SYBR® Safe (CAS# 1030826-36-8) GreenScreen® for Safer Chemicals (GreenScreen®) Assessment

Prepared for:

State of Washington

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TOXSERVICES
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GreenScreen® Executive Summary for SYBR® Safe (CAS #1030826-36-8)

SYBR® Safe is a chemical that functions as a DNA/RNA gel stain. Limited data were available for SYBR® Safe and therefore this evaluation is heavily based upon modeling. ToxServices evaluated this compound as a pure substance rather than in highly diluted, commercially available preparations for which the exact dilution factors were not known.

SYBR® Safe was assigned a GreenScreen® Benchmark Score of 1 ("Avoid – Chemical of High Concern") as it is predicted to have Very High Ecotoxicity (acute aquatic toxicity (AA) and chronic aquatic toxicity (CA)) and Very High persistence (P). This corresponds to GreenScreen® benchmark classification 1c in CPA 2011. Data gaps (dg) exist for reproductive toxicity (R), developmental toxicity (D), endocrine activity (E), acute toxicity (AT), systemic toxicity repeated exposure (STr*), neurotoxicity (single and repeated exposure (Ns and Nr*)) and respiratory sensitization (SnR*). As outlined in CPA (2013) Section 12.2 (Step 8 – Conduct a Data Gap Analysis to assign a final Benchmark score), SYBR® Safe meets requirements for a GreenScreen® Benchmark Score of 1 despite the hazard data gaps.

GreenScreen® Benchmark Score for Relevant Route of Exposure:
As a standard approach for GreenScreen® evaluations, all exposure routes (oral, dermal and inhalation) were evaluated together, so the GreenScreen® Benchmark Score of 1 ("Avoid – Chemical of High Concern") is applicable for all routes of exposure.

GreenScreen® Hazard Ratings for SYBR® Safe

Group I Human					Group II and II* Human									Ecotox		Fate		Physical	
C	M	R	D	E	AT	ST		N		SnS*	SnR*	IrS	IrE	AA	CA	P	B	Rx	F
						single	repeated*	single	repeated*										
L	**M**	DG	DG	DG	DG	*M*	DG	DG	DG	*L*	DG	*M*	*M*	*vH*	*vH*	*vH*	*L*	*L*	*L*

Note: Hazard levels (Very High (vH), High (H), Moderate (M), Low (L), Very Low (vL)) in *italics* reflect estimated (modeled) values, authoritative B lists, screening lists, weak analogues, and lower confidence. Hazard levels in **BOLD** font are used with good quality data, authoritative A lists, or strong analogues. Group II Human Health endpoints differ from Group II* Human Health endpoints in that they have four hazard scores (i.e., vH, H, M, and L) instead of three (i.e., H, M, and L), and are based on single exposures instead of repeated exposures. Please see Appendix A for a glossary of hazard acronyms.

GreenScreen® Assessment for SYBR® Safe (CAS #1030826-36-8)

Method Version: GreenScreen® Version 1.2[1]
Assessment Type[2]: Certified

Chemical Name: SYBR® Safe

CAS Number: 1030826-36-8

GreenScreen® Assessment Prepared By:
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Organization: ToxServices LLC
Date: August 29, 2014
Assessor Type: Licensed GreenScreen® Profiler

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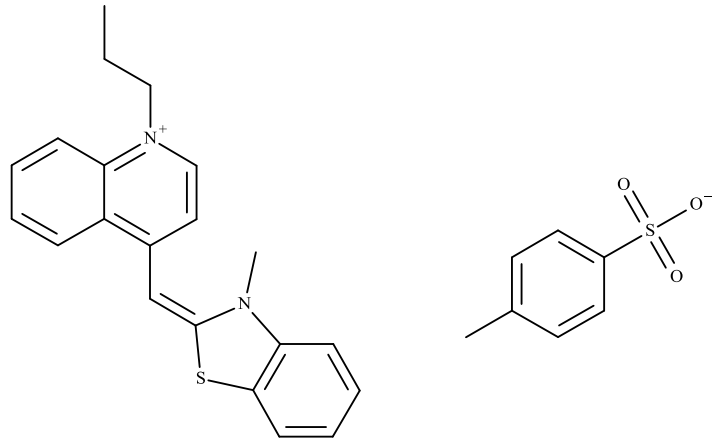
Title: Managing Director and Chief Toxicologist
Organization: ToxServices LLC
Date: October 27, 2014

Confirm application of the *de minimus* rule[3]: N/A

Chemical Structure(s):



(Evenson et al. 2012, ChemIDPlus 2014)

Also called: No synonyms identified.

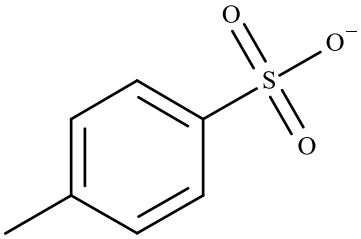
Chemical Structure(s) of Chemical Surrogates Used in the GreenScreen™:
An incomplete toxicological dataset was identified for SYBR Safe. Therefore, analogs were sought for with available tools. AIM software did not identify any analogs even after including "pass two", a less stringent search strategy for analogs, compared to the default "pass one" search. SYBR® Safe

[1] Use GreenScreen® Assessment Procedure (Guidance) V1.2

[2] GreenScreen® reports are either "UNACCREDITED" (by unaccredited person), "AUTHORIZED" (by Authorized GreenScreen® Practitioner), "CERTIFIED" (by Licensed GreenScreen® Profiler or equivalent) or "CERTIFIED WITH VERIFICATION" (Certified or Authorized assessment that has passed GreenScreen® Verification Program)

[3] Every chemical in a material or formulation should be assessed if it is:
1. intentionally added and/or
2. present at greater than or equal to 100 ppm

differs from the dye thiazole orange by having a propyl group instead of a methyl group substitution at the quinolinium ring nitrogen (Evenson et al. 2012). Therefore, ToxServices evaluated thiazole orange (CAS # 107091-89-4) as a potential surrogate to fill data gaps, but no relevant data were identified for thiazole orange where there are data gaps for SYBR® Safe. Based on the structure of thiazole orange, ToxServices deduced that SYBR® Safe is also a 1:1 salt of 4-methylbenzenesulfonic acid (CAS # 104-15-4) (ChemIDplus 2014). Therefore, data on the salt moiety 4-methyl benzenesulfonic acid were used to evaluate the toxicity of SYBR® Safe. Due to lack of data on the other dye moiety (i.e., (2E)-3-methyl-2-[(1-propylquinolin-1-ium-4-yl)methylene]-1,3-benzothiazole), 4-methyl benzenesulfonic acid is considered a weak surrogate. In addition, as it is a strong acid while SYBR® Safe is a relatively neutral product as sold (Life Technologies 2012a), data on 4-methylbenzenesulfonic acid are less useful to assess point-of-entry endpoints such as irritation. Acidity is known to cause irritating effects, and therefore, the irritating effects of 4-methyl benzenesulfonic acid may be attributed to acidity rather than the irritation potential of SYBR® Safe at neutral pH.



Chemical surrogate: 4-Methylbenzenesulfonic Acid (CAS #104-15-4)

Identify Applications/Functional Uses:
DNA/RNA gel stain (Life Technologies 2014)

GreenScreen® Summary Rating for SYBR® Safe[4]: SYBR® Safe was assigned a GreenScreen® Benchmark Score of 1 ("Avoid – Chemical of High Concern") as it is predicted to have Very High Ecotoxicity (acute aquatic toxicity (AA) and chronic aquatic toxicity (CA)) and Very High persistence (P). This corresponds to GreenScreen® benchmark classification 1c in CPA 2011. Data gaps (dg) exist for reproductive toxicity (R), developmental toxicity (D), endocrine activity (E), acute toxicity (AT), systemic toxicity repeated exposure (STr*), neurotoxicity (single and repeated exposure (Ns and Nr*)) and respiratory sensitization (SnR*). As outlined in CPA (2013) Section 12.2 (Step 8 – Conduct a Data Gap Analysis to assign a final Benchmark score), SYBR® Safe meets requirements for a GreenScreen® Benchmark Score of 1 despite the hazard data gaps.

Figure 1: GreenScreen® Hazard Ratings for SYBR® Safe

Group I Human					Group II and II* Human									Ecotox		Fate		Physical	
C	M	R	D	E	AT	ST		N		SnS*	SnR*	IrS	IrE	AA	CA	P	B	Rx	F
						single	repeated*	single	repeated*										
L	**M**	DG	DG	DG	DG	*M*	DG	DG	DG	*L*	DG	*M*	*M*	*vH*	*vH*	*vH*	*L*	*L*	*L*

Note: Hazard levels (Very High (vH), High (H), Moderate (M), Low (L), Very Low (vL)) in *italics* reflect estimated (modeled) values, authoritative B lists, screening lists, weak analogues, and lower confidence. Hazard levels in

[4] For inorganic chemicals with low human and ecotoxicity across all hazard endpoints and low bioaccumulation potential, persistence alone will not be deemed problematic. Inorganic chemicals that are only persistent will be evaluated under the criteria for Benchmark 4.

BOLD font are used with good quality data, authoritative A lists, or strong analogues. Group II Human Health endpoints differ from Group II* Human Health endpoints in that they have four hazard scores (i.e., vH, H, M, and L) instead of three (i.e., H, M, and L), and are based on single exposures instead of repeated exposures. Please see Appendix A for a glossary of hazard acronyms.

Transformation Products and Ratings:

Identify feasible and relevant fate and transformation products (i.e., dissociation products, transformation products, valence states) **and/or moieties of concern**[5]

No transformation products were identified. The OECD Toolbox predicted no transformation products from autoxidation, dissociation or hydrolysis under acidic, basic and neutral conditions (OECD 2013).

Introduction

SYBR® Safe is a fluorescent nucleic acid stain that contains a cyanine-based cationic core structure identical to the thiazole orange dye (CAS # 107091-89-4). It differs from thiazole orange by having a propyl group instead of a methyl group substitution at the quinolinium ring nitrogen (Evenson et al. 2012). Therefore, it is expected to bind to double stranded DNA the same way as thiazole orange, which stacks between bases as a monomer and binds externally as a dimer (Nygren et al. 1998). Based on the structure of thiazole orange, it is deduced that SYBR® Safe is also a 1:1 salt of 4-methylbenzenesulfonic acid (CAS # 104-15-4) (ChemIDplus 2014). SYBR® Safe is marketed in different sizes, concentrations and solvents. A product MSDS of SYBR® Safe in 0.5x Tris/ Borate/EDTA (TBE) buffer indicates that it contains 0.1 – 1.0% SYBR® Safe (Life Technologies 2012a). An MSDS of the 10,000X concentrate in dimethylsulfoxide (DMSO) indicates that it also contains SYBR® Safe at 0.1 – 1% (Life Technologies 2012b). ToxServices assumes that the 10,000 X concentrate is the most concentrated form of SYBR® Safe on the market.

ToxServices assessed SYBR® Safe against GreenScreen® Version 1.2 (CPA 2013) following procedures outlined in ToxServices' SOP 1.37 (GreenScreen® Hazard Assessment) (ToxServices 2013).

GreenScreen® List Translator Screening Results

The GreenScreen® List Translator identifies specific authoritative or screening lists that should be searched to identify GreenScreen® benchmark 1 chemicals (CPA 2012b). Pharos (Pharos 2014) is an online list-searching tool that is used to screen chemicals against the List Translator electronically. The output indicates benchmark or possible benchmark scores for each human health and environmental endpoint. SYBR® Safe is not found in Pharos database.

PhysicoChemical Properties of SYBR® Safe

SYBR® Safe is sold in liquid form at 0.1 – 1% in 0.5x Tris/Borate/EDTA (TBE) buffer. It is estimated to have a low vapor pressure, indicating low potential to exist as a vapor at room temperature. The commercial SYBR® Safe in TBE is soluble in water. The estimated partition coefficient of 3.37 suggests that it has a low bioaccumulation potential, as substances with log K_{ow} of less than 4 are assigned the score of Very Low for bioaccumulation under GreenScreen® Criteria (CPA 2012c). Table 1 summarizes the physiochemical properties of SYBR® Safe.

[5] A moiety is a discrete chemical entity that is a constituent part or component of a substance. A moiety of concern is often the parent substance itself for organic compounds. For inorganic compounds, the moiety of concern is typically a dissociated component of the substance or a transformation product.

Table 1: Physical and Chemical Properties of SYBR® Safe (CAS #1030826-36-8)		
Property	**Value**	**Reference**
Molecular formula	$C_{28}H_{28}N_2O_3S_2$	Evenson et al. 2012, Symyx Draw 2010
SMILES Notation	CCC[n+]1ccc(c2c1cccc2)/C=C/3\N(c4ccccc4S3)C.Cc1ccc(cc1)S(=O)(=O)[O-]	Evenson et al. 2012, Symyx Draw 2010
Molecular weight	504	Evenson et al. 2012, Symyx Draw 2010
Physical state	Liquid	Life Technologies 2012a
Appearance	Red – orange in TBE/DMSO	Molecular Probes Undated
Melting point	197.64ºC (est. for the cation)	U.S. EPA 2012a
Vapor pressure	2.66×10^{-9} mm Hg (est. for the cation)	U.S. EPA 2012a
Water solubility	Soluble (in 0.5x TBE)	Life Technologies 2012a
Dissociation constant	N/A	
Density/specific gravity	N/A	
Partition coefficient	3.37 (est. for the cation)	U.S. EPA 2012a

Hazard Classification Summary Section:

Group I Human Health Effects (Group I Human)

Carcinogenicity (C) Score (H, M, or L): *L*
SYBR® Safe was assigned a score of Low for carcinogenicity based on *in vitro* data and modeled results. GreenScreen® criteria classify chemicals as a Low hazard for carcinogenicity when adequate data are available and negative, there are no structural alerts, and they are not classifiable under GHS (CPA 2012a).

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists
  - *Screening:* Not present on any screening lists
- Molecular Probes Undated
  - An *in vitro* carcinogenicity assay (cell transformation assay) was conducted with SYBR® Safe in Syrian hamster embryo (SHE) cells (GLP status not reported) at concentrations of 0.200, 0.400 and 0.700 µg/mL in trial 1 and 0.0500, 0.150 and 0.300 µg/mL in trial 2 for an exposure period for 7 days (GLP status not reported). Trial 1 failed due to excess cytotoxicity. In trial 2, no cytotoxicity, slight cytotoxicity and moderate cytotoxicity were observed at the low, mid and high concentrations, respectively. There was no significant increase in the frequency of morphological transformation under the test conditions. This indicates negative carcinogenic potential *in vivo* both through genotoxic and non-genotoxic mechanisms (OECD 2012).
- U.S. EPA 2013
  - No data were identified for this endpoint. An attempt was made to use OncoLogic to evaluate the carcinogenic potential of SYBR® Safe. However, the current version (v.8.0) could not evaluate the ring structure of this chemical. See Appendix C for model output.
- Toxtree 2013
  - The Toxtree program predicted that SYBR® Safe is negative for genotoxic

carcinogenicity. However, it has a structural alert for nongenotoxic carcinogenicity: benzenesulfonic ethers. This was due to the 4-methylbenzenesulfonic acid anion. See Appendix D for model output.

- VEGA 2012
  - VEGA's carcinogenicity model CAESAR predicts that the cation of SYBR® Safe is carcinogenic while the anion is non-carcinogenic. However, the compound is out of the application domain of this program. Therefore, the results may not be reliable. See Appendix E for model output.
- Based on the weight of evidence, a score of Low was assigned for carcinogenicity. SYBR® Safe was not predicted to be carcinogenic by the genotoxic mechanism by Toxtree, but it has a structural alert for nongenotoxic carcinogenicity. It was predicted to be carcinogenic by VEGA, but the results may not be reliable. OncoLogic was not able to predict its carcinogenic potential. The cell biotransformation assay, which is an *in vitro* carcinogenicity assay, was negative, which suggests that SYBR® Safe is not carcinogenic either via genotoxic or nongenotoxic mechanisms. This *in vitro* evidence was considered to carry more weight than the presence of structural alert for nongenotoxic carcinogenicity. Confidence level in this hazard assignment was reduced due to lack of standard *in vivo* data.

Mutagenicity/Genotoxicity (M) Score (H, M, or L): M

SYBR® Safe was assigned a score of Moderate for mutagenicity/genotoxicity based on positive Ames assays and negative results in *in vitro* mammalian cell mutagenicity and chromosomal aberration tests. GreenScreen® criteria classify chemicals as a Moderate hazard for mutagenicity/genotoxicity when they are classified to GHS category 2 or there is limited or marginal evidence of mutagenicity in animals (CPA 2012a).

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists
  - *Screening:* Not present on any screening lists
- Molecular Probes Undated
  - *Mutagenicity:* An Ames test was conducted according to OECD guideline 471 in *Salmonella typhimurium* tester strains TA97a, TA98, TA100, TA102, TA1535, TA1537 and TA1538 (GLP status not reported). SYBR® Safe was tested at 0.100, 0.333, 1.00, 3.33, 10.0, 25.0 and 50.0 µg/plate with metabolic activation and 0.0100, 0.0333, 0.100, 0.333, 1.00, 3.33 and 10.0 µg/plate without metabolic activation. Increased mean number of revertants per plate (2.3 – 3.8 fold) was observed in TA97a, TA98, TA102 and TA1538 in the presence of S9. Cytotoxic (decreased number of revertant colonies with or without thinning of the bacterial background lawn) was observed at the two highest concentrations with and without metabolic activation.
  - *Mutagenicity:* A L5178Y TK+/- mouse lymphoma forward mutation screening assay was conducted with SYBR® Safe (GLP status not reported) at concentrations of 0.0313, 0.0625, 0.125, 0.250, 0.500, 0.750, 1.00 and 1.5 µg/mL without metabolic activation, and at 1.24, 2.47 and 4.93 µg/mL with metabolic activation. Cytotoxicity was observed at concentrations no less than 0.250 µg/mL (without activation) and 1.24 µg/mL (with activation). NO increase in the mutant frequency was observed under the experimental conditions.
  - *Transformation:* A transformation assay was conducted with SYBR® Safe in Syrian hamster embryo (SHE) cells (GLP status not reported) at concentrations of 0.200, 0.400 and 0.700 µg/mL in trial 1 and 0.0500, 0.150 and 0.300 µg/mL in trial 2 for an exposure period for 7 days (GLP status not reported). Trial 1 failed due to excess cytotoxicity. In

trial 2, no cytotoxicity, slight cytotoxicity and moderate cytotoxicity were observed at the low, mid and high concentrations, respectively. There was no significant increase in the frequency of morphological transformation under the test conditions.

- *Chromosomal aberration:* An *in vitro* chromosomal aberration test was conducted in human peripheral blood lymphocytes with SYBR® Safe concentrations of 0.500, 1.00, 2.00, 4.00, 6.00, 8.00 and 10.0 μg/mL without S9 and 7.81 μg/mL with S9 (higher concentrations were terminated due to excess cytotoxicity) (GLP status not reported). Cytotoxicity was observed at ≥ 2.00 μg/mL without S9 but not at 7.81 μg/mL with S9. There was no significant increase in the number of cells with structural aberrations, polyploidy or endoreduplication under the tested conditions.

- Based on the weight of evidence, a score of Moderate was assigned for mutagenicity. SYBR® Safe tested positive in an Ames assay, and negative for mutagenicity and chromosomal aberrations in mammalian cells. It also tested negative in a cell transformation assay, which indicates negative carcinogenic potential *in vivo* both through genotoxic and non-genotoxic mechanisms (OECD 2012). Although their GLP status of these studies was not reported, they were conducted by independent contract labs and the results appeared to be valid. Positive results in *S. typhimurium* tester strains TA97a, TA98 and TA102 suggests occurrence of G/C or A/T base pair damage, frameshift mutations and or small deletions while the mouse lymphoma TK gene mutation assay detects point mutations, chromosome deletions and other effects leading to loss of heterozygosity (Eastmond et al. 2009). Although mouse lymphoma gene mutation tests appear to be more relevant to humans than Ames tests as the former are mammalian cell assays while and latter bacterial assays, Ames tests have a strong statistically significant association with the 2-year rodent bioassay while the mouse lymphoma assays have no correlation with rodent bioassay results (Benigni and Bossa 2011). The ICH genotoxicity testing guidance and the WHO/IPCS recommend a follow-up *in vivo* genotoxicity test when Ames test was positive, regardless of the results of other genotoxicity tests (ICH 2012, Eastmond et al. 2009). Therefore, the genotoxic potential of SYBR® Safe cannot be ruled out.

Reproductive Toxicity (R) Score (H, M, or L): DG

SYBR® Safe was assigned a score of Data Gap for reproductive toxicity based on lack of data identified for this endpoint.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists
  - *Screening:* Not present on any screening lists
- No data were identified.
- Surrogate 4-methylbenzenesulfonic acid
  - U.S. EPA 2007
    - No data regarding the reproductive toxicity of 4-methylbenzenesulfonic acid were identified. However, due to the corrosive nature of aromatic sulfonic acids, unnecessary harm would be caused to laboratory animals if a chronic toxicity study were performed. Further, because aromatic sulfonic acids are highly polar and highly water soluble, they are not expected to be absorbed into systemic circulation. As a result, reproductive and developmental testing was not performed.

Developmental Toxicity incl. Developmental Neurotoxicity (D) Score (H, M, or L): DG

SYBR® Safe was assigned a score of Data Gap for developmental toxicity based on lack of data for this endpoint and inconclusive modeling results.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists
  - *Screening:* Not present on any screening lists
- U.S. EPA 2012b
  - As no data were identified, ToxServices performed modeling using T.E.S.T. software. SYBR® Safe was predicted to be a non-developmental toxicant, but only one chemical each was identified in the external test set and in the training set. Therefore, the reliability of the predicted result is questionable. See Appendix F for model output.
- Surrogate 4-Methylbenzenesulfonic acid
  - U.S. EPA 2007
    - No data regarding the developmental toxicity of 4-methylbenzenesulfonic acid were identified. However, due to the corrosive nature of aromatic sulfonic acids, unnecessary harm would be caused to laboratory animals if a chronic toxicity study were performed. Further, because aromatic sulfonic acids are highly polar and highly water soluble, they are not expected to be absorbed into systemic circulation. As a result, reproductive and developmental testing was not performed.

Endocrine Activity (E) Score (H, M, or L): DG
SYBR® Safe was assigned a score of Data Gap for endocrine disruption based on lack of data for this endpoint.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists
  - *Screening:* Not present on any screening lists
- Not listed as a potential endocrine disruptor on the EU Priority List of Suspected Endocrine Disruptors.
- Not listed as a potential endocrine disruptor on the OSPAR List of Chemicals of Possible Concern.
- No data were identified.

Group II and II* Human Health Effects (Group II and II* Human)

Note: Group II and Group II* endpoints are distinguished in the v 1.2 Benchmark system. For Systemic Toxicity and Neurotoxicity, Group II and II* are considered sub-endpoints and test data for single or repeated exposures may be used. If data exist for single OR repeated exposures, then the endpoint is not considered a data gap. If data are available for both single and repeated exposures, then the more conservative value is used.

Acute Mammalian Toxicity (AT) Group II Score (vH, H, M, or L): DG
SYBR® Safe was assigned a score of Data Gap for acute toxicity based on insufficient data available.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists
  - *Screening:* Not present on any screening lists
- Molecular Probes Undated
  - In a limit screen test, a single oral dose of 5,000 mg/kg SYBR® Safe in 0.5x TBE was given to female Sprague-Dawley rats, and no mortality, weight changes or toxic signs were observed for two weeks after exposure. Therefore, the oral LD_{50} for this SYBR® Safe + TBE buffer is > 5,000 mg/kg.

        - As this product contains 0.1 – 1% pure SYBR® Safe stain (Life Technologies 2012a), the LD_{50} of pure SYBR® Safe is > 5 – 50 mg/kg.
- U.S EPA 2012b
    - T.E.S.T. estimated an oral LD_{50} of 172.8 mg/kg in rats for the active moiety of the SYBR® Safe salt. However, there were no chemicals with a minimum similarity coefficient of 0.5 in the external test set, and the mean absolute error (MAE) for the training set chemicals with similarity coefficient ≥ 0.05 is greater than the MAE of the entire training set, indicating poor confidence. See Appendix F for model output.
- Surrogate 4-methylbenzenesulfonic acid
    - ECHA 2014
        - An acute oral toxicity study was performed according to OECD Guideline 401 to determine the oral LD_{50} of toluene sulfonic acid. The test substance was administered to groups of 5 male and 5 female Wistar rats via gavage at doses of 1,250, 1,600 and 2,000 mg/kg for females and 2,000 mg/kg for males. The authors identified an oral LD_{50} value of 1,410 mg/kg for both males and females
    - ESIS 2000
        - An oral LD_{50} of 2,480 mg/kg was determined in the rat; however, no details on the test substance (i.e., whether it was toluene sulfonic acid or a surrogate) or the study were provided, and the study was not identified as GLP.
        - Oral LD_{50} values ranging from 400 to 3,200 mg/kg were established in the mouse; however, no details on the test substance (i.e., whether it was toluene sulfonic acid or a surrogate) or the study were provided, and the study was not identified as GLP.
- Based on the weight of evidence, a score of Data Gap was assigned. Although the 0.1 – 1% SYBR® Safe preparation has an oral LD_{50} of > 5,000 mg/kg, there is insufficient information on the pure SYBR® Safe at GHS guidance values. The assessment of this endpoint could not be based on the 0.1 – 1% SYBR® Safe preparation, as SYBR® Safe is also sold as 10,000 concentrate in DMSO (Molecular Probes Undated). Although 4-methylbenzenesulfonic acid has oral LD_{50} values as low as 400 mg/kg/day, it is a strong acid and the acute toxicity may be attributed to, at least partially, its acidity. The pH value of the 0.1 – 1% SYBR® Safe preparation is 8.3 (Life Technologies 2012a), which is approximately neutral. Further, T.E.S.T. modeling results were not reliable. Therefore, there is insufficient information to assign a hazard for this endpoint.

Systemic Toxicity/Organ Effects incl. Immunotoxicity (ST)
***Group II Score (single dose)* (vH, H, M, or L):** *M*
SYBR® Safe was assigned a score of Moderate for systemic toxicity (single dose) based on self-classification to H335 for the most concentrated product of SYBR® Safe.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists
    - *Screening:* Not present on any screening lists
- Molecular Probes Undated
    - In a limit screen test, a single oral dose of 5,000 mg/kg SYBR® Safe in 0.5x TBE was given to female Sprague-Dawley rats, and no mortality, weight changes or toxic signs were observed for two weeks after exposure. Therefore, the NOAEL for this SYBR® Safe + TBE buffer is > 5,000 mg/kg.
        - As this product contains 0.1 – 1% pure SYBR® Safe stain (Life Technologies 2012a), the NOAEL of pure SYBR® Safe is > 5 – 50 mg/kg.

- Life Technologies 2012b
    - A 10,000x SYBR® Safe preparation in DMSO containing 0.1 – 1.0% pure SYBR® Safe was self-classified to the hazard phrase H335: May cause respiratory irritation.
- Based on the weight of evidence, insufficient data are available at GHS guidance values of 300 and 2,000 mg/kg for oral exposure. The self-classification to H335 in the MSDS of the most concentrated SYBR® Safe in DMSO on the market was used to assign a Moderate score. Confidence level was reduced due to lack of measured data and uncertainty regarding if the claimed respiratory irritation is attributable to the solvent DMSO rather than SYBR® Safe.

Group II* Score (repeated dose) **(H, M, or L):** DG

SYBR® Safe was assigned a score of Data Gap for systemic toxicity (repeated dose) based on lack of sufficient data.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists
    - *Screening:* Not present on any screening lists
- Surrogate 4-methylbenzenesulfonic acid
    - U.S. EPA 2007
        - 4-Methylbenzenesulfonic acid was administered orally at doses of 0, 4, 20, 100, and 500 mg/kg/day to male and female Wistar rats 7 days per week for 28 days according to OECD Guideline 407. It should be noted that it was not specified whether the route was feed or gavage; however, as the administration was seven times per week, the route of administration is assumed to be in feed. The only effects reported were that the urine of both sexes in the high dose group was acidic and a higher saliva production was observed at the end of the study in males (it was no clear if the higher saliva production was in all males or only males receiving the high dose). However, these findings were not considered toxicologically relevant; therefore, the authors identified the NOEL as 100 mg/kg/day, which indicates that the NOAEL was 500 mg/kg/day.
            - Because this study is a 28-day study, the guidance values are tripled; therefore, the cutoff for repeated dose toxicity for this study is 300 mg/kg/day. Because the NOAEL is 500 mg/kg/day, the LOAEL is >500 mg/kg/day and therefore, 4-methylbenzenesulfonic acid is not classifiable under GHS.
- Based on the weight of evidence, a score of Data Gap was assigned. Although 4-methylbenzenesulfonic acid is not classifiable under GHS, information is not available regarding the toxicity of the other moiety (the active moiety as a dye) of the SYBR® Safe salt.

Neurotoxicity (N)

Group II Score (single dose) **(vH, H, M, or L):** DG

SYBR® Safe was assigned a score of Data Gap for neurotoxicity (single dose) due to lack of data.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists
    - *Screening:* Not present on any screening lists
- Not classified as a developmental neurotoxicant (Grandjean and Landrigan 2006, 2014).
- No data were identified.

Group II* Score (repeated dose) **(H, M, or L):** DG
SYBR® Safe was assigned a score of Data Gap for neurotoxicity (repeated dose) based on lack of data.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists
  - *Screening:* Not present on any screening lists
- Not classified as a developmental neurotoxicant (Grandjean and Landrigan 2006, 2014).
- No data were identified.

Skin Sensitization (SnS) Group II* Score (H, M, or L): *L*
SYBR® Safe was assigned a score of Low for skin sensitization based on modeled data on the cation and measured data on the anion. GreenScreen® criteria classify chemicals as a Low hazard for skin sensitization when adequate data are available and negative, there are no structural alerts, and they are not classifiable under GHS (CPA 2012a).

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists
  - *Screening:* Not present on any screening lists
- Surrogate 4-methylbenzenesulfonic acid
  - ECHA 2014
    - 4-Methylbenzenesulfonic acid was administered to female guinea pigs at a 0.2% dose as an intradermal induction followed by a 20% topical induction dose. A topical challenge dose of 10% was administered; no sensitization reaction was observed.
  - ESIS 2000
    - A guinea pig maximization test was performed according to OECD Guideline 406 and was determined to be negative for sensitization.
- OECD 2013
  - OECD Toolbox was used to predict the skin sensitization potential of SYBR® Safe. However, no appropriate surrogates were identified that have data on skin sensitization.
- Payne and Walsh 1994
  - SYBR® Safe does not have any known structural alerts for skin sensitization. See Appendix G for a full list of structural alerts for skin sensitization.
- Toxtree 2013
  - SYBR® Safe does not have any skin sensitization reactivity domain alerts. See Appendix D for model output.
- VEGA 2012
  - VEGA predicted that the SYBR® Safe cation was a skin sensitizer. However, the compound was out of the model application domain. Therefore, the predicted results are not reliable. See Appendix E for model output.
- Based on the weight of evidence, a score of Low was assigned. 3-Methylbenzenesulfonic acid was not a dermal sensitizer as demonstrated by measured data. The cation does not have any known structural alert or reactivity domain alerts for skin sensitization. Although VEGA predicted it to be a dermal sensitization, the prediction was not reliable due to lack of close structural analogs with relevant data. Confidence level was reduced due to the reliance on modeled data.

Respiratory Sensitization (SnR) Group II* Score (H, M, or L): DG
SYBR® Safe was assigned a score of Data Gap for respiratory sensitization based on a lack of data.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists
  - *Screening:* Not present on any screening lists
- No data were identified.

Skin Irritation/Corrosivity (IrS) Group II Score (vH, H, M, or L): *M*
SYBR® Safe was assigned a score of Moderate for skin irritation/corrosivity based on the most concentrated SYBR® Safe in DMSO on the market being self-classified as category 3 skin irritant. GreenScreen® criteria classify chemicals as a Moderate hazard for skin irritation/corrosivity when classified to GHS category 3 (CPA 2012a). Confidence level was adjusted due to lack of measured data and uncertainty with the presence of DMSO solvent on this classification.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists
  - *Screening:* Not present on any screening lists
- Life Technologies 2012b
  - A 10,000x SYBR® Safe concentrate in DMSO was self-classified as H316: Causes mild skin irritation, a category 3 skin irritant.

Eye Irritation/Corrosivity (IrE) Group II Score (vH, H, M, or L): *M*
SYBR® Safe was assigned a score of Moderate for eye irritation/corrosivity based on the most concentrated SYBR® Safe in DMSO on the market being self-classified as H320 eye irritant. GreenScreen® criteria classify chemicals as a Moderate hazard for eye irritation/corrosivity when associated with H320 (CPA 2012a). Confidence level was adjusted due to lack of measured data and uncertainty with the presence of DMSO solvent on this classification.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists
  - *Screening:* Not present on any screening lists
- Life Technologies 2012b
  - A 10,000x SYBR® Safe concentrate in DMSO was self-classified as H320: Causes eye irritation.

Ecotoxicity (Ecotox)

Acute Aquatic Toxicity (AA) Score (vH, H, M, or L): *vH*
SYBR® Safe was assigned a score of Very High for acute aquatic toxicity based on predicted 48h L/EC_{50} of 0.072 mg/L in daphnia for the SYBR® Safe cation. GreenScreen® criteria classify chemicals as a Very High hazard for acute aquatic toxicity when acute toxicity values are less than 1 mg/L (CPA 2012a).

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists
  - *Screening:* Not present on any screening lists
- Molecular probes Undated
  - An acute aquatic toxicity screening test was conducted with fathead minnow on SYBR® Safe (0.1 – 1% in 0.5x TBE). The LC_{50} (duration not reported but appears to be 96h) > 500 mg/L.
- U.S. EPA 2012c

    - The SYBR® Safe cation was classified to Neutral Organics ECOSAR classes. The most conservative L/EC_{50} values are 0.088 mg/L in fish (96h), 0.072 mg/L in daphnia (48h) and 0.236 mg/L in green algae (72h). However, the chemical may not be soluble enough to measure the predicted values for fish and green algae. See Appendix H for model output.
- Surrogate: 4-methylbenzenesulfonic acid
    - U.S. EPA 2007
        - A 96-hr LC_{50} of >325 mg/L was determined in *Leuciscus idus melanotus* (fish)
        - A 48-hr EC_{50} of >100 mg/L was determined in *Daphnia magna* (aquatic invertebrate)
        - A 72-hr EC_{50} of 73 mg/L was determined in *Selenastrum capricornutum* (green algae)
- Based on the weight of evidence, a score of Very High was assigned. Measured data were only available for fish, and the study was conducted with a 0.1 – 1% SYBR® Safe preparation. Therefore, the acute aquatic toxicity of the pure substance is unknown. ECOSAR predicted the lowest L/EC_{50} of 0.072 mg/L in daphnia, which does not exceed its predicted water solubility. Measured data on 4-methylbenzenesulfonic acid reported the lowest EC_{50} of 73 mg/L in algae. Therefore, the evaluation of this endpoint was based on the most conservative ECOSAR predictions. Confidence level was reduced due to the reliance on modeled data.

Chronic Aquatic Toxicity (CA) Score (vH, H, M, or L): *vH*
SYBR® Safe was assigned a score of Very High for chronic aquatic toxicity based on the predicted ChV of 0.013 – 0.138 mg/L for the cation. GreenScreen® criteria classify chemicals as a Very High hazard for chronic aquatic toxicity when chronic NOECs are no greater than 0.1 mg/L (CPA 2012a).

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists
    - *Screening:* Not present on any screening lists
- U.S. EPA 2012c
    - The SYBR® Safe cation was classified to Neutral Organics ECOSAR classes. The most conservative ChV values are 0.013 mg/L in fish, 0.019 mg/L in daphnia and 0.138 mg/L in green algae. However, the chemical may not be soluble enough to measure the predicted value for green algae. See Appendix H for model output.
- Based on the weight of evidence, a score of Very High was assigned. No measured data were available either for the whole SYBR® Safe chemical or for the anion 4-methylbenzenesulfonic acid. Measured and predicted acute toxicity values discussed above indicate that the cation is much more toxic to the aquatic environment than the anion. Therefore, ECOSAR modeling was only performed with the cation. Confidence level was reduced due to the reliance on modeled data.

Environmental Fate (Fate)

Persistence (P) Score (vH, H, M, L, or vL): *vH*
SYBR® Safe was assigned a score of Very High for persistence based on predicted degradation half-life of 542 days in sediment (its major partitioning compartment) for the cation. GreenScreen® criteria classify chemicals as a Very High hazard for persistence when the degradation half-life is greater than 180 days in soil or sediment (CPA 2012a).

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists

- *Screening:* Not present on any screening lists
- U.S. EPA 2012a
  - The BIOWIN model predicted that the SYBR® Safe cation is not readily biodegradable. Fugacity model predicted that 50.5% would partition to sediment with a half-life of 542 days, 47.5% would partition to soil with a half-live of 120 days, and 1.97% would partition to water with a half-life of 60 days. See Appendix I for model output.
- Surrogate: 4-methylbenzenesulfonic acid
  - ESIS 2000
    - An aerobic biodegradation test was performed according to OECD Guideline 302B and the chemical was determined to be 90% degraded after 10 days
    - A second aerobic biodegradation test was performed according to OECD Guideline 302 B and the chemical was determined to be 79% degraded after 25 days
- Based on the weight of evidence, although the anion 4-methylbenzenesulfonic acid was demonstrated to be readily biodegradable passing the 10-day window criteria, the cation was not predicted to be readily biodegradable. The cation was expected to mainly partition to the sediment and soil compartments with a degradation half-life of 542 days in sediment. Therefore, assessment was based on this value. Confidence level was reduced due to the reliance on modeled data.

Bioaccumulation (B) Score (vH, H, M, L, or vL): *L*
SYBR® Safe was assigned a score of Low for bioaccumulation based on the biggest predicted BCF of 165.2 for the cation. GreenScreen® criteria classify chemicals as a Low hazard for bioaccumulation when BCF values are between 100 and 500 (CPA 2012a).

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists
  - *Screening:* Not present on any screening lists
- U.S. EPA 2012a
  - The cation has a predicted BCF of 165.2 based on an estimated log K_{ow} of 3.37. See Appendix I for model output.
  - The anion has a predicted BCF of 0.9086 based on an estimated log K_{ow} of -0.62. See Appendix I for model output.

Physical Hazards (Physical)

Reactivity (Rx) Score (vH, H, M, or L): *L*
SYBR® Safe was assigned a score of Low for reactivity based on measured data on SYBR® Safe (0.1 – 1%) in 0.5x TBE and HMIS rating of 0 on SYBR® Safe 10,000 concentrate in DMSO (0.1 – 1% active). GreenScreen® criteria classify chemicals as a Low hazard for reactivity when they are not explosive, and there are no data indicating that they are reactive otherwise (CPA 2012a).

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists
  - *Screening:* Not present on any screening lists
- Molecular Probes Undated
  - SYBR® Safe (0.1 – 1%) in 0.5x TBE was not reactive (no reactivity detected for both cyanide and sulfide, EPA 9010B/9040A Method)) or corrosive (detected by Corrositex Test method)
- Life Technologies 2012a
  - SYBR® Safe (0.1 – 1%) in 0.5x TBE has an HMIS reactivity score of 0 ("Materials that

are normally stable, even under fire conditions, and will not react with water, polymerize, decompose, condense, or self-react. Non-explosives")

- Life Technologies 2012b
  - SYBR® Safe 10,000 concentrate in DMSO (0.1 – 1% active) has an HMIS reactivity score of 0 ("Materials that are normally stable, even under fire conditions, and will not react with water, polymerize, decompose, condense, or self-react. Non-explosives")
- Based on the weight of evidence, a score of Low as assigned. The presumed most concentrated form of SYBR® Safe on the market was not considered to be reactive. Confidence was reduced as no data were available for the pure SYBR® Safe compound.

Flammability (F) Score (vH, H, M, or L): *L*

SYBR® Safe was assigned a score of Low for flammability based on lack of flammability for the SYBR® Safe (0.1 – 1%) in 0.5x TBE. GreenScreen® criteria classify chemicals as a Low hazard for flammability when the flash point of a liquid is greater than 93ºC, making it not classifiable as a flammable liquid under GHS (CPA 2012a).

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists
  - *Screening:* Not present on any screening lists
- Life Technologies 2012a
  - SYBR® Safe (0.1 – 1%) in 0.5x TBE has an HMIS flammability score of 0 ("Materials that will not burn").
- Life Technologies 2012b
  - SYBR® Safe 10,000 concentrate in DMSO (0.1 – 1% active) has an HMIS flammability score of 2 ("Materials which must be moderately heated or exposed to high ambient temperatures before ignition will occur. Includes liquids having a flashpoint at or above 100ºF but below 200ºF"). This is most likely due to the flammable solvent DMSO.
- Based on the weight of evidence, a score of Low was assigned. Confidence level was reduced as no data were available for the pure SYBR® Safe compound

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APPENDIX A: Hazard Benchmark Acronyms
(in alphabetical order)

(AA) Acute Aquatic Toxicity

(AT) Acute Mammalian Toxicity

(B) Bioaccumulation

(C) Carcinogenicity

(CA) Chronic Aquatic Toxicity

(Cr) Corrosion/ Irritation (Skin/ Eye)

(D) Developmental Toxicity

(E) Endocrine Activity

(F) Flammability

(IrE) Eye Irritation/Corrosivity

(IrS) Skin Irritation/Corrosivity

(M) Mutagenicity and Genotoxicity

(N) Neurotoxicity

(P) Persistence

(R) Reproductive Toxicity

(Rx) Reactivity

(SnS) Sensitization- Skin

(SnR) Sensitization- Respiratory

(ST) Systemic/Organ Toxicity

APPENDIX B: Results of Automated GreenScreen® Score Calculation for SYBR® Safe (CAS #1030826-36-8)

TOXSERVICES
TOXICOLOGY RISK ASSESSMENT CONSULTING

GREEN SCREEN FOR SAFER CHEMICALS

GreenScreen® Score Inspector

Table 1: Hazard Table

			Group I Human					Group II and II* Human								Ecotox		Fate		Physical	
			Carcinogenicity	Mutagenicity/Genotoxicity	Reproductive Toxicity	Developmental Toxicity	Endocrine Activity	Acute Toxicity	Systemic Toxicity		Neurotoxicity		Skin Sensitization*	Respiratory Sensitization*	Skin Irritation	Eye Irritation	Acute Aquatic Toxicity	Chronic Aquatic Toxicity	Persistence	Bioaccumulation	Reactivity	Flammability
Table 2: Chemical Details									S	R *	S	R *	*	*								
Inorganic Chemical?	**Chemical Name**	**CAS#**	**C**	**M**	**R**	**D**	**E**	**AT**	**STs**	**STr**	**Ns**	**Nr**	**SNS***	**SNR***	**IrS**	**IrE**	**AA**	**CA**	**P**	**B**	**Rx**	**F**
No	**SYBR® Safe**	**1030826-36-8**	*L*	**M**	DG	DG	DG	DG	*M*	DG	DG	DG	*L*	DG	*M*	*M*	*vH*	*vH*	*vH*	*L*	*L*	*L*

Table 3: Hazard Summary Table

Benchmark	a	b	c	d	e	f	g
1	No	No	Yes	No	No		
2	STOP						
3	STOP						
4	STOP						

Table 4

Chemical Name	Preliminary GreenScreen® Benchmark Score
SYBR® Safe	1

Note: Chemical has not undergone a data gap assessment. Not a Final GreenScreen™ Score

Table 6

Chemical Name	Final GreenScreen® Benchmark Score
SYBR® Safe	1

After Data gap Assessment
Note: No Data gap Assessment Done if Preliminary GS Benchmark Score is 1.

Table 5: Data Gap Assessment Table

Datagap Criteria	a	b	c	d	e	f	g	h	i	j	bm4	End Result
1												1
2												
3												
4												

APPENDIX C: OncoLogic Output for SYBR® Safe (CAS #1030826-36-8)

OncoLogic Justification Report

SUMMARY :

CODE NUMBER : SYBRSafe

SUBSTANCE ID :

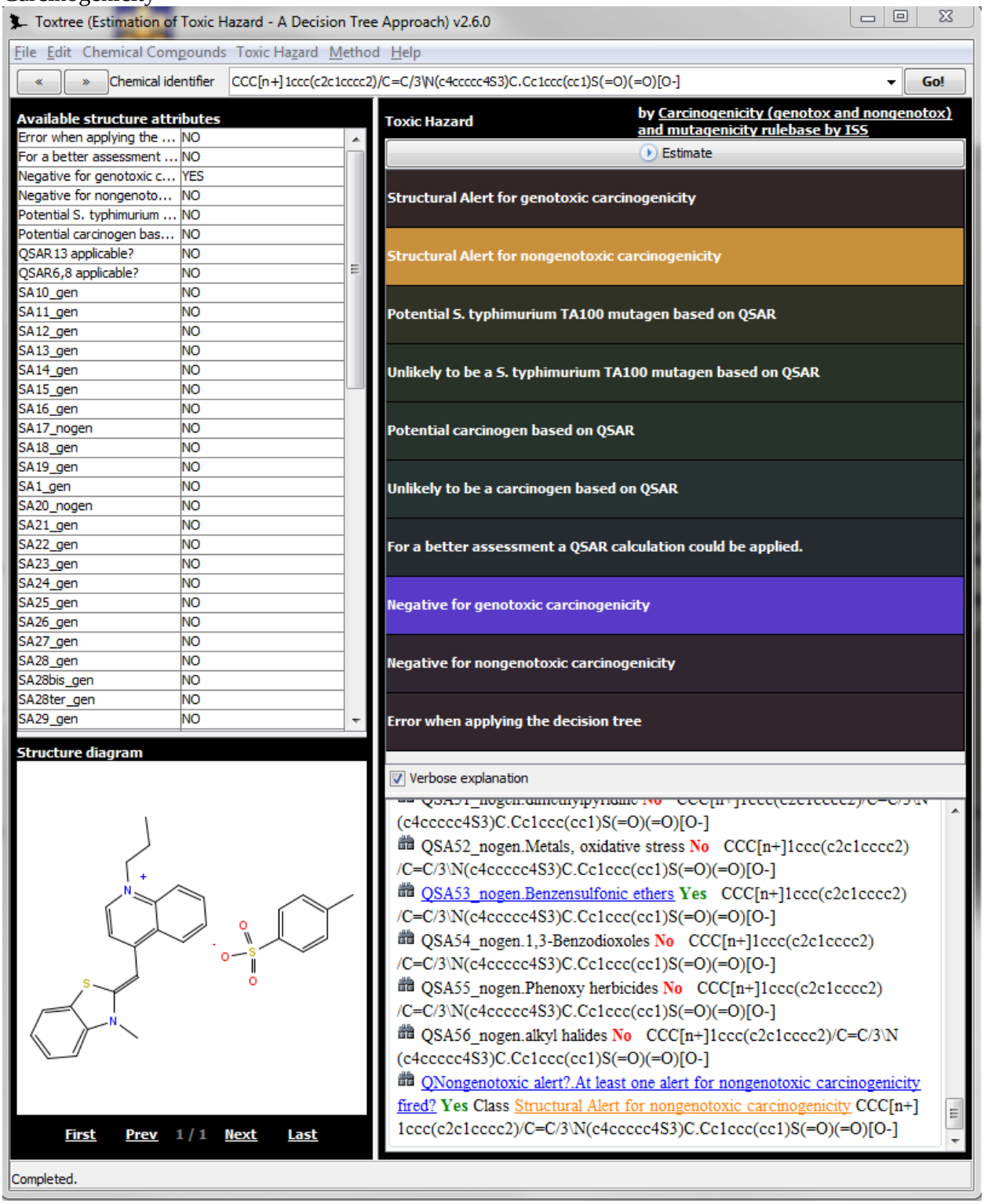
JUSTIFICATION:

The current version of OncoLogic can only evaluate aromatic amines with the particular ring skeleton you have described for which carcinogenicity concern levels have been established based on actual carcinogenicity data, supportive evidence (such as mutagenicity), and/or structure-activity relationship (SAR) analysis. These compounds can be identified by their name or CAS number.

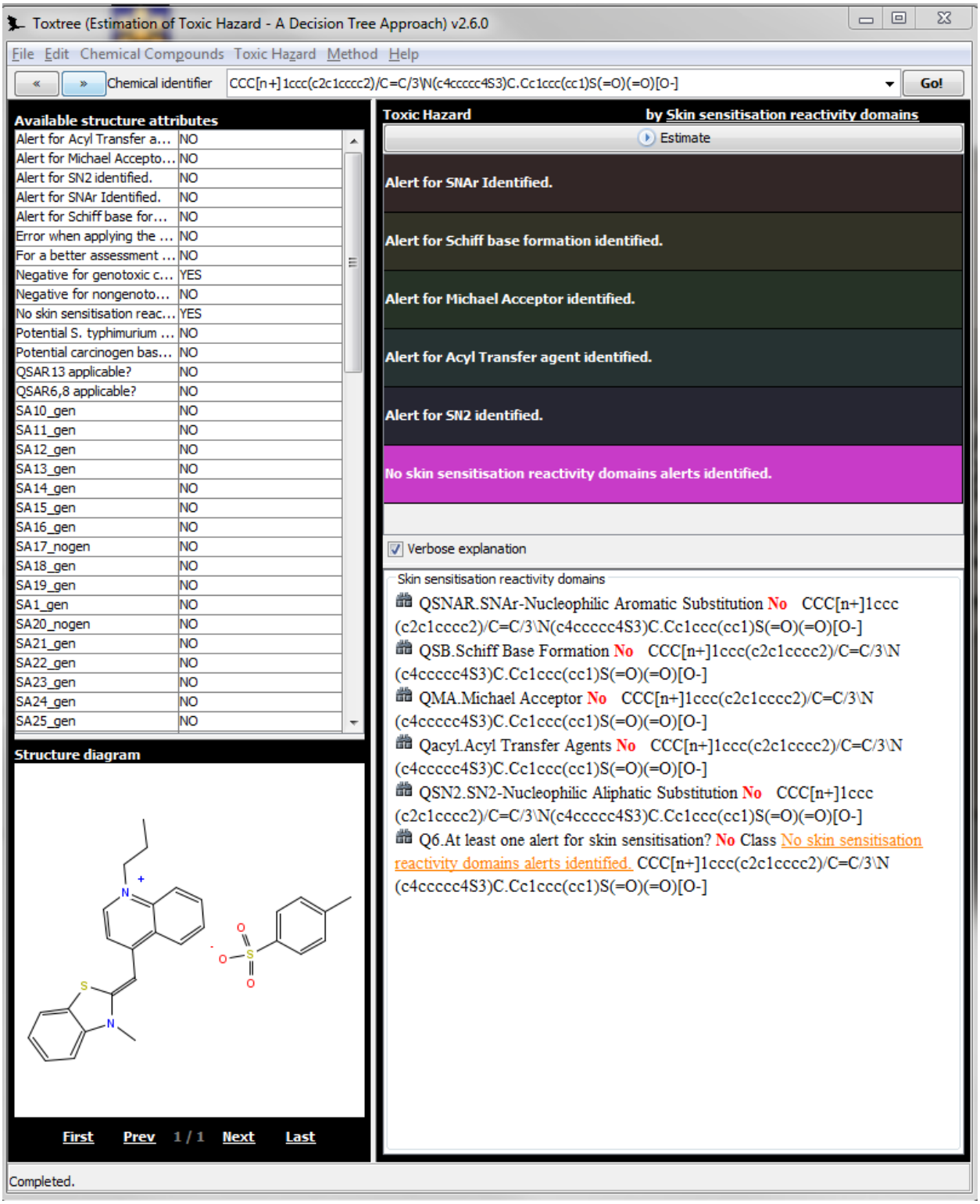
If the name or CAS number of the compound you wish to evaluate cannot be found, then it cannot be evaluated at this time.

APPENDIX D: Toxtree Output for SYBR® Safe (CAS #1030826-36-8)

Carcinogenicity



Skin Sensitization



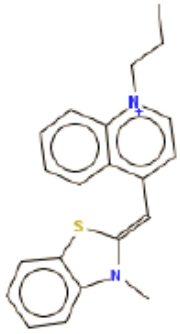
APPENDIX E: VEGA Output for SYBR® Safe (CAS #1030826-36-8)

Carcinogenicity CAESAR
Cation:

VEGA Carcinogenicity model (CAESAR) (version 2.1.8) page 1

1. Prediction Summary

Prediction for compound 1 (Molecule 1)



Prediction: Reliability:

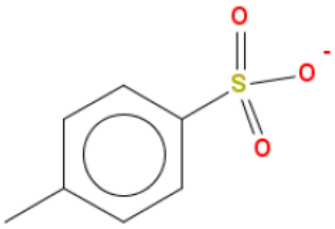
Model assessment: Prediction is Carcinogen, but the result may be not reliable. Careful check of the information given in the following section should be done, paying particular attention to the following issues:
- only moderately similar compounds with known experimental value in the training set have been found
- some similar molecules found in the training set have experimental values that disagree with the predicted value
- accuracy of prediction for similar molecules found in the training set is not optimal
- a prominent number of atom centered fragments of the compound have not been found in the compounds of the training set or are rare fragments

Compound: 1
Compound SMILES: c1ccc2c(c1)c(cc[n+]2CCC)C=C4N(c3ccccc3S4)C
Experimental value: -
Prediction: Carcinogen
Carcinogen: 0.76
NON-Carcinogen: 0.24
Structural Alerts: -
Reliability: Compound is out of model Applicability Domain
Remarks for the prediction:
none

Anion:

1. Prediction Summary

Prediction for compound 1 (Molecule 1)



Prediction: Reliability:

Model assessment: Prediction is NON-Carcinogen, but the result may be not reliable. Careful check of the information given in the following section should be done, paying particular attention to the following issues:
- similar molecules found in the training set have experimental values that disagree with the predicted value
- accuracy of prediction for similar molecules found in the training set is not adequate
- a prominent number of atom centered fragments of the compound have not been found in the compounds of the training set or are rare fragments
- predicted value disagrees with experimental values of training set compounds laying in the same neuron

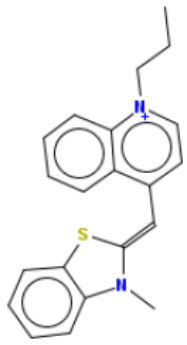
Compound: 1
Compound SMILES: O=S(=O)([O-])c1ccc(cc1)C
Experimental value: -
Prediction: NON-Carcinogen
Carcinogen: 0.36
NON-Carcinogen: 0.64
Structural Alerts: -
Reliability: Compound is out of model Applicability Domain
Remarks for the prediction:
none

Skin sensitization

Note: only the cationic structure was evaluated, as the program does not allow simultaneous evaluation of both ions and the anionic ion was not a skin sensitizer based on measured data.

1. Prediction Summary

Prediction for compound 1 (Molecule 1)



Prediction: Reliability:

Model assessment: Prediction is Sensitizer, but the result may be not reliable. Careful check of the information given in the following section should be done, paying particular attention to the following issues:
- only moderately similar compounds with known experimental value in the training set have been found
- similar molecules found in the training set have experimental values that disagree with the predicted value
- accuracy of prediction for similar molecules found in the training set is not adequate
- a prominent number of atom centered fragments of the compound have not been found in the compounds of the training set or are rare fragments

Compound: 1
Compound SMILES: c1ccc2c(c1)c(cc[n+]2CCC)C=C4N(c3ccccc3S4)C
Experimental value: -
Prediction: Sensitizer
O(Active): 1
O(Inactive): 0
Reliability: Compound is out of model Applicability Domain
Remarks for the prediction:
none

APPENDIX F: T.E.S.T. Output for SYBR® Safe (CAS #1030826-36-8)

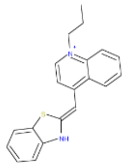
Only the cation was evaluated, as the program does not allow evaluation of both ions simultaneously and data were available for the anion.

Predicted Developmental Toxicity for C20H19N2S_1409243699985 from Consensus method

Prediction results		
Endpoint	**Experimental value**	**Predicted value**
Developmental Toxicity value	N/A	0.35
Developmental Toxicity result	N/A	Developmental NON-toxicant

Individual Predictions	
Method	**Predicted value**
Hierarchical clustering	0.40
Single model	0.63
FDA	0.02
Nearest neighbor	N/A

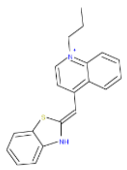
Test chemical

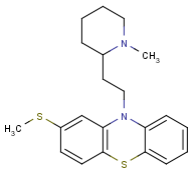


Descriptor values for test chemical

Predictions for the test chemical and for the most similar chemicals in the external test set
If *similar* test set chemicals were predicted well relative to the entire test set, one has greater confidence in the predicted value.

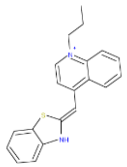
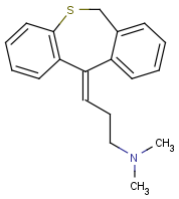
Prediction statistics for similar chemicals		
Concordance	**Sensitivity**	**Specificity**
1.00 (1 out of 1)	1.00 (1 out of 1)	N/A

CAS	Structure	Similarity Coefficient	Experimental value	Predicted value
C20H19N2S_1409243699985 (test chemical)			N/A	0.35

50-52-2		0.64	1.00	0.88

Predictions for the test chemical and for the most similar chemicals in the training set
If the predicted value matches the experimental values for similar chemicals in the training set (and the similar chemicals were predicted well), one has greater confidence in the predicted value.

Prediction statistics for similar chemicals		
Concordance	**Sensitivity**	**Specificity**
1.00 (1 out of 1)	1.00 (1 out of 1)	N/A

CAS	Structure	Similarity Coefficient	Experimental value	Predicted value
C20H19N2S_1409243699985 (test chemical)			N/A	0.35
113-53-1		0.59	1.00	0.79

Only the cation was evaluated, as the program does not allow both ions to be evaluated simultaneously, and data were available for the anion.

Predicted Oral rat LD50 for C21H21N2S_1409253289765 from Consensus method

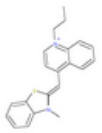
Prediction results

Endpoint	Experimental value	Predicted value
Oral rat LD_{50} -Log10(mol/kg)	N/A	3.29
Oral rat LD_{50} mg/kg	N/A	172.80

Individual Predictions

Method	Predicted value -Log10(mol/kg)
Hierarchical clustering	3.04
FDA	3.92
Nearest neighbor	2.89

Test chemical



Descriptor values for test chemical

Predictions for the test chemical and for the most similar chemicals in the external test set

Note: No chemicals in the test set exceed a minimum similarity coefficient of 0.5 for comparison purposes

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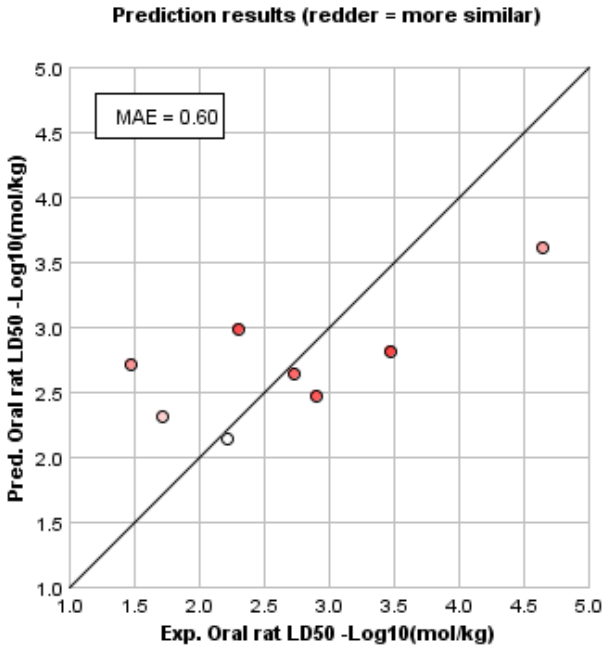
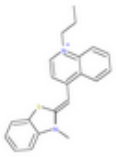
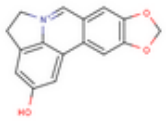
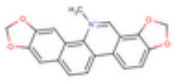
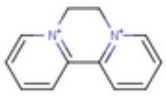
Predictions for the test chemical and for the most similar chemicals in the training set

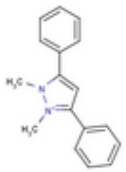
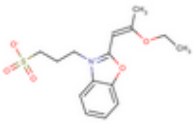
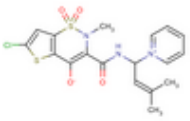
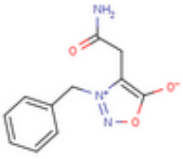
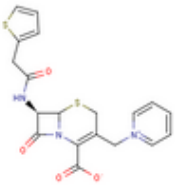
If the predicted value matches the experimental values for similar chemicals in the training set (and the similar chemicals were predicted well), one has greater confidence in the predicted value.



Test set chemicals	MAE*
Entire set	0.34
Similarity coefficient ≥ 0.5	0.60

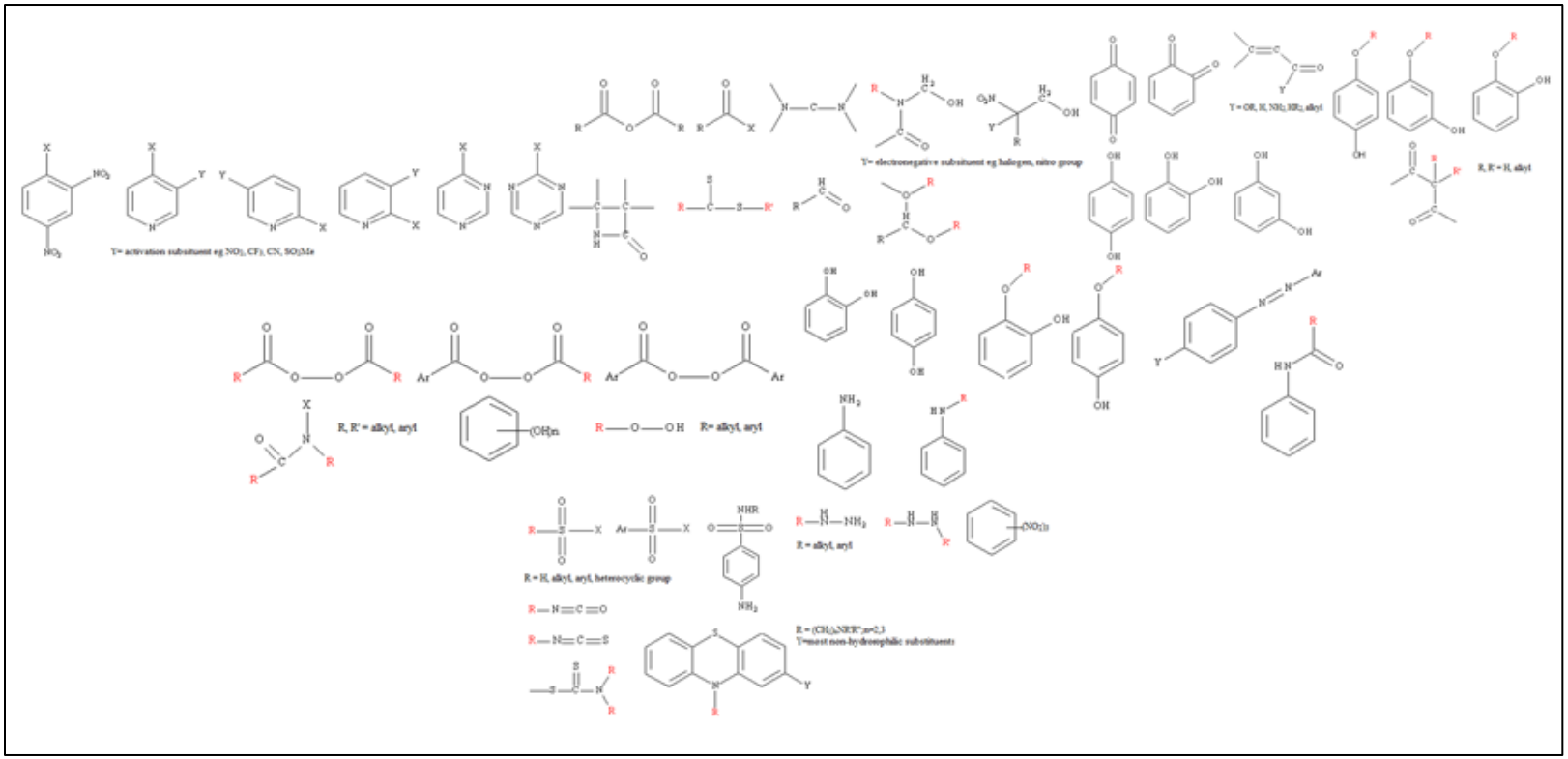
*Mean absolute error in -Log10(mol/kg)

CAS	Structure	Similarity Coefficient	Experimental value -Log10(mol/kg)	Predicted value -Log10(mol/kg)
C21H21N2S_1409253289765 (test chemical)			N/A	3.29
2121-12-2		0.86	3.47	2.81
2447-54-3		0.85	2.30	2.98
2764-72-9		0.82	2.90	2.47

49866-87-7		0.79	2.72	2.64
86701-22-6		0.71	1.47	2.72
123252-99-3		0.68	4.64	3.61
14504-15-5		0.59	1.72	2.32
50-59-9		0.50	2.22	2.14

APPENDIX G: Known Structural Alerts for Skin Sensitization

Below are known structural alerts for skin sensitizers (Payne and Walsh 1994). SYBR® does not possess any known structural alert.



APPENDIX H: ECOSAR Modeling Results for SYBR® Safe (CAS #1030826-36-8)

Note: Only the cation was evaluated, as the anion has measured data as 4-methylbenzenesulfonic acid.

ECOSAR Version 1.11 Results Page

SMILES : c15c(c(C=C3N(C)c4c(cccc4)S3)ccn1CCC)cccc5
CHEM :
CAS Num:
ChemID1:
MOL FOR: C21 H21 N2 S1
MOL WT : 333.47
Log Kow: 5.887 (EPISuite Kowwin v1.68 Estimate)
Log Kow: (User Entered)
Log Kow: (PhysProp DB exp value - for comparison only)
Melt Pt: (User Entered for Wat Sol estimate)
Melt Pt: (deg C, PhysProp DB exp value for Wat Sol estimate)
Wat Sol: 0.07308 (mg/L, EPISuite WSKowwin v1.43 Estimate)
Wat Sol: (User Entered)
Wat Sol: (PhysProp DB exp value)

Values used to Generate ECOSAR Profile

Log Kow: 5.887 (EPISuite Kowwin v1.68 Estimate)
Wat Sol: 0.07308 (mg/L, EPISuite WSKowwin v1.43 Estimate)

Available Measured Data from ECOSAR Training Set

No Data Available

ECOSAR v1.1 Class-specific Estimations

Neutral Organics

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Neutral Organics	: Fish	96-hr	LC50	0.088 *
Neutral Organics	: Daphnid	48-hr	LC50	0.072
Neutral Organics	: Green Algae	96-hr	EC50	0.236 *
Neutral Organics	: Fish		ChV	0.013
Neutral Organics	: Daphnid		ChV	0.019
Neutral Organics	: Green Algae		ChV	0.138 *
Neutral Organics	: Fish (SW)	96-hr	LC50	0.114 *
Neutral Organics	: Mysid	96-hr	LC50	0.006
Neutral Organics	: Fish (SW)		ChV	0.133 *
Neutral Organics	: Mysid (SW)		ChV	0.000166
Neutral Organics	: Earthworm	14-day	LC50	229.177 *

Note: * = asterisk designates: Chemical may not be soluble enough to measure this predicted effect. If the effect level exceeds the water solubility by 10X, typically no effects at saturation (NES) are reported.

Class Specific LogKow Cut-Offs

If the log Kow of the chemical is greater than the endpoint specific cut-offs presented below, then no effects at saturation are expected for those endpoints.

Neutral Organics:

Maximum LogKow: 5.0 (Fish 96-hr LC50; Daphnid LC50, Mysid LC50)
Maximum LogKow: 6.0 (Earthworm LC50)
Maximum LogKow: 6.4 (Green Algae EC50)
Maximum LogKow: 8.0 (ChV)

APPENDIX I: EPISuite Modeling Results for SYBR® Safe (CAS #1030826-36-8)

Cation:
CAS Number: (null)
SMILES : CCCn1(H)ccc(c2c1cccc2)C=C3N(c4ccccc4S3)C
CHEM :
MOL FOR: C21 H22 N2 S1
MOL WT : 334.48
---------- EPI SUMMARY (v4.11) ----------

Physical Property Inputs:
Log Kow (octanol-water): ------
Boiling Point (deg C) : ------
Melting Point (deg C) : ------
Vapor Pressure (mm Hg) : ------
Water Solubility (mg/L): ------
Henry LC (atm-m3/mole) : ------

Log Octanol-Water Partition Coef (SRC):
Log Kow (KOWWIN v1.68 estimate) = 3.37

Boiling Pt, Melting Pt, Vapor Pressure Estimations (MPBPVP v1.43):
Boiling Pt (deg C): 467.98 (Adapted Stein & Brown method)
Melting Pt (deg C): 197.64 (Mean or Weighted MP)
VP(mm Hg,25 deg C): 2.66E-009 (Modified Grain method)
VP (Pa, 25 deg C) : 3.55E-007 (Modified Grain method)
Subcooled liquid VP: 1.75E-007 mm Hg (25 deg C, Mod-Grain method)
: 2.34E-005 Pa (25 deg C, Mod-Grain method)

Water Solubility Estimate from Log Kow (WSKOW v1.42):
Water Solubility at 25 deg C (mg/L): 10.18
log Kow used: 3.37 (estimated)
no-melting pt equation used

Water Sol Estimate from Fragments:
Wat Sol (v1.01 est) = 0.013193 mg/L

ECOSAR Class Program (ECOSAR v1.11):
Class(es) found: Neutral Organics

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:
Bond Method: 4.89E-013 atm-m3/mole (4.96E-008 Pa-m3/mole)
Group Method: Incomplete
For Henry LC Comparison Purposes:
User-Entered Henry LC: not entered
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:
HLC: 1.150E-010 atm-m3/mole (1.165E-005 Pa-m3/mole)
VP: 2.66E-009 mm Hg (source: MPBPVP)
WS: 10.2 mg/L (source: WSKOWWIN)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:
Log Kow used: 3.37 (KowWin est)
Log Kaw used: -10.699 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate): 14.069
Log Koa (experimental database): None

Probability of Rapid Biodegradation (BIOWIN v4.10):
Biowin1 (Linear Model) : 0.3830
Biowin2 (Non-Linear Model) : 0.0186
Expert Survey Biodegradation Results:
Biowin3 (Ultimate Survey Model): 2.2052 (months)
Biowin4 (Primary Survey Model) : 3.0772 (weeks)
MITI Biodegradation Probability:
Biowin5 (MITI Linear Model) : -0.1798
Biowin6 (MITI Non-Linear Model): 0.0029
Anaerobic Biodegradation Probability:
Biowin7 (Anaerobic Linear Model): -1.3738
Ready Biodegradability Prediction: NO

Hydrocarbon Biodegradation (BioHCwin v1.01):
Structure incompatible with current estimation method!

Sorption to aerosols (25 Dec C)[AEROWIN v1.00]:
Vapor pressure (liquid/subcooled): 2.33E-005 Pa (1.75E-007 mm Hg)
Log Koa (Koawin est): 14.069
Kp (particle/gas partition coef. (m3/ug)):
Mackay model : 0.129
Octanol/air (Koa) model: 28.8
Fraction sorbed to airborne particulates (phi):
Junge-Pankow model : 0.823
Mackay model : 0.911
Octanol/air (Koa) model: 1

Atmospheric Oxidation (25 deg C) [AopWin v1.92]:
Hydroxyl Radicals Reaction:
OVERALL OH Rate Constant = 84.4012 E-12 cm3/molecule-sec
Half-Life = 0.127 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life = 1.521 Hrs
Ozone Reaction:
OVERALL Ozone Rate Constant = 2.100000 E-17 cm3/molecule-sec
Half-Life = 0.546 Days (at 7E11 mol/cm3)
Half-Life = 13.097 Hrs
Fraction sorbed to airborne particulates (phi):
0.867 (Junge-Pankow, Mackay avg)
1 (Koa method)
Note: the sorbed fraction may be resistant to atmospheric oxidation

Soil Adsorption Coefficient (KOCWIN v2.00):
Koc : 6.169E+005 L/kg (MCI method)

Log Koc: 5.790 (MCI method)
Koc : 529.6 L/kg (Kow method)
Log Koc: 2.724 (Kow method)

Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]:
Rate constants can NOT be estimated for this structure!

Bioaccumulation Estimates (BCFBAF v3.01):
Log BCF from regression-based method = 0.500 (BCF = 3.162 L/kg wet-wt)
Log Biotransformation Half-life (HL) = 0.0732 days (HL = 1.184 days)
Log BCF Arnot-Gobas method (upper trophic) = 2.218 (BCF = 165.2)
Log BAF Arnot-Gobas method (upper trophic) = 2.218 (BAF = 165.3)
log Kow used: 3.37 (estimated)

Volatilization from Water:
Henry LC: 4.89E-013 atm-m3/mole (estimated by Bond SAR Method)
Half-Life from Model River: 2.19E+009 hours (9.124E+007 days)
Half-Life from Model Lake : 2.389E+010 hours (9.953E+008 days)

Removal In Wastewater Treatment:
Total removal: 10.40 percent
Total biodegradation: 0.16 percent
Total sludge adsorption: 10.23 percent
Total to Air: 0.00 percent
(using 10000 hr Bio P,A,S)

Removal In Wastewater Treatment:
Total removal: 21.44 percent
Total biodegradation: 11.82 percent
Total sludge adsorption: 9.62 percent
Total to Air: 0.00 percent
(using Biowin/EPA draft method)

Level III Fugacity Model:

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	9.2e-006	2.47	1000
Water	1.97	1.44e+003	1000
Soil	47.5	2.88e+003	1000
Sediment	50.5	1.3e+004	0

Persistence Time: 5.54e+003 hr

Anion:

CAS Number: (null)
SMILES : Cc1ccc(cc1)S(=O)(=O)O
CHEM :
MOL FOR: C7 H8 O3 S1
MOL WT : 172.20
---------- EPI SUMMARY (v4.11) ----------

Physical Property Inputs:
Log Kow (octanol-water): ------
Boiling Point (deg C) : ------
Melting Point (deg C) : ------
Vapor Pressure (mm Hg) : ------
Water Solubility (mg/L): ------
Henry LC (atm-m3/mole) : ------

Log Octanol-Water Partition Coef (SRC):
Log Kow (KOWWIN v1.68 estimate) = -0.62

Boiling Pt, Melting Pt, Vapor Pressure Estimations (MPBPVP v1.43):
Boiling Pt (deg C): 331.69 (Adapted Stein & Brown method)
Melting Pt (deg C): 101.76 (Mean or Weighted MP)
VP(mm Hg,25 deg C): 2.9E-006 (Modified Grain method)
VP (Pa, 25 deg C) : 0.000386 (Modified Grain method)
MP (exp database): 104.5 deg C
BP (exp database): 140 @ 20 mm Hg deg C
Subcooled liquid VP: 1.74E-005 mm Hg (25 deg C, Mod-Grain method)
: 0.00231 Pa (25 deg C, Mod-Grain method)

Water Solubility Estimate from Log Kow (WSKOW v1.42):
Water Solubility at 25 deg C (mg/L): 2.023e+005
log Kow used: -0.62 (estimated)
no-melting pt equation used
Water Sol (Exper. database match) = 6.2e+005 mg/L (deg C)
Exper. Ref: BUDAVARI,S (1989)

Water Sol Estimate from Fragments:
Wat Sol (v1.01 est) = 1e+006 mg/L

ECOSAR Class Program (ECOSAR v1.11):
Class(es) found: Neutral Organics-acid

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:
Bond Method : 2.78E-009 atm-m3/mole (2.81E-004 Pa-m3/mole)
Group Method: Incomplete
For Henry LC Comparison Purposes:
User-Entered Henry LC: not entered
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:

HLC: 3.248E-012 atm-m3/mole (3.291E-007 Pa-m3/mole)
VP: 2.9E-006 mm Hg (source: MPBPVP)
WS: 2.02E+005 mg/L (source: WSKOWWIN)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:
Log Kow used: -0.62 (KowWin est)
Log Kaw used: -6.944 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate): 6.324
Log Koa (experimental database): None

Probability of Rapid Biodegradation (BIOWIN v4.10):
Biowin1 (Linear Model) : 0.4965
Biowin2 (Non-Linear Model) : 0.5280
Expert Survey Biodegradation Results:
Biowin3 (Ultimate Survey Model): 2.8860 (weeks)
Biowin4 (Primary Survey Model) : 3.5524 (days-weeks)
MITI Biodegradation Probability:
Biowin5 (MITI Linear Model) : 0.2963
Biowin6 (MITI Non-Linear Model): 0.2729
Anaerobic Biodegradation Probability:
Biowin7 (Anaerobic Linear Model): -0.2942
Ready Biodegradability Prediction: NO

Hydrocarbon Biodegradation (BioHCwin v1.01):
Structure incompatible with current estimation method!

Sorption to aerosols (25 Dec C)[AEROWIN v1.00]:
Vapor pressure (liquid/subcooled): 0.00232 Pa (1.74E-005 mm Hg)
Log Koa (Koawin est): 6.324
Kp (particle/gas partition coef. (m3/ug)):
Mackay model : 0.00129
Octanol/air (Koa) model: 5.18E-007
Fraction sorbed to airborne particulates (phi):
Junge-Pankow model : 0.0446
Mackay model : 0.0938
Octanol/air (Koa) model: 4.14E-005

Atmospheric Oxidation (25 deg C) [AopWin v1.92]:
Hydroxyl Radicals Reaction:
OVERALL OH Rate Constant = 1.3643 E-12 cm3/molecule-sec
Half-Life = 7.840 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life = 94.080 Hrs
Ozone Reaction:
No Ozone Reaction Estimation
Fraction sorbed to airborne particulates (phi):
0.0692 (Junge-Pankow, Mackay avg)
4.14E-005 (Koa method)
Note: the sorbed fraction may be resistant to atmospheric oxidation

Soil Adsorption Coefficient (KOCWIN v2.00):
Koc : 16.06 L/kg (MCI method)
Log Koc: 1.206 (MCI method)
Koc : 3.821 L/kg (Kow method)
Log Koc: 0.582 (Kow method)

Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]:
Rate constants can NOT be estimated for this structure!

Bioaccumulation Estimates (BCFBAF v3.01):
Log BCF from regression-based method = 0.500 (BCF = 3.162 L/kg wet-wt)
Log Biotransformation Half-life (HL) = -1.4151 days (HL = 0.03845 days)
Log BCF Arnot-Gobas method (upper trophic) = -0.042 (BCF = 0.9086)
Log BAF Arnot-Gobas method (upper trophic) = -0.042 (BAF = 0.9086)
log Kow used: -0.62 (estimated)

Volatilization from Water:
Henry LC: 2.78E-009 atm-m3/mole (estimated by Bond SAR Method)
Half-Life from Model River: 2.764E+005 hours (1.152E+004 days)
Half-Life from Model Lake : 3.015E+006 hours (1.256E+005 days)

Removal In Wastewater Treatment:
Total removal: 1.85 percent
Total biodegradation: 0.09 percent
Total sludge adsorption: 1.76 percent
Total to Air: 0.00 percent
(using 10000 hr Bio P,A,S)

Removal In Wastewater Treatment:
Total removal: 75.06 percent
Total biodegradation: 74.45 percent
Total sludge adsorption: 0.62 percent
Total to Air: 0.00 percent
(using Biowin/EPA draft method)

Level III Fugacity Model:

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	0.0596	188	1000
Water	28	360	1000
Soil	71.9	720	1000
Sediment	0.0701	3.24e+003	0

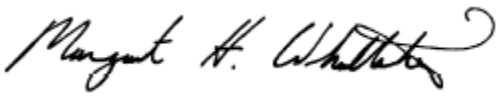
Persistence Time: 658 hr

Licensed GreenScreen® Profilers

SYBR® Safe GreenScreen® Evaluation Prepared By:

Bingxuan Wang, Ph.D.
Toxicologist
ToxServices LLC

SYBR® Safe GreenScreen® Evaluation QC'd By:



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