Screening Assessment for the Challenge

Phenol, 4,4'- (3*H*-2,1-benzoxathiol-3-ylidene)bis[2,5-dimethyl-, *S*,*S*-dioxide (Xylenol Blue)

Chemical Abstracts Service Registry Number 125-31-5

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 of Phenol, 4,4'- (3*H*-2,1-benzoxathiol-3-ylidene)bis[2,5-dimethyl-, *S*,*S*-dioxide (Xylenol Blue), Chemical Abstracts Service Registry Number 125-31-5. This substance was identified as a high priority for screening assessment and included in the Challenge because it had originally been 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 xylenol blue 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.

Xylenol blue is an organic substance used mainly as an analytical reagent in laboratories. This substance was likely not in commerce in Canada in 2006, indicating that its release to the Canadian environment is likely very low. Based on possible uses of this substance, it could end up in water bodies or landfills. Since xylenol blue is expected to be highly soluble in water, is not volatile and does not have a tendency to bind to particles (based on data for an analogue chemical); if released to water or soil, xylenol blue could be found in surface water and possibly in groundwater following leaching through soil.

Based on its physical and chemical properties, xylenol blue does not degrade quickly in the environment and is expected to be persistent in water and soil. Based on currently available information, xylenol blue does not have the potential to accumulate in organisms. This substance has been determined to meet the persistence criteria but not the bioaccumulation criteria as set out in the *Persistence and Bioaccumulation Regulations*. In addition, currently available information indicates that it is not highly hazardous to aquatic organisms ($LC_{50}/EC_{50} > 1$ mg/L).

For this screening assessment, a highly conservative exposure scenario was selected in which a facility (user of the substance) discharges xylenol blue into the aquatic environment. The predicted environmental concentration (PEC) in water was many orders of magnitude below predicted no-effect concentrations (PNECs) calculated for fish, daphnids and algae. Thus, this substance is not believed to cause ecological harm in the aquatic environment.

This substance will be included in the upcoming *Domestic Substances List* inventory update initiative. In addition and where relevant, research and monitoring will support verification of assumptions used during the screening assessment.

Based on the information available, it is concluded that Xylenol Blue does not meet any of the criteria set out in section 64 of CEPA 1999.

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 human health. Based on the results of a screening assessment, the Ministers can propose to take no further action with respect to the substance, to add the substance to the Priority Substances List (PSL) for further assessment, or to recommend that the substance be added to the List of Toxic Substances in Schedule 1 of the Act and, where applicable, the implementation of virtual elimination.

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 Phenol, 4,4'- (3*H*-2,1-benzoxathiol-3-ylidene)bis[2,5-dimethyl-, *S*,*S*-dioxide 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 was believed to be in commerce in Canada. The Challenge for this substance was published in the *Canada Gazette* on November 17, 2007 (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, no submissions of information were received for this substance.

Although Phenol, 4,4'- (3*H*-2,1-benzoxathiol-3-ylidene)bis[2,5-dimethyl-, *S*,*S*-dioxide 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 was not 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. Therefore, this assessment focuses principally on information relevant to the evaluation of ecological risks.

Under CEPA 1999, screening assessments focus on information critical to determining whether a substance meets the criteria for defining a chemical as toxic as set out in section 64 of the Act, where

> "64. [...] a substance is toxic if it is entering or may enter the environment in a quantity or concentration or under conditions that
>
> (*a*) have or may have an immediate or long-term harmful effect on the environment or its biological diversity;
>
> (*b*) constitute or may constitute a danger to the environment on which life depends; or
>
> (*c*) constitute or may constitute a danger in Canada to human life or health."

Screening assessments examine scientific information and develop conclusions by incorporating a weight-of-evidence approach and precaution.

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 May 2008. Key studies were critically evaluated; modelling results may have been used to reach conclusions. When available and relevant, information presented in hazard assessments from other jurisdictions was considered. 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 Program at Health Canada and Environment Canada and it incorporates input from other programs within these departments. This assessment has undergone external written peer review/consultation. Additionally, a draft of this screening assessment was subject to a 60-day public comment period. While 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. The critical information and considerations upon which the assessment is based are summarized below.

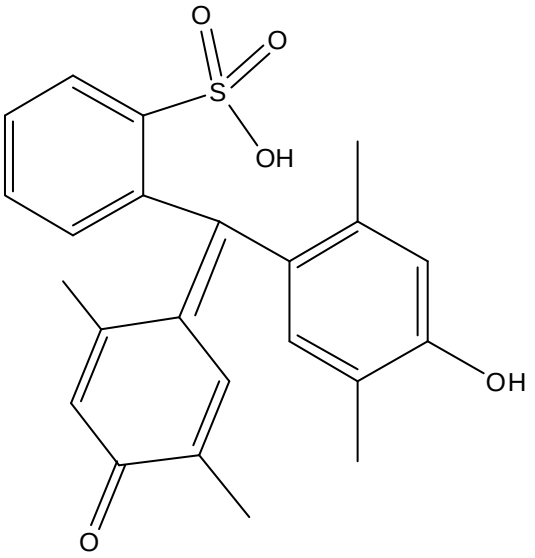
Substance Identity

Substance Name

For the purposes of this document, this substance will be referred to as xylenol blue, derived from the common name.

Table 1. Substance identity for xylenol blue

Chemical Abstracts Service Registry Number (CAS RN)	**125-31-5**
Domestic Substances List (DSL) name	**Phenol, 4,4'- (3*H*-2,1-benzoxathiol-3-ylidene)bis[2,5-dimethyl-, *S*,*S*-dioxide**
National Chemical Inventories (NCI) names[1]	*Phenol, 4,4'-(1,1-dioxido-3H-2,1-benzoxathiol-3-ylidene)bis[2,5-dimethyl-* (TSCA, PICCS, ASIA-PAC, NZIoC) *4,4'-(3H-2,1-benzoxathiol-3-ylidene)bis[2,5-dimethylphenol] S,S-dioxide* (EINECS) *XYLENOL BLUE* (PICCS)
Other names	*1,4-Dimethyl-5-hydroxybenzenesulfonphthalein* *2,5-Xylenol, 4,4'-(3H-2,1-benzoxathiol-3-ylidene)di-, S,S-dioxide* *NSC 10471* *p-Xylenesulfonephthalein* *p-Xylenol blue* *p-Xylenolsulfonephthalein* *p-Xylenolsulfonphthalein* *p-Xylenolsulfophthalein*
Chemical group (DSL Stream)	Discrete organics
Major chemical class or use	Triarylmethane dyes
Major chemical sub-class	Phenolsulfophthaleins
Chemical formula	$C_{23}H_{22}O_5S$

Chemical structure	
SMILES	OS(C1=CC=CC=C1/C(C2=C(C)C=C(O)C(C)=C2)=C(C(C)=C3)/C=C(C)C3=O)(=O)=O
Molecular mass	410.49 g/mol

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

The structure of xylenol blue is often shown with a closed benzoxathiole ring. However, once in water, this ring will hydrolyze to form an ionic sulfonate group. Since the hydrolyzed form is more environmentally relevant, the SMILES for this form was used as input to the various models used in this assessment.

Physical and Chemical Properties

Table 2 contains experimental and modelled physical and chemical properties of xylenol blue that are relevant to its environmental fate. Given the scarcity of experimental data for this substance, a search was conducted and a few close structural analogues were identified. The only analogue for which some experimental data could be found is bromophenol blue (CAS RN 115-39-9), a substance that is also assessed as part of the Challenge. Information on the chemical identity of bromophenol blue is provided in Table 3.

Key studies reporting experimental data for some physical and chemical properties of xylenol blue and its analogue bromophenol blue were critically reviewed for validity. These reviews (Robust Study Summary) are included in Appendix 1. The studies were found as a result of recent literature searches.

When available, experimental data from analogues are preferred to modelled data for the substance being assessed, especially if the model predictions are deemed unreliable. Given the scarcity of experimental data for both xylenol blue and its analogue bromophenol blue, quantitative structure-activity relationship (QSAR) models were used to generate data for physical and chemical properties of xylenol blue. These models (except WSKOWWIN 2000) are mainly based on fragment addition methods, i.e., they rely on the structure of a chemical. Since these models only accept the neutral form of a chemical as input (in SMILES form), the modelled values shown in Table 2 are for the neutral form of xylenol blue.

Table 2. Physical and chemical properties for the neutral form of xylenol blue

Property	Type	Value	Temperature (°C)	Reference
Physical state	Experimental	Brown crystals	N/A	O'Neil 2001
Decomposition point (ºC)	Modelled	263.1		MPBPWIN 2000
Boiling point (ºC)	Modelled	608.0		MPBPWIN 2000
Vapour pressure (Pa)	Modelled	1.71×10^{-14} (1.29×10^{-16} mm Hg)	25	MPBPWIN 2000
Henry's Law constant ($Pa\cdot m^3/mol$)	Modelled	1.76×10^{-15} (1.742×10^{-20} $atm\cdot m^3/mol$)	25	HENRYWIN 2000
Log K_{ow} (Octanol-water	Modelled	2.54		KOWWIN 2000

Property	Type	Value	Temperature (°C)	Reference
partition coefficient) (dimensionless)	Analogue[1] (ionized form)	-3.07	25	Franco et al. 1999
Log K_{oc} (Organic carbon-water partition coefficient – L/kg) (dimensionless)	Modelled	2.692		KOCWIN 2000 (K_{ow} method)
	Analogue[1,2] (ionized form)	-2.91 to -2.02	25	Franco et al. 1999
Water solubility (mg/L)	Modelled[3]	1 000 000	25	WSKOWWIN 2000
	Analogue[1]	4 000	NA	O'Neil 2001
pK_a (Acid dissociation constant) (dimensionless)	Experimental	~2.0 and 9.33	NA	Expert judgment; Kulichenko et al. 2001
	Modelled pKa_1 pKa_2	9.79 -0.90[4]		ACD 2005

NA: not available
[1] Experimental data from the structural analogue bromophenol blue (CAS RN 115-39-9).
[2] Calculated by Environment Canada based on Freundlich adsorption coefficients and percent organic carbon reported in Tables 3 and 1, respectively, in Franco et al.(1999).
[3] Using the experimental data found for log K_{ow} (ionized form) for the analogue as model input.
[4] A negative value indicates complete ionization.

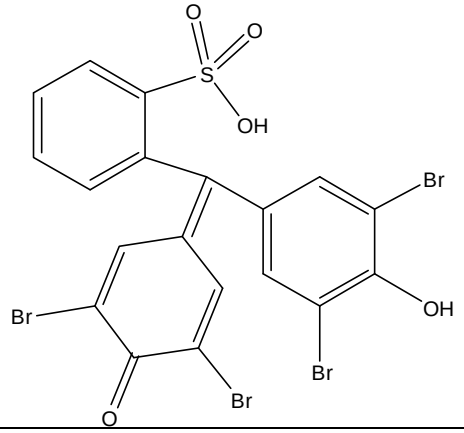
An experimental pK_a value for xylenol blue of 9.33 has been reported by Kulichenko et al. (2001). This value is consistent with the reported use of xylenol blue as a pH indicator (see Uses section) in the pH range 8.0–9.6 (pH-meter.Info 2005). However, xylenol blue is also reported to change colour in the pH range 1.2–2.8 (pH-meter.Info 2005), indicating that there is another much lower pK_a of approximately 2.0 for this compound. Since environmentally relevant pH values fall in the range of 6 to 9, the lower pK_a indicates that essentially all of this substance will be ionized in the environment. The ionization of this compound does not only involve the dissociation of the hydroxyl groups, but also occurs due to the hydrolysis of the ester link located between the central carbon and the oxygen atom in the benzoxathiole ring. Following this reaction, a sulfonic acid group is formed, which is expected to greatly increase the water solubility of this substance. These pK_a values for xylenol blue were recently identified and were not considered for categorization.

Based on the quantitative solubility value available for a structural analogue (4000 mg/L; bromophenol blue) and the evidence for a low pK_a (of approximately 2.0), it is believed that xylenol blue is highly soluble in water. In addition, xylenol blue is likely even more soluble than bromophenol blue given the absence of bromide atoms.

Regarding other physical and chemical properties, the analogue values shown in Table 2 suggest that xylenol blue does not bioaccumulate in organisms, does not bind to particles and is highly mobile in soil. Its volatility cannot be assessed because the modelled values for vapour pressure and Henry's Law constant are probably not reliable (see below). However, given its ionized state under environmental pH, this substance will likely have limited volatility. A more detailed discussion of how physical and chemical properties influence the environmental fate of this substance is presented later in this report.

As seen from Table 2, many models do not perform well in estimating the physical and chemical properties of xylenol blue. In particular, the modelled log K_{ow} and log K_{oc} values differ from the experimental values measured for an analogue by a few orders of magnitude. This is most likely because the chemical structures of ionisable substances like xylenol blue are poorly represented in the training set of some the models used. As modelled data are not reliable, the analogue data presented in Table 2 for bromophenol blue were considered more appropriate in this assessment.

Table 3. Substance identity for bromophenol blue, a structural analogue of xylenol blue

Chemical Abstracts Service Registry Number (CAS RN)	**115-39-9**
Domestic Substances List (DSL) name	**Phenol, 4,4'- (3*H*-2,1-benzoxathiol-3-ylidene)bis[2,6-dibromo-, *S*,*S*-dioxide**
Major chemical class or use	Triarylmethane dyes
Major chemical sub-class	Phenolsulfophthaleins
Chemical formula	$C_{19}H_{10}Br_4O_5S$
Chemical structure	
SMILES	OS(C1=CC=CC=C1/C(C2=CC(Br)=C(O)C(Br)=C2)=C(C=C3Br)/C=C(Br)C3=O)(=O)=O
Molecular mass	669.96 g/mol

Sources

Xylenol blue is not reported to be naturally produced in the environment.

Information gathered through a CEPA section 71 notice for the 2006 calendar year indicates that xylenol blue was not manufactured in, imported into or used in Canada in a quantity exceeding the prescribed thresholds (i.e., 100 kg for manufacture/import and 1000 kg for use). Three Canadian companies identified themselves as stakeholders, i.e., as having an interest in this substance (Environment Canada 2007). A similar CEPA section 71 notice for the 2005 calendar year also indicated that xylenol blue was not in commerce at the reporting threshold (i.e., 100 kg for manufacture/import). For that year, one Canadian company had identified itself as a stakeholder (Canada 2006b).

The quantity reported to be manufactured, imported or in commerce in Canada during the calendar year 1986 was 100 kg. The number of notifiers for the 1984 to 1986 calendar years was fewer than four.

Uses

No information on uses was received in response to the CEPA section 71 notice for the 2006 calendar year. The only uses that were identified for xylenol blue in the open literature are as an acid-base indicator (pH range 1.2 to 2.8 and 8.0 to 9.6) and as an absorbance dye in spectrophotometry.

Releases to the Environment

Since there were no reports of use, import or manufacture of xylenol blue in Canada in 2006 at or above the reporting thresholds specified in the CEPA section 71 notice (Environment Canada 2007), releases of this substance to the Canadian environment are expected to be very low.

Environmental Fate

Based on its physical and chemical properties (Table 2) and potential uses, xylenol blue, if released into the environment, would be expected to be found mainly in surface water, and possibly in soil and groundwater.

Given its dissociation constants (pK_a), most of any xylenol blue released to water bodies with environmentally relevant pH (6–9) would be ionized. The main ionization step is

due to the hydrolysis of the ester link located between the central carbon and the oxygen atom in the benzoxathiole ring. Because of this, the log K_{ow} and log K_{oc} values available for the ionized form of the analogue (bromophenol blue) are expected to be indicative of the partitioning behaviour of xylenol blue (Table 2; analogue data).

If xylenol blue is used as a liquid pH indicator in laboratories or for any other undocumented use that would result in disposal down the drain, it will reach sewage treatment plants (STP). Assuming no degradation in these plants, it would stay in wastewater and would not partition to sewage sludge based on its expected high water solubility (Table 2; analogue data) and low log K_{ow} and log K_{oc} values for ionized forms (Table 2; <1, analogue data). Similarly, once released to receiving water, it would mainly remain in the water column rather than partition into the sediments. Xylenol blue would not volatilize to air from water, based on its tendency to ionize at ambient pH in water.

If xylenol blue is used as a paper form pH indicator or for any other use that would generate solid wastes, it will end up in landfills through waste disposal. Assuming no degradation once in these sites, xylenol blue would likely leach through soil layers or undergo surface run-off given its expected high solubility in water and low affinity for soil constituents, as indicated by the very low log K_{oc} value for the ionized form of the analogue. The substance could eventually reach groundwater and/or local surface waters. Again, xylenol blue would not volatilize to air from soil, as indicated by its tendency to ionize at ambient pH. This substance is not expected to be released directly into air. The assumptions of no degradation in water and soil for this substance are supported by the assessment of its persistence in these media, as presented below.

Persistence and Bioaccumulation Potential

Environmental Persistence

The preceding information on the uses, likely pattern of release and subsequent environmental fate of xylenol blue suggests that, if released, it would mostly be found in surface water and possibly in soil and groundwater, but not in sediments. This substance is also not expected to be present in air.

There are no empirical degradation data available for xylenol blue or for a structural analogue. ETAD (1995) states that, with some exceptions, dyes may be considered essentially non-biodegradable under aerobic conditions. Repeated evaluation of ready- and inherent-biodegradability using accepted screening tests (e.g., OECD tests) have confirmed this characteristic (Pagga and Brown 1986, ETAD 1992). Based on the chemical structure of xylenol blue, there is no reason to suspect that its biodegradation would be significantly different from that of other dyes described in ETAD (1995).

Due to the lack of experimental data, the persistence of xylenol blue was examined using the predictive QSAR models for biodegradation shown in Table 4. Given the ecological importance of the water compartment, the fact that most of the available models apply to water and the fact that xylenol blue is expected to be released and remain in this compartment, biodegradation in water was primarily examined. Although the degradation models are structure-based and only consider the neutral form of xylenol blue, most of the modelled values (Table 4) are considered to be reliable, as some chemicals of structural comparability to xylenol blue are contained in their training sets.

Table 4. Modelled data for degradation of xylenol blue

Fate process	Model and model basis	Result	Interpretation	Extrapolated half-life (days)	Extrapolation reference and/or source
WATER					
Biodegradation (aerobic)	BIOWIN 2000 Sub-model 1: Linear probability	0.56	Biodegrades fast in water	n/a	n/a
Biodegradation (aerobic)	BIOWIN 2000 Sub-model 2: Non-linear probability	0.10	Does not biodegrade fast in water	n/a	n/a
Biodegradation (aerobic)	BIOWIN 2000 Sub-model 3: Expert Survey (ultimate biodegradation)	2.32	Ultimate biodegradation in months in water	60 120	US EPA 2002 Aronson et al. 2006
Biodegradation (aerobic)	BIOWIN 2000 Sub-model 4: Expert Survey (primary biodegradation)	3.16	Primary biodegradation in weeks in water	15	US EPA 2002, Aronson et al. 2006
Biodegradation (aerobic)	BIOWIN 2000 Sub-model 5: MITI linear probability	-0.16	Does not biodegrade fast in water	> 60	Aronson et al. 2006
Biodegradation (aerobic)	BIOWIN 2000 Sub-model 6: MITI non-linear probability	0.00	Does not biodegrade fast in water	> 60	Aronson et al. 2006
Biodegradation (anaerobic)	BIOWIN 2000 Sub-model 7: Linear probability	-1.47	Does not biodegrade fast	n/a	n/a
Biodegradation	BIOWIN 2000 Overall conclusion	no	Not ready-biodegradable in water	n/a	n/a
Biodegradation (aerobic)	TOPKAT 2004 Probability (MITI 1)	Out of acceptable domain	Persistent in water	n/a	n/a
Biodegradation (aerobic)	CATABOL 2004–2008 % BOD* (OECD 301C)	Out of acceptable domain	n/a	n/a	n/a

* BOD: biological oxygen demand.

The results from Table 4 show that most of the probability models (BIOWIN 1, 2, 5, 6 and 7) suggest that xylenol blue does not biodegrade rapidly. In fact, half of the probability results are less than 0.3, the cut-off suggested by Aronson et al. (2006) to identify substances as having a half-life >60 days (based on the MITI probability models), and all probability results except one are less than 0.5, the cut-off suggested by the model developers for slow biodegradation. Both the US EPA (2002) and Aronson et al. (2006) suggest that the half-life result from the primary survey model (BIOWIN 4) of "weeks" corresponds to a half-life of approximately 15 days. It is suggested by the US EPA (2002) that the ultimate survey model (BIOWIN 3) result of "months" corresponds to a half-life of approximately 60 days and by Aronson et al. (2006) that it corresponds to a half-life of 120 days. The substance is also not expected to degrade rapidly under anaerobic conditions. The overall conclusion from BIOWIN is "not ready-biodegradable."

Another ultimate degradation model (TOPKAT) predicted that xylenol blue does not undergo mineralization in a 28-day timeframe with probability of biodegradation in the range of very persistent chemicals. TOPKAT, which simulates the Japanese MITI I 28-day biodegradation test, produced a probability of zero, which is less than the suggested cut-off for persistent substances in this model (< 0.3) (TOPKAT 2004). CATABOL did not produce any acceptable results, as the substance was out of its domain of applicability.

When the results of the probability models, the overall BIOWIN conclusion and ultimate degradation models are considered, the preponderance of evidence (especially that relating to ultimate degradation) suggests that the biodegradation half-life of xylenol blue in water is >182 days.

Using an extrapolation ratio of 1:1 for a water:soil biodegradation half-life (Boethling et al.1995), the biodegradation half-life in soil is also >182 days. This indicates that xylenol blue is expected to be persistent in soil.

Overall, the empirical data on dyes from ETAD (1992, 1995) as well as the modelled data (Table 4) demonstrate that xylenol blue meets the persistence criteria in water and soil (half-lives ≥ 182 days) as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000). This substance is not expected to be found in air or sediments.

Potential for Bioaccumulation

There are no experimental bioaccumulation factor (BAF) or bioconcentration factor (BCF) data available for xylenol blue or for a structural analogue. Since xylenol blue has a low pK_a value (approximately 2.0), it is expected that this substance will exist almost exclusively in ionized form at environmentally relevant pH (6–9). The experimental log K_{ow} value for the ionized form of the analogue bromophenol blue is -3.07 (Table 2), indicating a very low potential for bioaccumulation. Ionization was not considered during categorization with respect to bioaccumulation potential.

A predictive approach was applied using available BAF and BCF models as shown in Table 5. The experimental log K_{ow} value for the ionized form of the analogue bromophenol blue was used as input for the models. The modelled bioaccumulation values do not take into account the metabolic potential of the substance; therefore the bioaccumulation potential may be over-estimated.

The modified Gobas BAF middle trophic-level model for fish predicted a BAF of < 1 L/kg, indicating that xylenol blue does not have the potential to bioconcentrate and biomagnify in the environment.

The results of BCF model calculations provide additional evidence to support the low bioconcentration potential of this substance. The very low BCF value of 3.16 is a default value recommended by the BCFWIN model for compounds, such as xylenol blue, which have a log K_{ow} < 1.

Table 5. Fish BAF and BCF predictions for xylenol blue

Test organism	Endpoint	Value wet weight (L/kg)	Reference
Fish	BAF	< 1	Gobas BAF middle trophic level (Arnot and Gobas 2003)
Fish	BCF	< 1	Gobas BCF middle trophic level (Arnot and Gobas 2003)
Fish	BCF	3.16	BCFBAF 2000
Fish	BCF	Out of acceptable domain	Baseline BCF model (Dimitrov et al. 2005)

Based on the experimental log K_{ow} data for the analogue bromophenol blue and modelled BAF and BCF values for xylenol blue, it is concluded that xylenol blue does not meet the bioaccumulation criteria (BCF or 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

As mentioned earlier, xylenol blue would tend to remain in water if it was released in this environmental compartment. In addition, it is expected to be persistent in this

compartment. Therefore, this substance could be of concern for ecological effects in aquatic ecosystems.

Depending on releases to the environment, aquatic organisms could be exposed to xylenol blue, given this substance's tendency to ionize and its expected high water solubility. However, since it is believed to have low affinity for lipids (see Table 5), it should not accumulate to a significant extent in tissues of exposed organisms.

There are no acceptable experimental aquatic toxicity data available for xylenol blue or for a structural analogue. Therefore, modelled data were used to estimate its potential for aquatic toxicity (Table 6). The ECOSAR model was run using the experimental analogue values shown in Table 2 for water solubility and log K_{ow} (ionized form). It was not possible to enter these values in the other models used (OASIS Forecast and AIEPS) as these models only accept chemical structure as input data. OASIS Forecast predicts a log K_{ow} value based on the structure entered (neutral form) and uses it to estimate toxicity. AIEPS is a probabilistic neural network based predictive model that uses structural fragments and presence or absence of atoms to determine similarity between the substance being modeled and those in the training set. It then calculates a prediction for three acute endpoints (fathead minnow, *Daphnia magna* and *Pseudokirchneriella subcapitata*).

Table 6. Modelled data for aquatic toxicity

Organism	Type of test	Endpoint	Value (mg/L)	Reference
Fish	Acute (96 hours)	LC_{50}[1]	> water solubility limit[3]	ECOSAR 2004
			0.116	OASIS Forecast 2005
			14.8	AIEPS 2003–2007
Daphnia	Acute (48 hours)	LC_{50}[1]	> water solubility limit[3]	ECOSAR 2004
			3.08	AIEPS 2003-2007
Algae	Acute (96 hours)	EC_{50}[2]	> water solubility limit[3]	ECOSAR 2004
			28.3	AIEPS 2003-2007

[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] Water solubility for the analogue = 4000 mg/L (see Table 2).

The aquatic toxicity predictions obtained from the ECOSAR model are reliable to some extent. Indeed, both the log K_{ow} (analogue data) and molecular weight of xylenol blue are covered by the domain of this model (cut-off of 7.0 for log K_{ow} and 1000 g/mol for molecular weight). However, the closest analogues contained in the training set of ECOSAR are chlorophenols, suggesting that the values predicted for the toxicity of xylenol blue are uncertain. Nevertheless, these modelled results suggest that xylenol blue is not highly hazardous to aquatic organisms (acute $LC/EC_{50} > 1.0$ mg/L), as it is observed for numerous substances having a very low log K_{ow}.

The results from the OASIS Forecast and AIEPS models suggest that xylenol blue is moderately to highly toxic ($LC_{50} \leq 1.0–28.3$ mg/L); however, these values are not considered reliable. The discrepancy between the OASIS and ECOSAR predictions is in part due to the fact that a modelled log K_{ow} value for the neutral molecule is used by OASIS Forecast in calculations. However, as noted previously, this molecule is expected to exist in ionized form at ambient pH. In addition, the chemical structure of xylenol blue is not well covered by the training set used by OASIS. Similarly, the AIEPS model did not provide reliable predictions as the similarity index indicated that most of chemicals in the training set were less than 60% similar to xylenol blue.

Given the high persistence of xylenol blue in the environment (see Table 4), chronic exposure is likely to occur. However, considering the likely moderate acute toxic effects of this substance and its low bioaccumulation potential, its chronic aquatic toxicity is also expected to be low.

B - In Other Environmental Compartments

No ecological effects studies were found for this compound in media other than water (e.g., sediment, soil). There are no QSAR models that generate toxicity data for these other media.

Ecological Exposure Assessment

There are no environmental monitoring data available for this substance. Based on the submissions received in response to the CEPA section 71 notice (Environment Canada 2007), releases of xylenol blue to the Canadian environment are believed to be very low. Accordingly, concentrations of this substance in environmental media are also expected to be very low.

While xylenol blue does not meet the bioaccumulation potential criteria prescribed by the *Persistence and Bioaccumulation Regulations* (Canada 2000), it can persist in the environment and, depending on the level of exposure, could potentially cause harm to the environment. To investigate this, a quantitative evaluation of exposure associated with the release of this chemical to aquatic ecosystems was conducted.

Environmental concentrations were estimated from available information, including estimated substance quantities, potential release rates, and characteristics of possible receiving water bodies. Environment Canada's Industrial Generic Exposure Tool – Aquatic (IGETA) was employed to estimate the substance concentration in a generic watercourse receiving industrial effluents (Environment Canada 2008a). This tool represents a highly conservative scenario in which the substance is released by a single facility at a single point in a watercourse. The generic scenario is designed to provide estimates of concentrations based on conservative assumptions regarding the amount of

chemical processed and released, the number of processing days, the sewage treatment plant removal rate, and the size of the receiving watercourse. The tool models an industrial-release scenario using loading data based on information from sources such as industrial surveys and knowledge of the distribution of industrial discharges across the country. It calculates a predicted environmental concentration (PEC) assuming instantaneous dilution in a small receiving water. The equation and inputs used to calculate the PEC in the receiving watercourse are described in Environment Canada (2008b). There were no reports of uses for this substance above the reporting threshold (1000 kg) in response to the CEPA section 71 notice (Environment Canada 2007). The reporting threshold of 100 kg for manufacture and import was used as a highly conservative estimate of the quantity of xylenol blue released to sewers from an industrial facility. Other key parameter values were as follows: 261 processing days (working days only, based on expected uses), no removal at sewage treatment plants (worst-case scenario) and 0.65 m^3/s as the flow of the receiving watercourse (15th percentile of the distribution of receiving watercourse flows in the country). The resulting PEC was calculated to be 0.0064 mg/L.

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, inherent toxicity, sources and fate of the substance.

A risk quotient analysis, integrating conservative estimates of exposures with ecological effects information, was performed for the aquatic medium to determine whether there is potential for ecological harm in Canada. For this, a conservative predicted no-effect concentration (PNEC) was derived by selecting the lowest critical toxicity value (CTV) for aquatic organisms from the reliable model results (ECOSAR). In this case, the CTV was predicted to be above the water solubility limit of 4000 mg/L (see Table 6). This value was divided by an assessment factor of 1000 to account for uncertainties associated with interspecies and intraspecies variability in sensitivity, extrapolation from acute toxicity endpoints to chronic toxicity and use of modelled toxicity data to represent field conditions. This yielded a PNEC value of 4 mg/L. The PEC calculated with IGETA was then used together with the PNEC to calculate the risk quotient, i.e. PEC/PNEC = 0.0016.

Given that IGETA provides a conservative estimate of exposure and in view of the large assessment factor used to estimate chronic effect thresholds (PNECs), the results indicate a low potential for ecological harm resulting from local exposure to a hypothetical point-source industrial release of xylenol blue to the aquatic environment.

In summary, the information gathered suggests that xylenol blue would not be causing ecological harm if it were to be released into the Canadian environment. Information on importation, manufacture and use of xylenol blue in Canada suggests very low releases of

this chemical into the Canadian environment. Data collected for calendar years 1986, 2005 and 2006 do not indicate an increasing trend in usage.

If it were released into the environment, this substance would be found mainly in water and possibly also in soil. It would persist in these environmental compartments but it would not bioaccumulate in organisms. This substance has low acute toxicity to aquatic organisms. A risk quotient analysis based on a highly conservative industrial scenario shows that aquatic organisms would not be at risk if they were exposed to xylenol blue. The lack of toxicity data for terrestrial organisms prevented the calculation of risk quotients for the soil compartment. However, given the very low releases expected for this chemical, it is believed that it would not adversely affect terrestrial organisms.

Uncertainties in Evaluation of Ecological Risk

For xylenol blue, there are virtually no experimental data for physical and chemical properties, and there are no such data for degradation, bioaccumulation factors or ecotoxicity. Gaps in available experimental data were largely filled through the use of data from a structural analogue (bromophenol blue) as well as through the use of QSAR-based models. While there are uncertainties associated with the use of these models to estimate chemical and biological characteristics, the approaches used allowed meaningful interpretation of the information. It is also noted that, with regard to ecotoxicity, there are no QSAR models that generate toxicity data for terrestrial organisms. Therefore, a risk quotient analysis could not be performed for this compartment.

Conclusion

Based on the information presented in this screening assessment, it is concluded that xylenol blue is not 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 or that constitute or may constitute a danger to the environment on which life depends.

It is therefore concluded that xylenol blue does not meet the definition of toxic as set out in section 64 of CEPA 1999. Additionally, xylenol blue meets criteria the for persistence as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000) but does not meet the criteria for bioaccumulation potential as set out in the same regulations.

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Appendix 1 – Robust Study Summary

Evaluation of experimental data using Kollig's approach (Kollig 1998)

Item	Weight	Response	Mark
Reference: Franco I, Leita L, Vischetti C, de Nobili M. 1999. Adsoprtion of five model organic compounds on a peat at different stages of drying. J. Soil Contamination 8(4):423–440.			
Test substance: CAS RN 115-39-9; Bromophenol blue			
Physical and chemical properties measured: pK_a, K_{ow}, K_{oc}			
Could you repeat the experiment with available information?	5	Only partially, based on the information included in the paper. One of the study authors was contacted by Environment Canada and provided clarification on methods.	3
Is a clear objective stated?	1	Yes	1
Is water quality characterized or identified (distilled or deionized)?	2	Yes, distilled water	2
Are the results presented in detail, clearly and understandably?	3	Fair	1.5
Are the data from a primary source and not from a referenced article?	3	Yes, primary source	3
Was the chemical tested at concentrations below its water solubility?	5	Yes	5
Were particulates absent?	2	Not mentioned	0
Was a reference chemical of known constant tested?	3	No, but a comparison made by the Environment Canada evaluator between the data measured in this study and other published data for the same substances indicate that values found in this study are in the same order of magnitude. See the table below in the "Additional comments" section.	1.5
Were other fate processes considered?	5	Hydrolysis and photolysis were not considered; however, these processes are not likely to influence the fate of bromophenol blue in solution.	5
Was a control (blank) run?	3	Yes for the batch equilibrium experiment (K_{oc}); N/A for determination of pK_a and K_{ow}	3
Was temperature kept constant?	5	Yes for batch equilibrium experiment (K_{oc}); T^o not mentioned for K_{ow} and pK_a determination.	3
Was the experiment done near room temperature (15–30^{o}C)?	3	Yes (25^{o}C) for K_{oc}; T^o not mentioned for K_{ow} and pK_a determination	2

Is the purity of the test chemical reported (> 98%)?	3	Not reported	0
Was the chemical's identity proven?	3	Partially (the chemical name, molecular weight and absorption maximum provided corresponded to CAS RN 115-39-9; however, the chemical structure did not); the CAS RN of the substances tested were not provided. One of the study authors was contacted. This author indicated that the salt form of bromophenol blue (CAS 62625-28-9) was used. Environment Canada considers that the dissociated (ionized) form of the latter substance is equivalent to the dissociated form of CAS RN 115-39-9.	2
Is the source of the chemical reported?	1	No	0
Results:	pK_a = 4.0 K_{ow} (ionized form) = 0.00085 K_{oc} (for a series of peat samples - corrected by the Environment Canada evaluator for %OC): ionized form = 0.0012 to 0.0095		
Score:	33/47 or 70%		
EC Reliability code : Reliability category (high, satisfactory, low):	2 Satisfactory		
Note	Evaluated independently by three Environment Canada evaluators (May 2008)		

Additional comments (by the Environment Canada evaluators):

- The sorbent material used in this batch equilibrium experiment was peat rather than soil. Since adsorption-desorption characteristics are usually useful for assessing the behaviour of a substance in soils, soil samples would have ideally been used in this experiment. Indeed, the sorptive capacity of peat is expected to be much higher than for soils, given its high organic carbon content. However, given that adsorption could be described by Freundlich isotherms (as indicated by the 1/n values in Table 3) and given that %OC for each sample was provided, the assessor was able to calculate K_{oc} values based on the K_f measured by the authors.
- The fact that the K_{oc} values for bromophenol blue were very low, even when dried peat was used (very high sorptive capacity), indicates that the hydrophobicity of bromophenol blue is very low.
- For the batch equilibrium experiment, the test substances should have been dissolved in 0.01 M $CaCl_2$ rather than distilled water in order to improve centrifugation and minimize cation exchange (OECD TG No 106; 2000).
- The optimal sorbent/solute ratio was determined in preliminary experiments.

- The pH of the aqueous phase before and/or after adsorption was not reported. This factor has an important influence on adsorption for ionizable substances. One of the study authors was contacted to clarify this aspect. The clarification is as follows: Batch equilibrium experiments were conducted in peat/water suspensions whose pH was that imposed by the buffering action of the peat's functional groups (pH = 4.5), as it actually happens in the field where xenobiotics are present at low concentration and the pH is imposed by the soil. To ensure that pH did not vary, the compounds were dissolved in distilled water and the pH of the solution was adjusted to that of a compound free suspension of peat in water. The adsorption isotherm therefore refers to a situation where the compound was for the most part ionized.
- Although the authors mention that they measured the water solubility of the test compounds, it does not seem that they measured it for bromophenol blue because they cite the value from O'Neil (2001) for this property (Table 2).
- The authors did not mention how they were able to measure a K_{ow} for both non-ionized and ionized forms of bromophenol blue (e.g., use of a buffer). Also, they did not mention the value of the pH of the aqueous phase during the measurements. The OECD TG 107 – Partition Coefficient (n-octanol/water): Shake Flask Method states that "Dissociation or association of the dissolved molecules results in deviations from the partition law (OECD TG 107; 1995). Such deviations are indicated by the fact that the partition coefficient becomes dependent upon the concentration. Measurements should be made on ionizable substances only in their non-ionized form (free acid or free base) produced by the use of an appropriate buffer with a pH of at least one unit below (free acid) or above (free base) the pK." One of the study authors was contacted to clarify this methodological aspect. The clarification is as follows: The K_{ow} of the non-ionized form of bromophenol blue was determined in an unbuffered water solution acidified to pH 1–1.5 with 0.020 mL of concentrated HCl, while the K_{ow} of the ionized form was determined in a solution made alkaline with a similar amount of 0.5 M NaOH. The study author contacted recognized that this is not a standard procedure. However, this person considered that measurements of K_{ow} in buffers could be misleading because of possible formation of ionic couples, intermolecular associations at high ionic strength, etc. This person also believed that the environmental behavior of substances such as bromophenol blue, which can be expected to be present in their ionized form in the environment, cannot be predicted on the basis of the K_{ow} of the non-ionized molecule.
- UV-Vis seems like an appropriate method to measure the concentration of the chemicals in this study given their absorption maximum and chemical structure (numerous double bonds).
- Because reference chemicals of a known constant were not tested in this study, the Environment Canada evaluator conducted a search for published data in order to validate (or not) the results measured in this study. Experimental data were found in the literature for bromophenol blue and for other substances tested in this study. They show that the values found in this study for water solubility, pK_a and K_{ow} are within the same order of magnitude as those published (see table below). No data were found to validate the K_{oc} values measured; however, there are sufficient

methodological details provided in the paper and in the author's response to consider the value for the ionized form of bromophenol blue as reliable. In addition, even if an actual K_{oc} value is not provided, a paper by Zeroual et al. 2006 does indicate that bromophenol blue does not adsorb to organic matter (fungal biomass) at pH 6, i.e., when under an ionized form.

	Water solubility (mg/L at 25°C)	**pK_a**	**K_{ow}**
Acridine orange (CAS RN 494-38-2)	873 (this study) *vs* 700 (PhysProp 2006)	-	-
Dinitrobenzoic acid (CAS RN 99-34-3)	986 (this study) *vs* 1350 (PhysProp 2006)	3.4 (this study) *vs* 2.82 (PhysProp 2006)	11.22 (this study) *vs* 35.48 (PhysProp 2006)
Bromophenol blue (CAS RN 115-39-9)	-	4.0 (this study) *vs* 4.1 (Kulichenko et al. 2001) *vs* 4.0 (O'Neil 2001)	-

Evaluation of experimental data using Kollig's approach (Kollig 1998)

Item	Weight	Response	Mark
Reference: Kulichenko SA, Fesenko SA, Fesenko NI. 2001. Color indicator system for acid-base titration in aqueous micellar solutions of the cationic surfactant tridecylpyridinium. J. Anal. Chem. 56(11):1002–1006.			
Test substances: CAS RN 115-39-9 (Bromophenol blue), CAS RN 115-40-2 (Bromcresol purple) and CAS RN 125-31-5 (Xylenol blue)			
Physical and chemical property measured: pK_a			
Could you repeat the experiment with available information?	5	Yes, but there was no mention of how the indicator solutions were prepared.	4
Is a clear objective stated?	1	Yes	1
Is water quality characterized or identified (distilled or deionized)?	2	No	0
Are the results presented in detail, clearly and understandably?	3	Yes	3
Are the data from a primary source and not from a referenced article?	3	Yes	3
Was the chemical tested at concentrations below its water solubility?	5	Assumed	3
Were particulates absent?	2	Not mentioned	0
Was a reference chemical of known constant tested?	3	No. However, comparison with other published data confirms that values measured seem correct (see comment below).	3
Were other fate processes considered?	5	N/A	N/A
Was a control (blank) run?	3	N/A	N/A
Was temperature kept constant?	5	Temperature was not mentioned but this factor does not have a major influence on the pK_a (cf Yao and Byrne 2001).	N/A
Was the experiment done near room temperature (15–30^o C)?	3	Temperature was not mentioned but this factor does not have a major influence on the pK_a (cf Yao and Byrne 2001).	N/A
Is the purity of the test chemical reported (> 98%)?	3	Yes (analytical grade)	3
Was the chemical's identity proven?	3	Only the common name of the chemicals was provided but this is deemed sufficient (see comment below).	3
Is the source of the chemical reported?	1	No	0
Results:	pK_a bromophenol blue = 4.09 ± 0.03 pK_a bromcresol purple = 6.40 ± 0.02		

	pK_a xylenol blue = 9.33 ± 0.02
Score:	23/31 or 74%
EC Reliability code : **Reliability category (high, satisfactory, low):**	2 Satisfactory
Note	Evaluated by one Environment Canada evaluator (May 2008)

Additional comments (by the Environment Canada evaluator):

- The authors measured the pK_a of test substances in aqueous solutions. They also measured the so-called pK_{eff} for the same substances in aqueous micellar solutions containing the ionic surfactant tridecylpyridinium. The pK_a values for the aqueous solutions are the ones used for the risk assessment.
- Even though the CAS RN were not provided to check the identity of the test substances, a verification in the NCI confirms that there is only one CAS RN associated with the names bromcresol purple and xylenol blue. For bromophenol blue, there is only one CAS RN associated with the non-salt form.
- A comparison between the pK_a values measured in this study with other published data for the same substances indicates that all data are similar. Therefore, the values from this study are considered reliable.

  6.49 for bromcresol purple (Yao and Byrne 2001)
  vs
  6.40 (this study)

  8.03 and 8.08 for phenol red (Yao and Byrne 2001, PhysProp 2006)
  vs
  8.00 (this study)

  7.0 for bromothymol blue (O'Neil 2001)
  vs
  7.30 (this study)