Oral

Although limited information is available, the available information on the parent chemical indicates that the chemicals in this group are not considered to cause serious damage to health from repeated oral exposure.

In a 28-day oral study conducted according to OECD TG 407, Wistar rats (five/sex/dose) were administered (gavage) the parent chemical (CAS No. 104516-93-0) at doses of 100, 316 and 1000 mg/kg bw/day. Effects observed at the highest concentration (1000 mg/kg bw/day) included reduced red blood cell counts, haemoglobin concentrations and reduced uric acid content; increased bilirubin and polychromatic erythrocytes; increased absolute and relative spleen weights, although significantly only in males; spleen lymphoid depletion and congestion as well as induction of haematopoesis; and absolute liver weight was elevated in females only. A no observed adverse effect level (NOAEL) of 316 mg/kg bw/day was reported (SCCP, 2008).

In a 90-day repeated dose study (OECD TG 408), Wistar rats (15/sex/dose) were administered (gavage) the parent chemical at doses of 0, 15, 30 and 60 mg/kg bw/day. The highest dose tested, 60 mg/kg bw/day, was deemed an NOAEL due to the lack of adverse health effects at all doses (SCCP, 2008).

Dermal

No data are available.

Inhalation

No data are available.

Genotoxicity

Based on the weight of evidence from the available in vitro and in vivo genotoxicity studies, the chemicals are not considered to be genotoxic. While several in vitro tests produced positive results, all in vivo tests were negative.

The parent chemical was positive in a bacterial reverse mutation assay with *Salmonella typhimurium* TA98, TA100, and TA1538 strains, both in the absence and presence of metabolic activation. The hydrochloride salt tested positive in a bacterial reverse mutation assay with *S typhimurium* TA98 and TA1538 strains, both in the absence and presence of metabolic activation. The parent chemical tested weakly positive for gene mutations at the *tk* locus of mouse lymphoma cells, both in the absence and presence of metabolic activation. The parent chemical (CAS No. 104516-93-0) induced an increase in micronucleated binucleated cells and was concluded to be genotoxic (clastogenic and/or aneugenic) in cultured human peripheral lymphocytes in vitro (SCCP, 2008).

The parent chemical did not induce:

- micronuclei in the bone marrow of mice in a mammalian erythrocyte micronucleus test in NMRI male mice;
- unscheduled DNA synthesis in primary hepatocytes of treated rats in an unscheduled DNA synthesis (UDS) test with mammalian liver cells; or
- DNA damage in liver, stomach and urinary bladder of rats (comet assay) (SCCP, 2008).

Carcinogenicity

No animal toxicity data are available on the carcinogenicity of the parent base and the salt. Based on the available genotoxicity data and mechanistic information, the chemicals are not considered to be carcinogenic.

A structurally similar chemical, 1,4-benzenediamine, 2-nitro- (CAS No. 5307-14-2), has been characterised for carcinogenic potential (NICNASa). The chemical 1,4-benzenediamine, 2-nitro- has been assessed as having no carcinogenic potential, indicating that the chemicals in this group might also not be carcinogenic.

Experimental in vitro genotoxicity data (refer to **Genotoxicity** section) showed that the chemical is not considered to be genotoxic. The QSAR modelling using OASIS–TIMES resulted in negative results for the chemicals for in vivo genotoxicity, and the result was within the applicability domain of the models.

Reproductive and Developmental Toxicity

Although data are not available for the parent chemical (CAS No. 104516-93-0), the available information on the hydrochloride salt (CAS No. 132885-85-9) indicates that the chemicals in this group are not likely to have any specific reproductive or developmental toxicity.

In a non-guideline study, the hydrochloride salt (CAS No. 132885-85-9) was administered (gavage) to pregnant Wistar rats (24/dose) from gestation day (GD) 5–15 at a dose of 15, 60 and 140 mg/kg bw/day. Food consumption and body weight changes were not significantly different from controls and no significant clinical or histopathological findings were noted. Offspring parameters including foetal weight, sex ratio, placental weight, number of resorptions and corpora lutea were not affected by the treatment. The NOAEL for maternal and developmental toxicity was established as 140 mg/kg bw/day (the highest dose (SCCP, 2008).

Risk Characterisation

Critical Health Effects

The critical health effect identified for risk characterisation is skin sensitisation (local effect). While data are not available for acute inhalation, or repeated dose dermal and repeated dose inhalation toxicity, these exposure routes are not considered relevant to the main use of the chemicals.

Public Risk Characterisation

The chemicals are reported to be used in in permanent and semi-permanent hair dyes in Australia (NICNAS, 2007).

New Zealand and the European Union have restricted the use of the chemicals in non-oxidative hair dye formulations with a maximum on-head concentration of 1.5 % and in oxidative hair dye formulations with a maximum on-head concentration of 0.75 %. The chemicals should also not be used in combination with nitrosating substances and the nitrosamine content should be <50 ppb (SCCP, 2008).

Currently, there are no restrictions in Australia, although an amendment to the SUSMP, in place from 1 June 2015, will apply to the chemicals (TGA, 2014). A number of warning statements, first aid instructions and safety directions relating to the chemicals will apply. The current controls are considered adequate to minimise the risk to public health posed by domestic and cosmetic products containing the chemicals; therefore, the chemicals are not considered to pose an unreasonable risk to public health.

Occupational Risk Characterisation

Given the critical local health effects (skin sensitisation), the chemicals may pose an unreasonable risk to workers unless adequate control measures to minimise dermal exposure to the chemicals 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 appropriate controls.

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

NICNAS Recommendation

Assessment of the chemicals is considered to be sufficient provided that the recommended classification is adopted, and labelling 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

Products containing the chemicals should be labelled in accordance with state and territory legislation (SUSMP, 2015).

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 or environmental hazards. This is the existing classification for the hydrochloride salt (CAS No. 132885-85-9).

Note: While the existing classification stated below is for the hydrochloride salt (CAS No. 132885-85-9), this should also apply to the parent chemical (CAS No. 104516-93-0).

Hazard	Approved Criteria (HSIS)[a]	GHS Classification (HCIS)[b]
Acute Toxicity	Harmful if swallowed (Xn; R22)*	Harmful if swallowed - Cat. 4 (H302)
Sensitisation	May cause sensitisation by skin contact (Xi; R43)*	May cause an allergic skin reaction - Cat. 1 (H317)

[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 dermal and oral 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:

- 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

European Commission Cosmetic Ingredients and Substances (CosIng) Database. Accessed March 2015 at **http://ec.europa.eu/consumers/cosmetics/cosing/**

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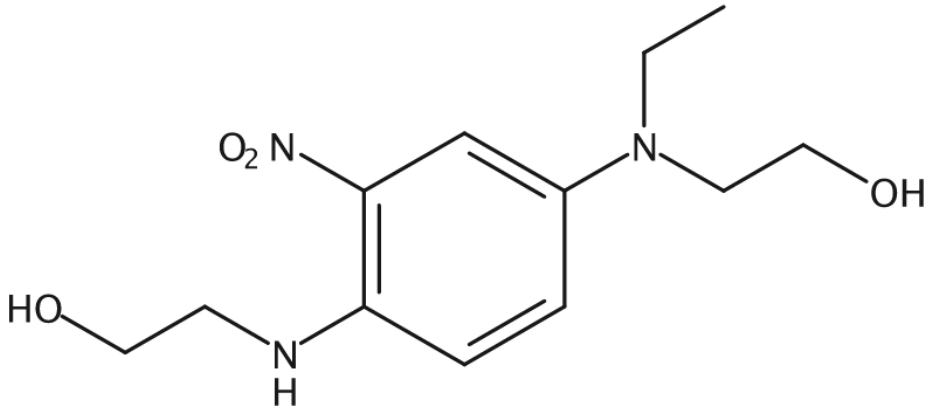
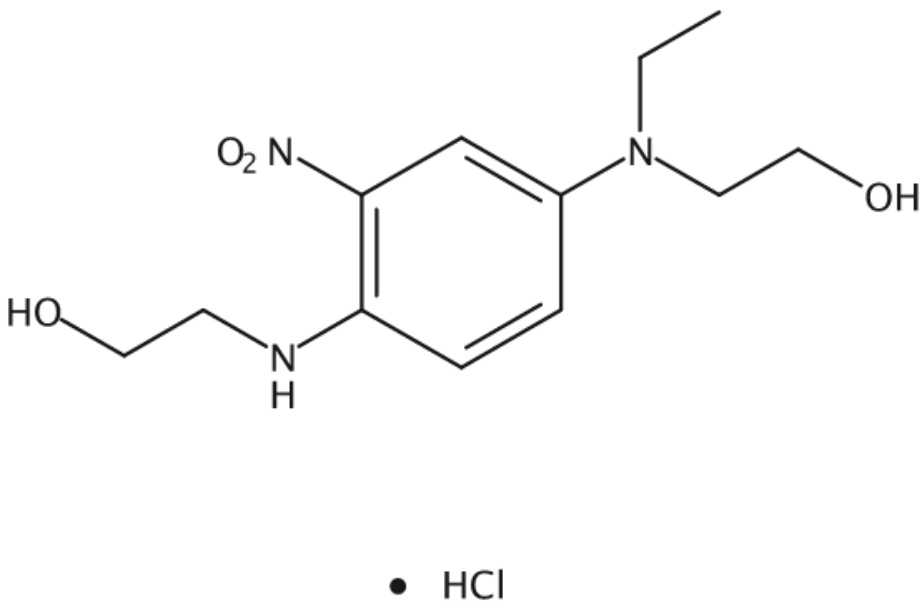
US National Library of Medicines, Household Products Database, Health& Safety Information on Household Products. Accessed March 2015 at **http://householdproducts.nlm.nih.gov/**

Last Update 24 April 2015

Chemical Identities

Chemical Name in the Inventory and Synonyms	**Ethanol, 2-[4-[ethyl[(2-hydroxyethyl)amino]-2-nitrophenyl]amino]-** 1-(beta-hydroxyethyl)amino-2-nitro-4-N-ethyl-N-(beta-hydroxyethyl)aminobenzene 2-[[4-[ethyl(2-hydroxyethyl)amino]-2-nitrophenyl]amino-ethanol 2-[Ethyl-4-[(2-hydroxyethyl)amino]-3-nitroanilino]ethanol HC Blue No. 12
CAS Number	104516-93-0

Structural Formula	
Molecular Formula	C12H19N3O4
Molecular Weight	269.25

Chemical Name in the Inventory and Synonyms	**Ethanol, 2-[[4-[ethyl(2-hydroxyethyl)amino]-2-nitrophenyl]amino]-, hydrochloride (1:1)** Ethanol, 2-[[4-[ethyl(2-hydroxyethyl)amino]-2-nitrophenyl]amino]-, hydrochloride (1:1) 2-[Ethyl-4-[(2-hydroxyethyl)amino]-3-nitroanilino]ethanol hydrochloride Ethanol, 2-[[4-[ethyl(2-hydroxyethyl)amino]-2-nitrophenyl]amino-, monohydrochloride 1-(beta-hydroxyethyl)amino-2-nitro-4-N-ethyl-N-(beta-hydroxyethyl)aminobenzene hydrochloride hydrochloride
CAS Number	132885-85-9
Structural Formula	
Molecular Formula	C12 H19 N3 O4 . Cl H

Molecular Weight	305.76

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