Screening Assessment for the Challenge

Methylium, [4-(dimethylamino)phenyl]bis[4-(ethylamino)-3-methylphenyl]-, acetate

Chemical Abstracts Service Registry Number
72102-55-7

Environment Canada
Health Canada

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Synopsis

Pursuant to section 74 of the *Canadian Environmental Protection Act, 1999* (CEPA 1999), the Ministers of the Environment and of Health have conducted a screening assessment on Methylium, [4-(dimethylamino)phenyl]bis[4-(ethylamino)-3-methylphenyl]- (MAPBAP acetate), Chemical Abstracts Service Registry Number 72102-55-7. This substance was identified as a high priority for screening assessment and included in the Challenge because it was found to meet the ecological categorization criteria for persistence, bioaccumulation potential and inherent toxicity to non-human organisms and is believed to be in commerce in Canada.

The substance MAPBAP acetate was not considered to be a high priority for assessment of potential risks to human health, based upon application of the simple exposure and hazard tools developed by Health Canada for categorization of substances on the Domestic Substances List. Therefore, this assessment focuses on information relevant to the evaluation of ecological risks.

MAPBAP acetate is an organic substance that is used in Canada and elsewhere as a cationic dye mainly in the production of paper products. The substance is not naturally produced in the environment. Between 10 000 and 100 000 kg of MAPBAP acetate were imported into Canada in 2006.

Based on reported use patterns and certain assumptions, most of the substance is expected to end up in wastewater, be disposed of in landfills, or be recycled. Based on its physical/chemical properties and partitioning behaviour, MAPBAP acetate could be found in water, sediments, and soil. It is not expected to be significantly present in air.

Based on its physical and chemical properties, MAPBAP acetate is not expected to degrade quickly in the environment. It is persistent in water, soil and sediments. MAPBAP acetate therefore meets the criteria for persistence, but not bioaccumulation potential, as set out in the Persistence and Bioaccumulation Regulations. The conclusion that MAPBAP acetate has low bioaccumulation potential is based on an experimental read-across result, as well as modelled data based on an experimental read-across logKow. In addition, modelled and read-across acute aquatic toxicity values indicate that MAPBAP acetate is potentially highly hazardous to aquatic organisms.

Although no releases of MAPBAP acetate were reported in response to a survey under Section 71 of CEPA, releases to the environment are expected to occur given the substance's current use as a paper dye. Concentrations of MAPBAP acetate in surface waters receiving effluents from 10 industrial sites were estimated. These exposure concentrations were found to exceed levels expected to cause harm to sensitive aquatic organisms at all of the sites considered.

The potential for exposure of the general population to MAPBAP acetate from environmental media is expected to be negligible. Exposure to MAPBAP acetate from consumer products (newsprint dye) is expected to be negligible for the intended purpose

of the product (negligible dermal exposure for reading activities) and low for incidental events such as mouthing exposure by toddlers.

As exposure of the general population in Canada based on the use of the substance as a paper dye is expected to be low to negligible, the risk to human health is considered to be low.

No empirical toxicity data were identified for MAPBAP acetate. The outputs of QSAR predictions for carcinogenicity and genotoxicity were mixed. Information from analogue substances suggests a possible concern for carcinogenicity, genotoxicity and developmental toxicity.

Based upon consideration of the available data on its potential to cause harm to human health, it is concluded that MAPBAP acetate is not a substance entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

Based upon consideration of the available data on its potential to cause ecological harm (i.e., read-across and modelled data for persistence and aquatic toxicity, and the characterization of risk to aquatic organisms), it is concluded that MAPBAP acetate is entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity.

Based on the information available, it is concluded that MAPBAP acetate meets one or more of the criteria set out in section 64 of the *Canadian Environmental Protection Act, 1999*. MAPBAP acetate meets the criteria for persistence but does not meet the criteria for bioaccumulation as set out in the *Persistence and Bioaccumulation Regulations*.

Where relevant, research and monitoring will support verification of assumptions used during the screening assessment and, where appropriate, the performance of potential control measures identified during the risk management phase.

Introduction

The *Canadian Environmental Protection Act, 1999* (CEPA 1999) (Canada 1999) requires the Minister of the Environment and the Minister of Health to conduct screening assessments of substances that have met the categorization criteria set out in the Act to determine whether these substances present or may present a risk to the environment or to human health.

Based on the information obtained through the categorization process, the Ministers identified a number of substances as high priorities for action. These include substances that

- met all of the ecological categorization criteria, including persistence (P), bioaccumulation potential (B) and inherent toxicity to aquatic organisms (iT), and were believed to be in commerce in Canada; and/or
- met the categorization criteria for greatest potential for exposure (GPE) or presented an intermediate potential for exposure (IPE) and had been identified as posing a high hazard to human health based on classifications by other national or international agencies for carcinogenicity, genotoxicity, developmental toxicity or reproductive toxicity.

The Ministers therefore published a notice of intent in the *Canada Gazette*, Part I, on December 9, 2006 (Canada 2006a), that challenged industry and other interested stakeholders to submit, within specified timelines, specific information that may be used to inform risk assessment, and to develop and benchmark best practices for the risk management and product stewardship of those substances identified as high priorities.

The substance Methylium, [4-(dimethylamino)phenyl]bis[4-(ethylamino)-3-methylphenyl]-, acetate was identified as a high priority for assessment of ecological risk as it had been found to be persistent, bioaccumulative and inherently toxic to aquatic organisms and is believed to be in commerce in Canada. The Challenge for this substance was published in the *Canada Gazette* on January 31, 2009 (Canada 2007). A substance profile was released at the same time. The substance profile presented the technical information available prior to December 2005 that formed the basis for categorization of this substance. As a result of the Challenge, submissions of information pertaining to the use of the substance were received.

Although Methylium, [4-(dimethylamino)phenyl]bis[4-(ethylamino)-3-methylphenyl]-, acetate was determined to be a high priority for assessment with respect to the environment, it did not meet the criteria for GPE or IPE and high hazard to human health based on classifications by other national or international agencies for carcinogenicity, genotoxicity, developmental toxicity or reproductive toxicity. Therefore, this assessment focuses principally on information relevant to the evaluation of ecological risks.

Screening assessments focus on information critical to determining whether a substance meets the criteria as set out in section 64 of CEPA 1999. Screening assessments examine scientific information and develop conclusions by incorporating a weight-of-evidence approach and precaution[1].

This screening assessment includes consideration of information on chemical properties, hazards, uses and exposure, including the additional information submitted under the Challenge. Data relevant to the screening assessment of this substance were identified in original literature, review and assessment documents, stakeholder research reports and from recent literature searches, up to April 2010 for ecological sections of the document. The screening assessment does not represent an exhaustive or critical review of all available data. Rather, it presents the most critical studies and lines of evidence pertinent to the conclusion.

This screening assessment was prepared by staff in the Existing Substances Programs at Health Canada and Environment Canada and incorporates input from other programs within these departments. The ecological portions of this assessment have undergone external written peer review/consultation. Additionally, the draft of this screening assessment was subject to a 60-day public comment period. Although external comments were taken into consideration, the final content and outcome of the screening risk assessment remain the responsibility of Health Canada and Environment Canada. Approaches used in the screening assessments under the Challenge have been reviewed by an independent Challenge Advisory Panel.

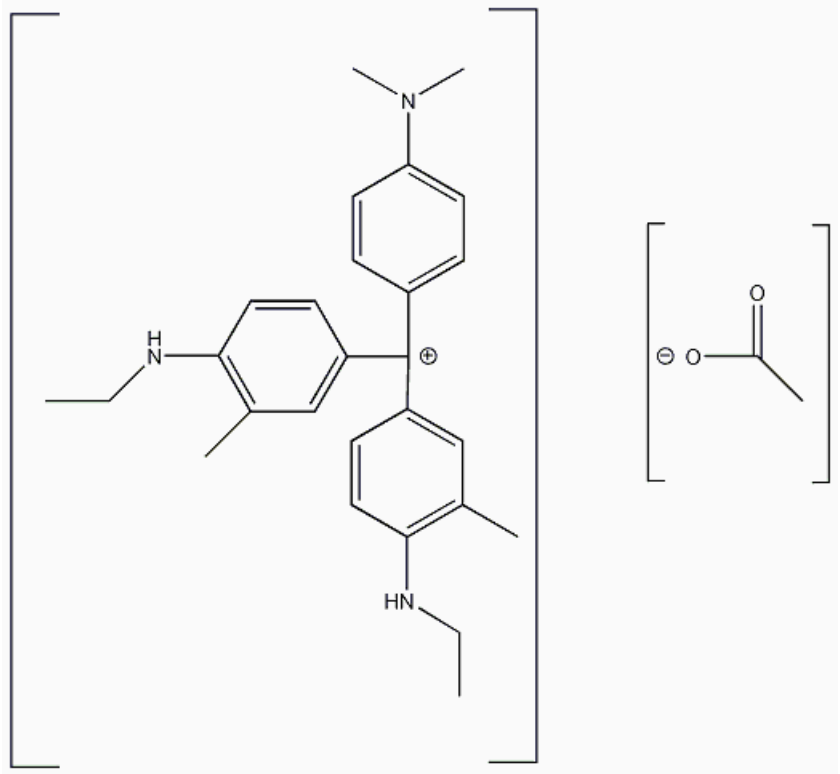
The critical information and considerations upon which the assessment is based are summarized below.

Substance Identity

For the purposes of this document, this substance will be referred to as MAPBAP acetate, derived from the DSL name. MAPBAP acetate belongs to a class of dyes known as cationic triarylmethanes. The class can be further sub-divided into those where the charge on the cation (triarylmethane moiety) is localized or delocalized. MAPBAP acetate belongs to the latter sub-category (Hunger 2003) implying that the bond holding the cationic and anionic components of the structure together is at least partly covalent.

[1] A determination of whether one or more of the criteria of section 64 are met is based upon an assessment of potential risks to the environment and/or to human health associated with exposures in the general environment. For humans, this includes, but is not limited to, exposures from ambient and indoor air, drinking water, foodstuffs, and the use of consumer products. A conclusion under CEPA 1999 on the substances in the Chemicals Management Plan (CMP) Challenge Batches 1-12 is not relevant to, nor does it preclude, an assessment against the hazard criteria specified in the Controlled Products Regulations, which is part of regulatory framework for the Workplace Hazardous Materials Information System [WHMIS] for products intended for workplace use

Table 1. Substance identity for MAPBAP acetate.

Chemical Abstracts Service Registry Number (CAS RN)	**72102-55-7**
DSL name	**Methylium, [4-(dimethylamino)phenyl]bis[4-(ethylamino)-3-methylphenyl]-, acetate**
National Chemical Inventories (NCI) names[1]	*Methylium, [4-(dimethylamino)phenyl]bis[4-(ethylamino)-3-methylphenyl]-, acetate (1:1)* (TSCA) *Methylium, [4-(dimethylamino)phenyl]bis[4-(ethylamino)-3-methylphenyl]-, acetate* (AICS, PICCS, ASIA-PAC, NZIoC)
Other names	*[4-(Dimethylamino)phenyl]bis[4-(ethylamino)-3-methylphenyl]methylium acetate*
Chemical group (DSL Stream)	Discrete organics
Major chemical class or use	Cationic triphenylmethanes; anilines;
Major chemical sub-class	Secondary Aromatic Amines, Secondary Amines, Tertiary Amines, Tertiary Aromatic Amines
Chemical formula	$C_{27}H_{34}N_3.C_2H_3O_2$
Chemical structure [2]	
SMILES [3]	CN(c2ccc(cc2)C[(OC(=O)C)](c3cc(c(cc3)NCC)C)c1cc(c(cc1)NCC)C)C
Molecular mass (g/mol)	459.64

[1] National Chemical Inventories (NCI). 2006: AICS (Australian Inventory of Chemical Substances); ASIA-PAC (Asia-Pacific Substances Lists); PICCS (Philippine Inventory of Chemicals and Chemical Substances); NZIoC (New Zealand Inventory of Chemicals) and TSCA (Toxic Substances Control Act Chemical Substance Inventory).

[2] This substance is an organic salt, comprising a carbocation and an acetate anion.

3 SMILES stands for : Simplified Molecular Line Input Entry System. This SMILES notation was used to generate predictions. It is for the neutral form of the molecule and indicates a covalent bond between the carbocation and acetate anion. This is typically how they are shown in EPIWIN. It is not fully established what effect using this SMILES will have on the predictions. The acetate part of the SMILES is placed in square brackets here to highlight the fact that the molecule is at least partly ionic.

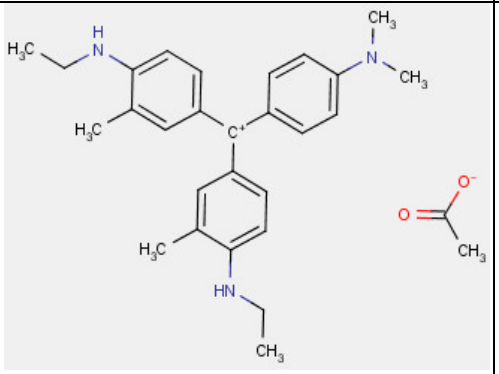
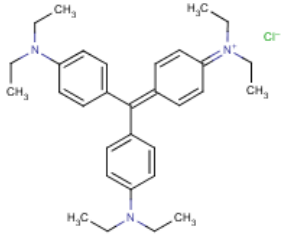
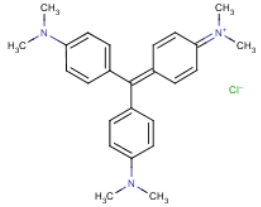
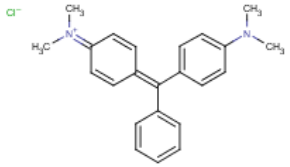
Physical and Chemical Properties

No experimental data are available for MAPBAP acetate. At the Environment Canada-sponsored Quantitative Structure-Activity Relationship (QSAR) Workshop in 1999 (Environment Canada 2000) modelling experts identified many structural classes of pigment and dyes as being “difficult to model” using QSARs. Some physical and chemical properties of many of the structural classes of dyes and pigments are not amenable to prediction by models. Under such circumstances, a "read-across" approach is considered which employs close analogues, to determine the approximate physical and chemical properties of MAPBAP acetate. A search of the ChemIDPlus (2009) database yielded a number of suitable analogues which are described in Table 2. Experimental data for these analogues, when available, were used as extrapolated (read-across) values for MAPBAP acetate or as supporting values for the weight of evidence.

A limited number of read-across data were found for the selected analogues and, therefore, predicted values are also used for MAPBAP acetate and the uncertainties of the predictions are noted.

Table 3 below contains predicted physical-chemical properties of the neutral form of MAPBAP acetate that are relevant to its environmental fate. Analogue data are available for water solubility and log K_{ow}. The water solubility of Ethyl Violet (CAS RN 2390-59-2) is 9000 mg/L (Green 1990). There is an indication that triphenylmethane acetates are more soluble than the chlorides (Pfenninger and Bruttel 1985) indicating the water solubility of MAPBAP acetate is high.

Table 2. MAPBAP acetate and its structural analogues

Methylium, [4-(dimethylamino)phenyl]bis[4-(ethylamino)-3-methylphenyl]-, acetate MAPBAP acetate (CAS RN 72102-55-7)	**Analogue 1** Ethanaminium, N-[4-[bis[4-(diethylamino)phenyl]methylene]-2,5-cyclohexadien-1-ylidene]-N-ethyl-, chloride Ethyl Violet (CAS RN 2390-59-2)	**Analogue 2** N-(4-(Bis(4-(dimethyl amino) phenyl) methylene) -2,5-cyclo hexadien-1-ylidene)-N-methyl methanaminium, Chloride Gentian violet (CAS RN548-62-9)	**Analogue 3** Methanaminium, N-(4-((4-(dimethylamino)phenyl)phenyl-methylene)-2,5-cyclohexadien-1-ylidene)-N-methyl-, chloride Malachite Green (CAS RN 569-64-2)
			

Comparative analysis:

The difference between the chemical structures of MAPBAP acetate (i) and analogues 1,2 and 3 are:

- the number and position of the methyl, or ethyl, groups;
- the counteranions: acetate, for MAPBAP acetate and chloride (Cl-) for the analogues.

For all substances, the charge on the cation is de-localized. Resonance hybrids can occur and these affect the position of the counteranion (acetate for (i) and chloride for the analogues).

Table 3. Physical and chemical properties for the neutral form of MAPBAP acetate and analogues

Property	Substance	Type	Value[1]	Temperature (°C)	Reference
Melting point (ºC)	MAPBAP acetate	Modelled	236.73	-	MPBPWIN 2008
Boiling point (ºC)	MAPBAP acetate	Modelled	551.67	-	MPBPWIN 2008
Vapour pressure (Pa)	MAPBAP acetate	Modelled	9.13×10^{-10} (6.85×10^{-12} mmHg)	25	EPIWIN 2004
Henry's Law constant ($Pa \cdot m^3/mol$)	MAPBAP acetate	Modelled	1.92×10^{-10} (1.895×10^{-15} $atm \cdot m^3/mole$)	25	HENRYWIN 2008
Log K_{ow} (Octanol-water partition coefficient) (dimensionless)	Analogue (C.I. Basic Violet 3 CAS RN 548-62-9)	Experimental	0.51	-	Tsai et al. 1991
K_{oc} (Organic carbon-water partition coefficient) (dimensionless)	MAPBAP acetate	Modelled	10.26 [2]	-	PCKOCWIN 2008
Water solubility [3] (mg/L)	Analogue (CAS RN 2390-59-2)	Experimental	9000	-	Green 1990

Property	Substance	Type	Value[1]	Temperature (°C)	Reference
	MAPBAP acetate	Modelled	475 [2]	25	WSKOWWIN 2008

[1] Values and units in brackets represent those originally reported by the authors or estimated by the models.
[2] This value was modelled using the experimental analogue logKow of 0.51 as input,.
[3] Importer of MAPBAP acetate has indicated that it is completely soluble at environmental pHs (eg. pH 7).

Sources

MAPBAP acetate is not reported to be naturally produced in the environment.

Information gathered from the CEPA 1999 Section 71 notices for the 2005 and 2006 calendar years indicates that MAPBAP acetate was not manufactured in Canada in a quantity meeting the 100 kg reporting threshold (Canada 2006b; Environment Canada 2009a). For the 2005 calendar year, fewer than four Canadian companies reported importing MAPBAP acetate (either alone, or contained in a mixture, product or manufactured item) and the total quantity imported was in the 1 001 to 100 000 kg/year range.

For the 2006 calendar year, fewer than four Canadian companies reported importing MAPBAP acetate (either alone, or contained in a mixture, product or manufactured item) and the total quantity imported was in the 10 000 to 100 000 kg/year range (Environment Canada 2009a). Fewer than twenty companies reported using MAPBAP acetate and/or were identified by importers as customers who used MAPBAP acetate, and the total quantity used was 10 000–100 000 in 2006 (Environment Canada 2009a).

During the 1986 calendar year, it was reported that approximately 100 000 kg of MAPBAP acetate was manufactured, imported or in commerce in Canada (Environment Canada 1988). Fewer than four companies reported using MAPBAP acetate during the calendar years 1984 to 1986.

Products containing MAPBAP acetate may enter the country even if they are not identified as such in the section 71 survey because they may be imported unknowingly in manufactured items, or in quantities below the 100 kg reporting threshold for the survey. Available information is currently not sufficient to derive a quantitative estimate of the importance of this source.

Uses

The main use reported in the responses to the CEPA 1999 Section 71 notices for the 2005 and 2006 calendar years (Canada 2006b; Environment Canada 2009a) is as a paper dye, principally in newsprint. Another use for MAPBAP acetate, reported in response to the section 71 survey for 2006, was indicated as Confidential Business Information (CBI) but has been taken into consideration in conducting this assessment.

The uses reported in the DSL nomination data were “Colourant - pigment/stain/dye/ink” and “Formulation Component” (Environment Canada 1988).

Releases to the Environment

Responses to a notice issued under section 71 of CEPA 1999 indicated no reported releases of MAPBAP acetate to the environment in the calendar year of 2006 (Environment Canada 2009a). MAPBAP acetate is not a reportable substance to the Canadian National Pollutant Release Inventory (NPRI 2008) or to the U.S. Toxics Release Inventory (TRI 2007); therefore no release data were available from these sources.

The losses of MAPBAP acetate via various routes during its lifecycle are estimated based on regulatory survey data, industry data and data published by different organizations. The losses are grouped into seven types: (1) discharge to wastewater; (2) emission to air; (3) loss to land; (4) chemical transformation; (5) disposal to landfill; (6) disposal by recycling; and (7) disposal by incineration. Losses may occur at one or more of the substance's lifecycle stages that include manufacture, industrial use, consumer/commercial use, and disposal. To assist in estimating these losses, a spreadsheet (Mass Flow tool) was used that incorporates all data and assumptions required for the estimation (Environment Canada 2009). Unless specific information on the rate or potential for release of the substance from landfills, recycling operations and incinerators is available, the Mass Flow tool does not quantitatively account for releases to the environment from these waste disposal methods.

In the context of the estimation assisted by the Mass Flow tool, the discharge to wastewater refers to wastewater prior to any treatment, either on-site industrial wastewater treatment or off-site municipal sewage treatment. The loss via chemical transformation refers to changes in substance identity that occur within the manufacture, industrial use, or consumer/commercial use stages, but excludes those during waste management operations such as incineration and wastewater treatment.

The losses estimated for MAPBAP acetate over its lifecycle are presented in Table 4 (Environment Canada 2009b). Taking into account its use as a paper dye, approximately 8 % of the total quantity of the substance in Canadian commerce is expected to be released to wastewater. In general, wastewater is a common source for releases to water and soil (e.g., application of biosolids) through wastewater treatment facilities.

Table 4. Mass Balance of MAPBAP acetate from Different Life Cycle Stages under a Realistic Worst Case Scenario

Loss Type	Proportion (percent)			
	Manufacture	**Industrial Use**	**Consumer / Commercial Use**	**Total**
Wastewater	0	7.72	0	7.72
Paved/unpaved surfaces	0	0	0	
Emission to Air	0	0	0	
Chemical Transformation	0	0	0	
Incineration	0	0	1.36	1.36
Landfill	0	0	43.82	43.82
Recycling	0	0	47.10	47.10
Total	0	7.72	92.28	100

MAPBAP acetate is also expected to be released to the environment via routes other than wastewater, such as by landfilling of pulp mill biosolids. MAPBAP acetate disposed of in a landfill will likely remain there because of its low vapour pressure.

MAPBAP acetate is expected to be used in some manufactured items and consumer products such as dyed paper. Although no information is available on the quantity of manufactured items and consumer products containing MAPBAP acetate that are imported into Canada, it is anticipated that the amount lost to wastewater (which results mainly from industrial use) would not be significantly different. However, the quantities sent for waste management would be higher if importation of these items were taken into consideration. Available information is currently not sufficient to derive a quantitative estimate for these losses.

Environmental Fate

According to its uses and the Mass Flow Tool results presented in Table 4, MAPBAP acetate is released to water during the processing and use stages, but could partition to sediments. It may also reside in soil as the result of biosludge application.

Due to its high water solubility, based on read-across data (see Table 3 above), MAPBAP acetate, once released to water, will tend to partition initially to that medium where it will ionize, and be dissociated, at environmental pHs. Dissociation of the MAPBAP acetate molecule will yield a reactive carbocation (MAPBAP), so MAPBAP acetate released to

water could react/bind with available dissolved and particulate organic and anionic material, including humic acid that is present in the water. The US EPA (2002) makes the following recommendation for the testing of new cationic dyes: "…two fish acute toxicity tests are recommended with known amounts of humic acid added to the dilution water. This testing is necessary to measure the mitigating effects of dissolved organic carbon (DOC) on the toxicity of the cationic dye."

MAPBAP acetate might enter soil with pulp mill biosolids, which may be used for soil enrichment. Volatilization from dry or moist soil surfaces is an unimportant fate process because MAPBAP acetate has low vapour pressure (see Table 2). If released to soil, MAPBAP acetate could be mobile based on the low modelled K_{oc} value (using a modelled read-across $\log K_{ow}$ as input). However, similar to the situation in surface water, dissociated cationic MAPBAP acetate could bind to anionic material in the soil and, therefore tend to remain in that medium.

MAPBAP acetate is a solid at room temperature and based on its vapour pressure and Henry's Law constant, it is not released to air.

Persistence and Bioaccumulation Potential

Environmental Persistence

No experimental biological degradation data for MAPBAP acetate have been identified. There are no suitable analogues with experimental data for degradation so a QSAR-based weight-of-evidence approach (Environment Canada 2007) was applied using the degradation models shown in Table 5 below. The models for persistence use chemical structure as the basis for their predictions. Chemical speciation (ionic *vs.* neutral form) is less relevant for biodegradation predictions than for bioaccumulation and toxicity predictions.

The BIOWIN models were used to estimate the substance's persistence in water, and these predictions were extrapolated to soil and sediment, where MAPBAP is expected to partition to some degree.

Table 5. Modelled data for degradation of MAPBAP acetate

Fate Process	Model and model details	Prediction	Extrapolated Half-life (days or hours)
AIR			
Atmospheric oxidation	AOPWIN 2000	$t_{1/2}$ = 0.048 days	< 2
Ozone reaction	AOPWIN 2000	n/a[1]	n/a
WATER			
Hydrolysis	HYDROWIN 2008	$t_{1/2}$ = 1059 days (pH7) $t_{1/2}$ = 106 days (pH8)	n/a
Biodegradation (aerobic)	BIOWIN 2008 Sub-model 3: Expert Survey (ultimate biodegradation)	1.44 [2] "biodegrades slowly"	> 182 [4]
Biodegradation (aerobic)	BIOWIN 2008 Sub-model 4: Expert Survey (primary biodegradation)	2.62 [2] "may biodegrade fast"	< 182 [4]
Biodegradation (aerobic)	BIOWIN 2008 Sub-model 5: MITI linear probability	-0.38 [3] "biodegrades very slowly"	> 182 [4]
Biodegradation (aerobic)	BIOWIN 2008 Sub-model 6: MITI non-linear probability	0.0002 [3] "biodegrades very slowly"	> 182 [4]
Biodegradation (aerobic)	CPOPs 2008 % BOD (biological oxygen demand)	% BOD = 4.4 "biodegrades very slowly"	> 182 [4]
Biodegradation (aerobic)	OECD Toolbox 2009a	% BOD = 0.19 "biodegrades very slowly"	> 182

[1] Model does not provide an estimate for this type of structure.
[2] Output is a numerical score from 0 to 5
[3] Output is a probability score
[4] Expected half-lives for BIOWIN and CATABOL models are determined based on Environment Canada 2009.

The structural components of the MAPBAP acetate carbocation are represented in the model fragment sets and predictions are consistent with what would be expected for the biodegradation potential of this tri-phenyl structure. However, there is some uncertainty as chemicals of overall structural similarity to MAPBAP acetate are not contained in their training sets.

In air, a predicted atmospheric oxidation half-life value of 0.048 days (see Table 5 above) indicates that MAPBAP acetate will be rapidly oxidized. The substance is not expected to react with other photo-oxidative species in the atmosphere, such as O_3 nor is it likely to degrade via direct photolysis. Therefore, it is expected that reactions with hydroxyl radicals will be the most important fate process in the atmosphere for MAPBAP acetate. With a half-life of 0.02 days via reactions with hydroxyl radicals, MAPBAP acetate is considered not persistent in air.

In water, a predicted hydrolysis half-life value of 1059 days at pH 7 (see Table 5 above) demonstrates that this chemical will be slowly hydrolysed. Other fate processes in water such as biodegradation, need to be considered to determine the overall level of persistence in this medium.

Results for the three BIOWIN ultimate biodegradation models (BIOWIN Sub-models 3, 5 and 6) indicate that biodegradation is slow and that the half-life in water would be > 182 days. Although the result from BIOWIN Sub-model 4 indicates that primary biodegradation may be faster and that the half-life from primary transformation in water would be <182 days, the identity of the degradation products is not known. In addition, the ultimate degradation predictions from CPOPs and the OECD Toolbox indicate a very slow rate of biodegradation.

According to the Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers, with some exceptions, dyes are considered essentially non-biodegradable under aerobic conditions (ETAD 1995). Repeated evaluation of ready and inherent biodegradability using accepted screening tests (see the *OECD Guidelines for the Testing of Chemicals* website) have confirmed this understanding (Pagga and Brown 1986; ETAD 1992). Based on the chemical structure of MAPBAP acetate, there is no reason to suspect that biodegradation will be other than that described for dyes generally (ETAD 1995).

Considering all model results and the structure of the MAPBAP acetate molecule (triphenylmethane), the ultimate biodegradation half-life of MAPBAP acetate is > 182 days in water.

Using an extrapolation ratio of 1:1:4 for a water: soil: sediment biodegradation half-life (Boethling et al 1995), the ultimate biodegradation half-life in soil is also >182 days and the half-life in sediments is >365 days. This indicates that MAPBAP Acetate is expected to be persistent in soil and sediments.

Based on the information presented above, MAPBAP acetate meets the persistence criteria in water, soil, and sediment (half-lives in soil and water ≥ 182 days and half-life in sediment ≥ 365 days), but does not meet the criteria for air (half-life in air ≥ 2 days) as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

Potential for Bioaccumulation

There are no empirical bioaccumulation data available for MAPBAP acetate. There are, however, empirical bioaccumulation data for Malachite Green (CAS RN 569-64-2), an analogue of MAPBAP acetate. This data is presented below in Table 6a.

Table 6a. Empirical data for bioaccumulation of Malachite Green (analogue of MAPBAP acetate)

Test organism	Endpoint	Value wet weight (L/kg)	Reference
Fish	BCF	36 - 91	NITE 2002

Since no experimental bioaccumulation factor (BAF) data and only one experimental bioconcentration factor (BCF) result were found for analogues of MAPBAP acetate, a predictive approach was applied using available BAF and BCF models as shown in Table 6b below. According to the *Persistence and Bioaccumulation Regulations* (Canada 2000) a substance is bioaccumulative if its BCF or BAF is $\geq$ 5000, however measures of BAF are the preferred metric for assessing bioaccumulation potential of substances. This is because BCF may not adequately account for the bioaccumulation potential of substances via the diet, which predominates for substances with log K_{ow} > ~4.0 (Arnot and Gobas 2003). Kinetic mass-balance modelling is in principle considered to provide the most reliable prediction method for determining the bioaccumulation potential because it allows for correction for metabolic transformation as long as the log K_{ow} of the substance is within the log K_{ow} domain of the model.

The mass-balance bioaccumulation model BCFBAFWIN was run for MAPBAP acetate. Read-across data for the logKow (0.51) was used for model input. MAPBAP acetate has high water solubility (based on read-across data) and is probably completely dissociated at environmental pHs. The BAF modelling considers a food web that includes benthic invertebrates (Arnot and Gobas 2003). The predicted BAF is 1.015 L/kg.(see Table 6b below).

Table 6b. Fish BAF and BCF predictions for MAPBAP acetate using the Arnot-Gobas kinetic model (2003) and considering metabolism.

Test organism	Endpoint	Value wet weight (L/kg)	Reference
Fish	BAF	1.015	Gobas BAF Middle Trophic Level (Arnot and Gobas 2003)
Fish	BCF	1.015	Gobas BCF Middle Trophic Level (Arnot and Gobas 2003)

Table 6c. Additional Modelled data for bioaccumulation that considers metabolism

Test organism	Endpoint	Value wet weight (L/kg)	Reference
Fish	BCF	65	CPOPs 2008

Ionic dyes are generally considered to have a very low bioaccumulation potential based on results of studies with various dyes (ETAD 1995).

Considering the available evidence, MAPBAP acetate does not meet the bioaccumulation criteria (BCF, BAF ≥ 5000) as set out in the Persistence and Bioaccumulation Regulations (Canada 2000).

Potential to Cause Ecological Harm

Ecological Effects Assessment

A - In the Aquatic Compartment

There are no empirical ecotoxicity data available for MAPBAP acetate and therefore data from chemical analogues are used. Based on the available experimental evidence (Table 7a), MAPBAP acetate is expected to cause harm to aquatic organisms at relatively low concentrations (i.e., acute LC_{50}s are < 1 mg/L).

Table 7a. Empirical data for aquatic toxicity of analogues of MAPBAP acetate

CAS RN (Common name)	Test organism	Type of test	Duration (hours)	End point	Value (mg/L)	Reference
548-62-9 (Basic Violet 3)	*Oryzias latipes* (Medaka fish)	Acute	48	LC_{50}[1]	0.1	Tonogai et al. 1982
569-64-2 (Malachite Green)	*Pseudokirchneriella subcapitata* (Green algae)	Chronic	72	Enzyme activity	10	Ericson 1977
	Palaemonetes kadiakensis (Grass shrimp)	Acute	96	LC_{50}[1]	1.9	Bills et al. 1977
	Lepomis macrochirus (Bluegill)	Acute	24	LC_{50}[1]	0.151	Bills et al. 1977
	Oncorhynchus mykiss (Rainbow trout)	Acute	24	LC_{50}[1]	0.332	Van Heerden et al. 1995

[1] LC_{50} – The median or nominal Lethal Concentration (LC_{50}) is the concentration of a substance that is estimated to be lethal to 50% of the test organisms.

The most reliable study for the acute aquatic toxicity, of a close analogue of MAPBAP acetate (Malachite Green, CAS RN 569-64-2) is the empirical study with Rainbow trout (Table 7a) with a 24-hour LC_{50} of 0.332 mg/L (Van Heerden et al. 1995). This result indicates that MAPBAP acetate is likely to be hazardous to aquatic organisms at relatively low concentrations (i.e., LC/EC_{50} s ≤ 1 mg/L). This conclusion is supported by empirical acute toxicity studies for other structural analogues of MAPBAP acetate.

A Robust Study Summary (RSS) for the Rainbow trout study can be found in Appendix 2. Neither of the two lower values (the LC_{50} values of 0.1 mg/L for medaka exposed to Basic Violet 3 (Tonogai et al. 1982) and 0.151 mg/L for bluegill exposed to Malachite Green (Bills et al. 1977) were used. There is insufficient information provided for the bluegill study to perform a Robust Study Summary. The medaka study is considered to have low reliability, based on a RSS.

Given that there are no empirical ecotoxicity data available for MAPBAP acetate, aquatic toxicity was also assessed using QSAR models.

Table 8. Modelled data for aquatic toxicity of neutral form of MAPBAP acetate

Test Organism	Type of Test	Endpoint	Value (mg/L)	Reference
Fish	Acute (96 hour)	LC_{50} [1]	0.082	ECOSAR 2008 [3]
Daphnid	Acute (48 hour)	LC_{50} [1]	0.061	ECOSAR 2008 [3]
Green Algae	Acute (96 hour)	EC_{50} [2]	0.608	ECOSAR 2008 [3]
Daphnia	Acute (48 hours)	EC_{50} [2]	0.00095	TOPKAT 2004

[1] LC_{50} – The concentration of a substance that is estimated to be lethal to 50% of the test organisms.
[2] EC_{50} – The concentration of a substance that is estimated to cause some toxic sublethal effect on 50% of the test organisms.
[3] ECOSAR Special Class for the model run: cationic delocalized (triphenylmethane) dyes

The US EPA has developed a list called the "TSCA New Chemicals Program (NCP) Chemical Categories." One of the categories is cationic dyes. MAPBAP acetate is a cationic dye. The EPA indicates that cationic dyes are water soluble substances that are toxic to fish, daphnids, and algae (US EPA 2002). It should be noted, however, that the EPA's ecotoxicity concerns about cationic dyes are based on QSAR predictions for delocalized cationic dyes.

The modelled data for aquatic toxicity is in good agreement with the analogue data and supports the conclusion that MAPBAP acetate is potentially highly hazardous to aquatic organisms (acute $LC/EC_{50} \leq 1.0$ mg/L).

B - In Other Environmental Compartments

Since MAPBAP acetate could partition to sediment and may potentially enter soil from biosludge which is commonly used for soil enrichment, as well as from the disposal of products that degrade and release these solvent dyes, it would be desirable to have toxicity data for sediment and soil organisms. However, no ecological effects studies were found in media other than water. Furthermore, the equilibrium partitioning approach (Di Toro et al. 1991) is meant for non-ionic substances. Consequently, effects data cannot be estimated reliably for soil-dwelling organisms.

Ecological Exposure Assessment

No data concerning concentrations of this substance in water in Canada have been identified.

Industrial Release

A site-specific exposure analysis was conducted for the aquatic compartment at a total of 10 sites where MAPBAP acetate was used as a dye in the production of paper products. The quantity of the substance used at each site was in the range of 1000 to 10 000 kg/year (Environment Canada 2009a). The fixation rate of the substance is a key parameter in estimating its exposure in receiving water. It can be increased with the use of a dye fixative agent and further increased via process water reuse. Two scenarios were therefore used: a worst case scenario which assumes no use of a fixative agent and a low degree of water reuse within a mill; and a best case scenario which assumes the use of a fixative agent and a high degree of water reuse. The straight fixation rates (80% for the worst case and 90 % for the best case, respectively) are taken from the OECD Emission Scenario Document (ESD) on non-integrated pulp mills (OECD 2006). The degree of water reuse, also taken from the ESD, varies from 65% for the worst case to 85% for the best case. After accounting for the influence of water reuse on the overall fixation rate and the container residue at 0.3% expected from the on-site cleaning of totes (OECD 2009b), the maximum fraction lost from the production processes to wastewater prior to any wastewater treatment was estimated to be 8.3 %. The wastewater containing MAPBAP acetate was then treated by on-site secondary wastewater treatment systems with a model predicted removal rate of 3.4% (ASTreat 1.0). The effluents from these treatment systems were then released to rivers, lakes or costal waters and a dilution factor up to 10 was used in deriving the predicted environmental concentrations (PECs) from the effluent concentrations. The estimated PECs for the 10 industrial sites ranged from 0.00575 mg/L to 0.0543 mg/L (Environment Canada 2009c). These PEC values represent the level of exposure in the receiving water near the point of the discharge from the wastewater treatment plant for each site.

Characterization of Ecological Risk

The approach taken in this ecological screening assessment was to examine various supporting information and develop conclusions based on a weight-of-evidence approach and using precaution as required under CEPA 1999. Lines of evidence considered include results from a conservative risk quotient calculation, as well as information on persistence, bioaccumulation, toxicity, sources and fate of the substance.

MAPBAP acetate is expected to be persistent in water, soil and sediment, but not in air. It is also expected to have a low bioaccumulation potential. The importation volumes of MAPBAP acetate into Canada, along with information on its uses, indicate potential for

some releases into the Canadian environment. Once released into the environment, MAPBAP acetate could be found in water, soil, or sediments depending on the medium of release. Based on read-across data, MAPBAP acetate is expected to have high potential for toxicity to aquatic organisms.

A site-specific risk quotient analysis, integrating conservative estimates of exposure with toxicity information, was performed for the aquatic medium at 10 pulp mills to determine whether there is potential for ecological harm in Canada. The estimated PECs for the 10 sites ranged from 0.00575 mg/L to 0.0543 mg/L (Environment Canada 2009c). The critical toxicity value (CTV) for this assessment, based on experimental read-across data, is the experimental LC_{50} of 0.332 mg/L for the rainbow trout. To derive the PNEC, this value is divided by an assessment factor of 100 (10 to account for interspecies and intraspecies variability in sensitivity and 10 to estimate a long-term no-effects concentration from a short-term LC_{50}) to give a value of 0.003 mg/L. The resulting risk quotients (PEC/PNEC) for the worst-case scenario ranged from 1.5 to 18.1. Even for the best-case scenario, 8 out of 10 RQs were above 1 (1.2 to 3.6).

This information indicates that MAPBAP acetate could be causing ecological harm in Canada.

As noted previously, when MAPBAP acetate is released into a water body, it partitions into suspended particulate matter and to bottom sediments, where sediment-dwelling organisms would be exposed to the substance. No environmental monitoring data or toxicity data specific to sediment-dwelling organisms were found, so a risk quotient based on exposure in sediment pore water may be calculated based on the aquatic compartment PEC and PNEC values presented above and used for sediment risk characterization. In the calculation, bottom sediment and its pore water are assumed to be in equilibrium with the overlying water and benthic and pelagic organisms are assumed to have similar sensitivities to the substance. Therefore the PEC and PNEC for sediment pore water is considered to be the same as for the aquatic compartment. This equilibrium approach would therefore result in risk quotients for the sediment compartment that are the same as for the aquatic compartment.

Uncertainties in Evaluation of Ecological Risk

There are no experimental data for MAPBAP acetate. There are no suitable read-across (analogue) data for persistence. This necessitated the use of model predictions for persistence. In addition, there is uncertainty with the model predictions for physical-chemical properties, and bioaccumulation potential because there are no triarylmethane dyes in the training sets for these models.

Read-across data was used for some physical-chemical properties (water solubility, logKow), bioaccumulation potential and aquatic toxicity. There is uncertainty with the

read-across experimental aquatic toxicity data used because the toxicity could be mitigated by DOC, which is not mentioned in the study.

Uncertainty exists because of the lack of information on environmental concentrations (e.g., monitoring data) of MAPBAP acetate in Canada, or elsewhere, so models were used to address this data gap.

There is uncertainty associated with the exposure scenario, especially with the fixation rate of MAPBAP acetate and the ensuing release of MAPBAP acetate to wastewater. There is uncertainty about the amount of MAPBAP acetate removed in sewage treatment sludge because models were used to predict the amount and they don't take into account partitioning due to electrostatic attraction. There is uncertainty about the conditions at specific pulp mills that would influence the amount of MAPBAP acetate released to wastewater, specifically, the degree of closure (whitewater recycling) and whether fixatives are used so the exposure modelling incorporated some conservative assumptions.

Although no information is available on the quantity of MAPBAP acetate that is imported in consumer products, it is anticipated that given the diffuse nature of the releases the concentrations of MAPBAP acetate in the various environmental media would not be significantly different. It is also recognised that potential releases from waste disposal sites could be possible and contribute to overall environmental concentration. However available information is currently not sufficient to derive a quantitative estimate for these releases.

Potential to Cause Harm to Human Health

Exposure Assessment

Environmental Media

There were no data identified for MAPBAP acetate in environmental media, regardless of location. In the absence of release data from publicly available inventories and since in responses to a notice issued under section 71 of CEPA 1999, no releases were reported, as a conservative approach, environmental concentrations were estimated using the loss percentages predicted by the Mass Flow tool by Environment Canada (see Table 4) (Environment Canada 2009c). The percentages were applied to the total quantity of MAPBAP acetate in Canadian commerce in 2006.

The total quantity in commerce was conservatively assumed to be up to 100 000 kg (Environment Canada 2009a). The corresponding loss quantities to water and soil are considered as overestimates.

The estimated losses were used in ChemCAN, a Canada-specific environmental exposure model, to estimate concentrations in various environmental media (ChemCAN 2003). This model differs from the point source models used in the ecological assessment section of the document, which provide estimates of exposure near release points, in that it is a regional far-field level III fugacity model that is used to estimate average concentrations in various media to inform human exposure estimates. The predicted environmental concentrations are presented in Appendix 3 and were used as surrogates for measured data in deriving intake estimates. The estimated concentration in ambient air was used as a surrogate for indoor air data. In addition, the estimated concentration in surface water was used as a surrogate for drinking water data. The intake estimates for each medium, in addition to total intake for each age group, are presented in Appendix 4. Soil is the estimated predominant source of environmental exposure, producing a maximum total daily intake of 0.039 μg/kg-bw per day for formula-fed infants aged 0 to 6 months.

Consumer Products

MAPBAP acetate is used as a basic dye for certain types of paper such as newsprint. In responses to a notice issued under section 71 of CEPA 1999, MAPBAP acetate was used in Canada in 2006 to dye newsprint at a concentration range of 0.06 to 0.12% by weight in the paper (Environment Canada 2009a). Unlike inks, dyes are contained within the matrix of the paper and would be anticipated to exhibit negligible migration in a dermal scenario. However, toddlers of age 0.5 to 4 years are known to ingest paper during mouthing behaviours, leading to potential oral exposure. The highly acidic environment of the stomach (pH ≈ 2) may affect the dye fastness in an unknown manner; therefore release and availability of the dye for absorption in the gastrointestinal tract was conservatively assumed to be 100% when modelling potential exposure from this route. A paper ingestion scenario produced an estimated oral acute intake of 0.067 mg/kg-bw per event and is presented in Appendix 5.

In the United States, MAPBAP acetate is permitted as an inert ingredient in pest control products for non-food uses by the U.S. EPA (US EPA 2009). However, MAPBAP acetate is not currently registered as a formulant or active ingredient in pest control products in Canada; therefore, this application is not considered a source of exposure for Canadians (September 2009 email from the Pest Management Regulatory Agency, Health Canada to Risk Assessment Bureau, Health Canada; unreferenced).

Health Effects Assessment

No empirical toxicity data were identified for MAPBAP acetate. Sources of health hazard information considered included examination of available international reviews, assessments or classifications, reviewing the available empirical data where available and the use of predictive models as appropriate. The outputs of predictive models were also considered using five different QSAR models: TOPKAT (2004), CASETOX (2008), Toxtree (2009), DEREK 2008, and Model Applier (2009).

Using the representative molecular structure of MAPBAP (with the acetic acid fragment (acetate) attached to the carbon atom (attached to three aromatic rings)), the following results were obtained. Positive predictions were obtained on five different genotoxicity endpoints and only one of these (i.e. rodent micronucleus assay) is corroborated by more than one model (CASETOX and Toxtree). The Benigni-Bossa model within the Toxtree also predicts it to be a *Salmonella typhimurium* TA100 mutagen with metabolic activation. On the other hand, the female rat cancer models of both CASETOX and Model Applier gave positive predictions. The male rat cancer model of Model Applier as well as both mice models (male and female) of CASETOX gave positive predictions. The presence of a structural alert indicative of genotoxic carcinogenicity is another piece of supporting information that has been obtained from the Benigni-Bossa model within Toxtree. Applying the OncoLogic model to a nearly similar structure containing hydroxyl group in place of the acetate group results in a positive carcinogenicity prediction. This prediction is based on presence of Nitrogen substituted groups on the aromatic rings.

It is important to note that the Toxtree micronucleus model is a coarse grain filter for preliminary screening of potential *in vivo* mutagens and the OncoLogic does not use the identical structure for prediction purposes. Also, the Ames point mutation models of CASETOX and Model Applier predict negative results whereas TOPKAT and DEREK fail to provide any information. However, in the case of cancer models, there are at least three models (CASETOX, Model Applier and Toxtree) that classify this chemical as a potential carcinogen. The CASETOX, Model Applier and the Toxtree models are based on unique methodologies for making predictions and since they point towards a similar outcome, it carries more weight.

Thus the model predictions were mixed for carcinogenicity (6 positive and 4 negative), genotoxicity (6 positive and 7 negative), developmental (2 positive; 18 negative and 10 no result) and reproductive toxicity (1 positive and 12 no result).

Potential structural analogues of MAPBAP acetate for the purposes of read-across for human health toxicity information were identified using Leadscope (Leadscope 2008) and ChemID (ChemIDPlus 2009) along with professional judgement. As the main structure would distribute the positive charge across the molecule through resonance structures, the acetate counter ion would likely interchange with other ions or substrates when the dye is used. Therefore, the moiety of interest from a human health toxicological perspective would be the parent molecule itself. Other similar triarylamine substances, that have empirical data, include gentian violet (CAS 548-62-9), malachite green (CAS 569-64-2), C.I. Basic Violet 4 (CAS 2390-59-2) and leucomalachite green (CAS 129-73-7) as shown in Appendix 7.

Gentian violet has been classified by the European Union as Carcinogenicity Category 2 (ECB 2002) based on carcinogenicity in experimental animals. One study did report negative *in vitro* genotoxicity for mutations in a reverse mutation assay in several *S. typhimurium* strains after exposure to gentian violet at concentrations ranging from

5 – 1000 µg/plate (NICNAS 1999). Malachite green has been classified by the European Union as Reproductive Toxicity Category 3(ECB 2003) based on developmental toxicity in experimental animals. Also, the U.S. NTP (2005) reported equivocal evidence of carcinogenicity in female rats and negative results for genotoxicity from an *in vivo* micronucleus assay and an *in vitro* assay in *S. typhimurium* (NTP 1997, 1994). C.I Basic Violet 4 had negative *in vitro* genotoxicity data for chromosomal aberrations in Chinese Hamster Ovary cells (NICNAS 1999) and was also found to be predominately negative *in vitro* in assays conducted in *S. typhimurium* and mouse lymphoma cells (CCRIS 2009). Leucomalachite green was found to have some evidence of carcinogenicity in female mice and had positive *in vivo* genotoxicity data (NTP 1996, 2005).

The information obtained from the QSAR models as well as potential analogues, suggest that there may be potential carcinogenic or developmental toxicity hazards associated with the substance.

The confidence in the toxicity database is considered to be low due to the lack of available data for MAPBAP acetate

Characterization of Risk to Human Health

The potential for exposure of the general population to MAPBAP acetate from environmental media is expected to be negligible. Exposure to MAPBAP acetate from consumer products (newsprint dye) is expected to be negligible for the intended purpose of the product (negligible dermal exposure for reading activities) and low for incidental events such as mouthing exposure by toddlers.

As exposure of the general population in Canada based on the use of the substance as a paper dye is expected to be low to negligible, the risk to human health is considered to be low.

No empirical toxicity data were identified for MAPBAP acetate. The outputs of QSAR predictions for carcinogenicity and genotoxicity were mixed. Information from analogue substances suggests a possible concern for carcinogenicity, genotoxicity and developmental toxicity.

Uncertainties in Evaluation of Risk to Human Health

Confidence in the environmental exposure estimates is moderate. Literature data were not identified for concentrations in environmental media. However, in light of no releases reported in response to a notice issued under section 71 of CEPA 1999, in conjunction with the conservative use of loss quantities predicted by the Mass Flow Tool to represent worst-case release estimates, it is unlikely that the intake values are underestimates. Confidence in the consumer product exposure estimate is moderate. While end products in current use in Canada are considered to have been comprehensively captured in the

responses to a notice issued under section 71 of CEPA 1999, the quantity of paper ingested by a toddler per mouthing event was a conservative estimate.

Due to the lack of data available for MAPBAP acetate, confidence in the toxicological database is very low.

Conclusion

Based on the information presented in this screening assessment, it is concluded that MAPBAP acetate is entering or may be entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity. Additionally, MAPBAP acetate meets the criteria for persistence as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

Based upon consideration of the available data, it is concluded that MAPBAP acetate is not a substance entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

It is therefore concluded that MAPBAP acetate meets one or more of the criteria set out in section 64 of CEPA 1999.

Where relevant, research and monitoring will support verification of assumptions used during the screening assessment and, where appropriate, the performance of potential control measures identified during the risk management phase.

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APPENDIX 1 - PBT Model Inputs Summary Table

	Phys-Chem/Fate	Fate	Fate	Fate	Fate	Fate	Fate	PBT Profiling	Ecotoxicity
Model Input Parameters	EPISuite (all models, including: AOPWIN, KOCWIN, BCFBAF BIOWIN and ECOSAR)	STP (1) ASTreat (2) SimpleTreat (3) (required inputs are different depending on model)	EQC (required inputs are different if Type I vs. Type II chemical)	TaPL3 (required inputs are different if Type 1 vs. Type 2 chemical)	OECD POPs Tool	Arnot-Gobas BCF/BAF Model	Gobas Wolf BMF Model	Canadian -POPs (including: Catabol, BCF Mitigating Factors Model, OASIS Toxicity Model)	Artificial Intelligence Expert System (AIES)/ TOPKAT/ ASTER
SMILES Code	CN(c2ccc(cc2)C(OC(=O)C)(c3cc(c(cc3)NCC)C)c1cc(c(cc1)NCC)C)C							CN(c2ccc(cc2)C(OC(=O)C)(c3cc(c(cc3)NCC)C)c1cc(c(cc1)NCC)C)C	CN(c2ccc(cc2)C(OC(=O)C)(c3cc(c(cc3)NCC)C)c1cc(c(cc1)NCC)C)C
Molecular weight (g/mol)		460 (1, 2, 3)	na	na	na				
Melting point (ºC)	236.73								
Boiling point (ºC)	551.67								
Data temperature (ºC)			na	na					
Density (kg/m^3)		1.3504 (2)							
Vapour pressure (Pa)	9.13E-10	9.1E-10 (1, 3)							
Henry's Law constant (Pa·m^3/mol)	1.92E-10	1.9E-10 (1) 7.9E-14(3)							
Log K_{aw} (Air-water partition coefficient; dimensionless)			na	na	na				
Log K_{ow} (Octanol-water partition coefficient; dimensionless)	0.51	0.51 (1)			na	0.51	na		
K_{ow} (Octanol-water partition coefficient;		0.51 (2, 3)							

	Phys-Chem/Fate	**Fate**	**Fate**	**Fate**	**Fate**	**Fate**	**Fate**	**PBT Profiling**	**Ecotoxicity**
Model Input Parameters	EPISuite (all models, including: AOPWIN, KOCWIN, BCFBAF BIOWIN and ECOSAR)	STP (1) ASTreat (2) SimpleTreat (3) (required inputs are different depending on model)	EQC (required inputs are different if Type I vs. Type II chemical)	TaPL3 (required inputs are different if Type 1 vs. Type 2 chemical)	OECD POPs Tool	Arnot-Gobas BCF/BAF Model	Gobas Wolf BMF Model	Canadian -POPs (including: Catabol, BCF Mitigating Factors Model, OASIS Toxicity Model)	Artificial Intelligence Expert System (AIES)/ TOPKAT/ ASTER
dimensionless)									
Log K_{oc} (Organic carbon-water partition coefficient – L/kg)									
Water solubility (mg/L)	9000	9000 (1, 3)		na					
Log K_{oa} (Octanol-air partition coefficient; dimensionless)							na		
Soil-water partition coefficient (L/kg)[1]			na	na					
Sediment-water partition coefficient (L/kg)[1]			na	na					
Suspended particles-water partition coefficient (L/kg)[1]		27 (2)	na	na					
Fish-water partition coefficient (L/kg)[2]			na	na					
Aerosol-water partition coefficient; dimensionless[3]			na	na					
Vegetation-water partition coefficient; dimensionless[1]				na					
Enthalpy (K_{ow})				na					
Enthalpy (K_{aw})				na					
Half-life in air (days)			na	na	na				

	Phys-Chem/Fate	**Fate**	**Fate**	**Fate**	**Fate**	**Fate**	**Fate**	**PBT Profiling**	**Ecotoxicity**
Model Input Parameters	EPISuite (all models, including: AOPWIN, KOCWIN, BCFBAF BIOWIN and ECOSAR)	STP (1) ASTreat (2) SimpleTreat (3) (required inputs are different depending on model)	EQC (required inputs are different if Type I vs. Type II chemical)	TaPL3 (required inputs are different if Type 1 vs. Type 2 chemical)	OECD POPs Tool	Arnot-Gobas BCF/BAF Model	Gobas Wolf BMF Model	Canadian -POPs (including: Catabol, BCF Mitigating Factors Model, OASIS Toxicity Model)	Artificial Intelligence Expert System (AIES)/ TOPKAT/ ASTER
Half-life in water (days)			na	na	na				
Half-life in sediment (days)			na	na					
Half-life in soil (days)			na	na	na				
Half-life in vegetation (days)[4]				na					
Metabolic rate constant (1/days)						96.23	na		
Biodegradation rate constant (1/days) or (1/hr) -specify		0.0130; 0.31 (3, 1/hr) (2, 1/days)							
Biodegradation half-life in primary clarifier ($t_{1/2\text{-}p}$) (hr)		533.08 (1)							
Biodegradation half-life in aeration vessel ($t_{1/2\text{-}s}$) (hr)		53.31 (1)							
Biodegradation half-life in settling tank ($t_{1/2\text{-}s}$) (hr)		53.31 (1)							

APPENDIX 2: Robust Study Summary

Robust Study Summaries : Aquatic iT				
No	**Item**	**Weight**	**Yes/No**	**Specify**
1	Reference: Van Heerden, E., J.H.J. Van Vuren, and G.J. Steyn. 1995 LC50 Determination for Malachite Green and Formalin on Rainbow Trout (*Oncorhynchus mykiss*) Juveniles Water S.A. 21(1):87-94. [cited in ECOTOX].			
2	**Substance identity: CAS RN 569-64-2**	n/a		
3	Substance identity: chemical name(s): **Malachite green (analogue of MAPBAP acetate (Batch 8, CAS # 72102-55-7)**	n/a		
4	Chemical composition of the substance	2	N	
5	Chemical purity	1	N	
6	Persistence/stability of test substance in aquatic solution reported?	1	N	
Method				
7	Reference	1	Y	See No. 8 below.
8	OECD, EU, national, or other standard method?	3	Y	Franson MAH. 1989. Standard Methods for the Examination of Water and Wastewater. AWWA.
9	Justification of the method/protocol if not a standard method was used	2		
10	GLP (Good Laboratory Practice)	3	N	
Test organism				
11	Organism identity: name	n/a	Y	Rainbow trout *(O. Mykiss)*
12	Latin or both Latin & common names reported?	1	Y	Same as No. 11 above
13	Life cycle age / stage of test organism	1	Y	juveniles
14	Length and/or weight	1	Y	Mean mass of 0.3755 g (range 0.233 – 0.531)
15	Sex	1	N	
16	Number of organisms per replicate	1	Y	10 fish
17	Organism loading rate	1	Y	
18	Food type and feeding periods during the acclimation period	1	Y	No feeding during acclimation period
Test design / conditions				
19	Test type (acute or chronic)	n/a	Y	acute
20	Experiment type (laboratory or field)	n/a	Y	laboratory
21	Exposure pathways (food, water, both)	n/a	Y	water
22	Exposure duration	n/a	Y	96 hours
23	Negative or positive controls (specify)	1	N	
24	Number of replicates (including controls)	1	Y	20 fish in total
25	Nominal concentrations reported?	1	N	

26	Measured concentrations reported?	3	N	Unclear if the concentrations are measured or nominal.
27	Food type and feeding periods during the long-term tests	1	Y	No feeding
28	Were concentrations measured periodically (especially in the chronic test)?	1		
29	Were the exposure media conditions relevant to the particular chemical reported? (e.g., for the metal toxicity - pH, DOC/TOC, water hardness, temperature)	3	Y	pH = 6.95; conductivity = 16.4 mS/ m; total alkalinity =52 mg/L CaCO3
30	Photoperiod and light intensity	1	Y	24 hours (12:12, L:D)
31	Stock and test solution preparation	1		
32	Was solubilizer/emulsifier used, if the chemical was poorly soluble or unstable?	1		
33	If solubilizer/emulsifier was used, was its concentration reported?	1		
34	If solubilizer/emulsifier was used, was its ecotoxicity reported?	1		
35	Monitoring intervals (including observations and water quality parameters) reported?	1		
36	Statistical methods used	1	Y	
Information relevant to the data quality				
37	Was the endpoint directly caused by the chemical's toxicity, not by organism's health (e.g. when mortality in the control >10%) or physical effects (e.g. 'shading effect')?	n/a		
38	Was the test organism relevant to the Canadian environment?	3	Y	
39	Were the test conditions (pH, temperature, DO, etc.) typical for the test organism?	1	Y	
40	Does system type and design (static, semi-static, flow-through; sealed or open; etc.) correspond to the substance's properties and organism's nature/habits?	2	Y	Flow-through
41	Was pH of the test water within the range typical for the Canadian environment (6 to 9)?	1	Y	6.95
42	Was temperature of the test water within the range typical for the Canadian environment (5 to 27°C)?	1	Y	$10 \pm 1\ ^{o}C$
43	Was toxicity value below the chemical's water solubility?	3	Y	Based on read-across solubility value of 9000 mg/L
Results				
44	Toxicity values (specify endpoint and value)	n/a	n/a	
45	Other endpoints reported - e.g. BCF/BAF, LOEC/NOEC (specify)?	n/a		
46	Other adverse effects (e.g. carcinogenicity, mutagenicity) reported?	n/a		
47	***Score*: ... %**			***67.5***
48	***EC Reliability code*:**			***2***

49	***Reliability category (high, satisfactory, low):***	***Satisfactory Confidence***
50	***Comments***	

Appendix 3: Estimated concentrations of MAPBAP acetate in environmental media using ChemCAN version 6.00 (ChemCAN 2003).[1, 2]

Medium[3]	Estimated concentration
Ambient air[4]	5.14×10^{-4} ng/m^3
Surface water[5]	364 ng/L
Soil[6]	0.158 ng/g solids
Sediment[6]	0.0321 ng/g solids

[1]For modeling purposes, the log K_{ow} and water solubility of the analogue CAS RN 548-62-9 were used.
[2]The concentrations were estimated for the area of southern Ontario.
[3]Default inflow concentrations of 2 ng/m^3 in air and 3 ng/L in water were specified by ChemCAN.
[4]The degradation half-life in air was assumed to be 0.02 days (AOPWIN 2000).
[5]The degradation half-life in water was assumed to be 1059 days at pH of 7 (HYDROWIN 2008).
[6]Degradation processes in soil and sediment were assumed to be negligible.

Appendix 4. Estimates of total and media-specific daily intakes of MAPBAP acetate for various age groups.

Route of exposure	Estimated intake (µg/kg-bw per day) of MAPBAP acetate by various age groups							
	0–0.5 years[1,2,3]			0.5–4 years[4]	5–11 years[5]	12–19 years[6]	20–59 years[7]	60+ years[8]
	Breast milk fed	Formula fed	Not formula fed					
Air[9]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Drinking water[10]	N/A	0.039	0.015	0.016	0.013	0.007	0.008	0.008
Food and beverages[11]	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Soil[12]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Total intake	<0.001	0.039	0.015	0.016	0.013	0.007	0.008	0.008
Maximum total intake from all routes of exposure: ~0.039 µg/kg-bw per day								

[1] No data were identified for concentrations of MAPBAP acetate in breast milk.
[2] Assumed to weigh 7.5 kg, to breathe 2.1 m^3 of air per day, to drink 0.8 L of water per day (formula fed) or 0.3 L/day (not formula fed) and to ingest 30 mg of soil per day (Health Canada 1998).
[3] For exclusively formula-fed infants, intake from water is synonymous with intake from food. The concentration of MAPBAP acetate in drinking water used to reconstitute formula was based on an estimated surface water concentration of 364 ng/L provided by ChemCAN version 6.00. For non-formula-fed infants, approximately 50% are introduced to solid foods by 4 months of age and 90% by 6 months of age (NHW 1990).
[4] Assumed to weigh 15.5 kg, to breathe 9.3 m^3 of air per day, to drink 0.7 L of water per day and to ingest 100 mg of soil per day (Health Canada 1998).
[5] Assumed to weigh 31.0 kg, to breathe 14.5 m^3 of air per day, to drink 1.1 L of water per day and to ingest 65 mg of soil per day (Health Canada 1998).
[6] Assumed to weigh 59.4 kg, to breathe 15.8 m^3 of air per day, to drink 1.2 L of water per day and to ingest 30 mg of soil per day (Health Canada 1998).
[7] Assumed to weigh 70.9 kg, to breathe 16.2 m^3 of air per day, to drink 1.5 L of water per day and to ingest 30 mg of soil per day (Health Canada 1998).
[8] Assumed to weigh 72.0 kg, to breathe 14.3 m^3 of air per day, to drink 1.6 L of water per day and to ingest 30 mg of soil per day (Health Canada 1998).

[9] The concentration of MAPBAP acetate in ambient air and indoor air were based upon an estimated concentration in ambient air of 5.14×10^{-4} ng/m^3 provided by ChemCAN version 6.00.
[10] The concentration of MAPBAP acetate in drinking water was based upon an estimated concentration in surface water of 364 ng/L provided by ChemCAN version 6.00.
[11] No data were identified upon which to estimate exposure from food.
[12] The concentration of MAPBAP acetate in soil was based upon an estimated concentration in soil of 0.158 ng/g solids provided by ChemCAN version 6.00.

Appendix 5: Exposure estimate for ingestion of multi-use paper by a toddler of age 0.5 to 4 years.

Assumptions	Exposure estimates
MAPBAP acetate has been identified in newsprint, a type of paper defined as weighing between 40 g/m^2 to 57 g/m^2 (NPA 2007) and used for newspaper printing presses in addition to some consumer products, such as student sketch pads. An assumption was made that MAPBAP acetate may be used in some sketch pads of letter size (8.5 × 11 inches) intended for children. As toddlers of age 0.5 to 4 years exhibit mouthing behaviours, ingestion exposure was estimated. The maximum basis weight of newsprint in inches squared: (57 g/m^2) × (0.00064516 m^2/1 in^2) = 0.037 g/in^2 The maximum weight of a piece of sketch paper (in letter size): (8.5 in × 11 in)(0.037 g/in^2) = 3.46 g It was very conservatively assumed that ¼ of all of the MAPBAP acetate in a sheet of sketch paper was ingested (0.87 g of paper). The maximum concentration of MAPBAP acetate in paper reported in responses to a notice issued under section 71 of CEPA 1999 was 0.12% by weight (Environment Canada 2009a). An oral uptake fraction of 1 was conservatively assumed. **Estimated oral intake:** Intake = [Concentration of MAPBAP acetate in paper × weight of paper eaten] / (body weight of toddler) For toddlers 0.5–4 years[1] Intake = [(1.2 mg/g) × (0.87 g)] / 15.5 kg = 0.067 mg/kg-bw	Event oral intake: 0.067 mg/kg-bw

[1]Toddlers (0.5-4 years) are assumed to weigh 15.5 kg (Health Canada 1998).

Appendix 6: Summary of (Q)SAR Results
(Q)SAR PREDICTIONS ON CARCINOGENICITY

Model/ Species	Mice		Rat		Rat	Mice	Rodent	Mammal
	Male	Female	Male	Female				
Model Applier	N	N	P	P	P	N	N	-
Multicase Casetox	P	P	ND*	P	-	-	-	-
Topkat	NR	NR	NR	NR	-	-	-	-
Derek	-	-	-	-	-	-	-	NR

** This one is weakly positive (30 case units & 81 % probability)*

(Q)SAR PREDICTIONS ON GENOTOXICITY

Model/endpoints	chrom. ab.	chrom. ab. other rodent	chrom. ab. rat	micronucleus mice	micronucleus rodent	drosophila	drosophila HT	drosophila SLRL	mam. mutation	mam. mutation DL	UDS	UDS human lymphocytes	UDS rat hepatocytes	mouse lymphoma mut	s. cerevisiae	yeast	hgprt	e. coli	e. coli w	microbial	salmonella	BB cancer alert
MA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	P	N	ND	-	N	N	ND	N	ND	P	N	-
CT	N	-	-	P	-	ND	-	-	-	-	NR	-	-	ND	-	-	-	-	-	-	N	-
TK	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NR	-
TT	-	-	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	P

Challenge SAR

(Q)SAR PREDICTIONS ON REPRODUCTIVE TOXICITY

Model Applier

Model/ endpoint	Female			Male		
Species	mice	rat	Rodent	mice	rat	rodent
repro	ND	ND	ND	ND	ND	ND
sperm	-	-	-	ND	ND	ND

Multicase Casetox

mice	rat	rabbit	human
NR	P	NR	NR

(Q)SAR PREDICTIONS ON DEVELOPMENTAL TOXICITY

Model Applier

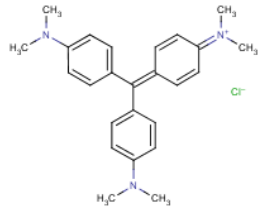
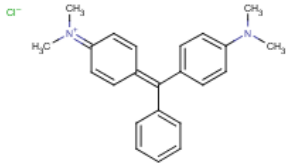
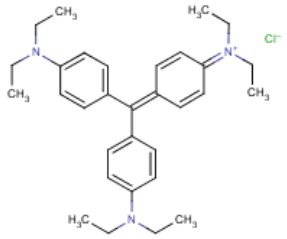
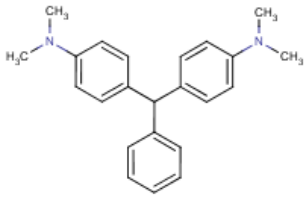
Endpoint/ Species	mice	rabbit	rat	rodent
Retardation	N	ND	N	N
Weight decrease	N	ND	N	N
Fetal death	N	ND	N	N
Post impl. loss	ND	ND	N	N
Pre impl. loss	P	ND	N	N
Structural	N	ND	ND	N
Visceral	N	-	N	N

Multicase Casetox

Endpoint/Species	Hamster	Mammal	Miscellaneous
Teratogenicity	-	P	NR
Developmental	NR	-	-

MA – model applier;
CT – Multicase Casetox;
TK – Topkat;
TT – Toxtree;
BB – Benigni-Bossa rule;
ND – not in domain;
'-' no model available in QSAR suite
NR – no result
P – positive
N – negative

Appendix 7: Analogues of MAPBAP acetate considered in Human Health portion of assessment

Name / CAS RN	Structure	Data/Classifications
Genitian violet 548-62-9		European Union Carcinogenicity Category 2 (ECB 2002) based on evidence in experimental animals **Genotoxicity** *In-vitro* reverse mutation: Negative in *S.typhimurium* TA98, TA100, TA1535, TA1537 with and without activation (NICNAS 1999).
Malachite green 569-64-2		European Union Reproductive Category 3 (ECB 2003) **Carcinogenicity** Equivocal evidence of cancer in female rats (NTP 2005) **Genotoxicity** *In-vitro* gene mutation: Negative in *S.typhimurium* TA97, TA98, TA100, TA102, TA104, TA1535 with and without activation (NTP 1994). Chromosome aberration: *In vivo*: Negative in mouse micronucleus bone marrow and peripheral blood (NTP 1997).
C.I Basic Violet 4 2390-59-2		**Genotoxicity** *In vitro* gene mutation: Negative in *S.typhimurium* TA98, TA100, TA1537, TA1538 with and without activation; TA1535 without activation (CCRIS 2009): Positive in TA1535 with S9 activation (CCRIS 2009) Negative in Mouse Lymphoma L5178Y with and without activation (CCRIS 2009) Chromosome aberration: Negative in Chinese Hamster Ovary Cells V79 with and without S9 activation (NICNAS 1999).
Leucomalachite green 129-73-7		**Carcinogenicity** Some evidence of carcinogenicity in female mice (NTP 2005) **Genotoxicity** *In vivo* chromosome aberration: Positive in female mouse micronucleus peripheral blood study (NTP 1996).