

**SCREENING-LEVEL HAZARD CHARACTERIZATION
OF HIGH PRODUCTION VOLUME CHEMICALS**

CHEMICAL CATEGORY NAME

Phenolic Benzotriazoles

SPONSORED CHEMICALS

2-(2'-Hydroxy-5'-methylphenyl) benzotriazole	(CAS No. 2440-22-4)
2-(2'-Hydroxy-5'-octylphenyl) benzotriazole	(CAS No. 3147-75-9)
2-(2'-Hydroxy-3',5'-di- <i>t</i> -amylphenyl) benzotriazole	(CAS No. 25973-55-1)
2-(2H-Benzotriazol-2-yl)-4,6-bis(1-methyl-1-phenylethyl) phenol	(CAS No. 70321-86-7)

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SCREENING-LEVEL HAZARD CHARACTERIZATION OF HIGH PRODUCTION VOLUME CHEMICALS

The High Production Volume (HPV) Challenge Program¹ is a voluntary initiative aimed at developing and making publicly available screening-level health and environmental effects information on chemicals manufactured in or imported into the United States in quantities greater than one million pounds per year. In the Challenge Program, producers and importers of HPV chemicals voluntarily sponsor chemicals; sponsorship entails the identification and initial assessment of the adequacy of existing toxicity data/information, conducting new testing if adequate data do not exist, and making both new and existing data and information available to the public. Each complete data submission contains data on 18 internationally agreed to “SIDS” (Screening Information Data Set^{1,2}) endpoints that are screening-level indicators of potential hazards (toxicity) for humans or the environment.

The Environmental Protection Agency’s Office of Pollution Prevention and Toxics (OPPT) is evaluating the data submitted in the HPV Challenge Program on approximately 1,400 sponsored chemicals. OPPT is using a hazard-based screening process to prioritize review of the submissions. The hazard-based screening process consists of two tiers described below briefly and in more detail on the Hazard Characterization website³.

Tier 1 is a computerized sorting process whereby key elements of a submitted data set are compared to established criteria to “bin” chemicals/categories for OPPT review. This is an automated process performed on the data as submitted by the sponsor. It does not include evaluation of the quality or completeness of the data.

In Tier 2, a screening-level hazard characterization is developed by EPA that consists of an objective evaluation of the quality and completeness of the data set provided in the Challenge Program submissions. The evaluation is performed according to established EPA guidance^{2,4} and is based primarily on hazard data provided by sponsors. EPA may also include additional or updated hazard information of which EPA, sponsors or other parties have become aware. The hazard characterization may also identify data gaps that will become the basis for a subsequent data needs assessment where deemed necessary. Under the HPV Challenge Program, chemicals that have similar chemical structures, properties and biological activities may be grouped together and their data shared across the resulting category. This approach often significantly reduces the need for conducting tests for all endpoints for all category members. As part of Tier 2, evaluation of chemical category rationale and composition and data extrapolation(s) among category members is performed in accord with established EPA² and OECD⁵ guidance.

The screening-level hazard characterizations that emerge from Tier 2 are important contributors to OPPT’s existing chemicals review process. These hazard characterizations are technical documents intended to support subsequent decisions and actions by OPPT. Accordingly, the documents are not written with the goal of informing the general public. However, they do provide a vehicle for public access to a concise assessment of the raw technical data on HPV chemicals and provide information previously not readily available to the public. The public, including sponsors, may offer comments on the hazard characterization documents.

The screening-level hazard characterizations, as the name indicates, do not evaluate the potential risks of a chemical or a chemical category, but will serve as a starting point for such reviews. In 2007, EPA received data on uses of and exposures to high-volume TSCA existing chemicals, submitted in accordance with the requirements of the Inventory Update Reporting (IUR) rule. For the chemicals in the HPV Challenge Program, EPA will review the IUR data to evaluate exposure potential. The resulting exposure information will then be combined with the screening-level hazard characterizations to develop screening-level risk characterizations^{4,6}. The screening-level risk characterizations will inform EPA on the need for further work on individual chemicals or categories. Efforts are currently underway to consider how best to utilize these screening-level risk characterizations as part of a risk-based decision-making process on HPV chemicals which applies the results of the successful U.S. High Production Volume Challenge Program and the IUR to support judgments concerning the need, if any, for further action.

¹ U.S. EPA. High Production Volume (HPV) Challenge Program; <http://www.epa.gov/chemrtk/index.htm>.

² U.S. EPA. HPV Challenge Program – Information Sources; <http://www.epa.gov/chemrtk/pubs/general/guidocs.htm>.

³ U.S. EPA. HPV Chemicals Hazard Characterization website (<http://www.epa.gov/hpvis/abouthc.html>).

⁴ U.S. EPA. Risk Assessment Guidelines; <http://cfpub.epa.gov/ncea/raf/rafguid.cfm>.

⁵ OECD. Guidance on the Development and Use of Chemical Categories; <http://www.oecd.org/dataoecd/60/47/1947509.pdf>.

⁶ U.S. EPA. Risk Characterization Program; <http://www.epa.gov/osa/spc/2riskchr.htm>.

SCREENING-LEVEL HAZARD CHARACTERIZATION Phenolic Benzotriazoles Category

Introduction

The sponsor, The Phenolic Benzotriazoles Association (Ciba Specialty Chemicals Corporation and Cytec Industries, Inc.), submitted a Test Plan and Robust Summaries to EPA for the Phenolic Benzotriazoles Category on October 26, 2001. EPA posted the submission on the ChemRTK HPV Challenge website on December 4, 2001 (<http://www.epa.gov/chemrtk/pubs/summaries/phenbenz/c13266tc.htm>). EPA comments on the original submission were posted to the website on June 19, 2002. Public comments were also received and posted to the website. The sponsor provided EPA with a response to comments on December 20, 2002 and submitted revised documents February 18, 2004, which were posted to the ChemRTK website on August 7, 2003 and April 7, 2004, respectively. The Phenolic Benzoate Category consists of the following four chemicals:

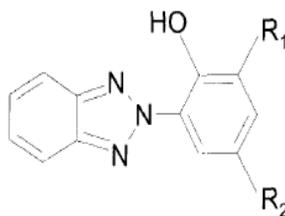
Sponsored Chemicals

2-(2'-Hydroxy-5'-methylphenyl) benzotriazole	CAS No. 2440-22-4
2-(2'-Hydroxy-5'-octylphenyl) benzotriazole	CAS No. 3147-75-9
2-(2'-Hydroxy-3',5'-di- <i>t</i> -amylphenyl) benzotriazole	CAS No. 25973-55-1
2-(2H-Benzotriazol-2-yl)-4,6-bis(1-methyl-1-phenylethyl) phenol	CAS No. 70321-86-7

This screening-level hazard characterization is based primarily on the review of the test plan and robust summaries of studies submitted by the sponsor(s) under the HPV Challenge Program. In preparing the hazard characterization, EPA considered its own comments and public comments on the original submission as well as the sponsor's responses to comments and revisions made to the submission. Structure(s) of the sponsored chemical(s) is included in the appendix. The screening-level hazard characterization for environmental and human health toxicity is based largely on SIDS endpoints and is described according to established EPA or OECD effect level definitions and hazard assessment practices.

Category Justification

The four members of the phenolic benzotriazoles category have the identical molecular base structure of a benzotriazole group. They also have in common a phenolic group attached to the benzotriazole structure at the same location but the substituents (R1 and R2) on the phenolic group vary.



The submitter's primary justification for the category is twofold: (1) the similarity of the structural backbone of all members (phenolic benzotriazoles), and (2) the similar or regular pattern of the chemical, physical and toxicological properties of the members. The submitter adequately supports the grouping of the category members with the information provided.

The submitter proposed to extrapolate existing mammalian and ecotoxicity data for two of the members (CAS Nos. 2440-22-4 and 70321-86-7) to the other two members of the category. No data were available on reproductive toxicity for these chemicals (CAS Nos. 2440-22-4 and 70321-86-7) and the submitter proposed to conduct such studies. Adequate developmental toxicity data are available for the same two category members (CAS Nos. 2440-22-4 and 70321-86-7) and can be interpolated to the remaining category members. In accordance with HPV Challenge Program guidance, EPA concluded that histological evaluation of reproductive organs from the available 90-day repeated-dose toxicity studies and the availability of developmental toxicity studies would address the

reproductive toxicity endpoints. Thus, the revised submission contains evaluation of reproductive organs from the 90-day studies for three chemicals. EPA also suggested the submitter demonstrate toxicokinetic similarities among the category members. EPA has not received the supporting toxicokinetic information.

Summary-Conclusion

The log K_{ow} of the phenolic benzotriazoles category members indicates that their potential to bioaccumulate is high. Members of this category are not readily biodegradable, indicating they have the potential to persist in the environment.

The evaluation of available aquatic toxicity data for fish indicates that the potential acute hazard of phenolic benzotriazoles category members to aquatic organisms is moderate.

The acute toxicity of phenolic benzotriazoles is low via oral, dermal and inhalation routes. Based on read-across values, the potential for the acute inhalation and dermal toxicity is low for the members of this category. Following repeated exposure to phenolic benzotriazoles via the oral route, the liver is the target organ in rodent and non-rodent studies with histopathological changes including hepatocellular hypertrophy and necrosis. Kidney toxicity was observed at higher dose levels. No reproductive toxicity studies were submitted. However, effects on gonads (increased testes weight in rats; atrophy of testes, epididymis, ovary, prostate and uterus and abnormal spermiogenesis in dogs) were seen following repeated exposures to the category members. The repeated-dose data indicate that the more hindered phenolic structures (CAS No. 25973-55-1 and 70321-86-7) were more toxic than the other two category members. Developmental toxicity data were available for two category members, those with the simplest and most complex structures. The developmental toxicity, a slight increase in fetal body weight and delayed skeletal maturation, was seen in rats at high doses; maternal toxicity was not evident up to 3000 mg/kg/day. The category members were not mutagenic at the concentrations tested in bacteria and did not induce chromosomal aberration when tested *in vivo*. Long-term studies available for one category member showed no evidence of carcinogenic potential in rats or mice at the doses tested.

The potential health hazard of the phenolic benzotriazoles category members is moderate based on repeated-dose toxicity. Other available data suggest the category members may have the potential to cause reproductive toxicity.

No data gaps have been identified under the HPV Challenge Program. All HPV Challenge Program endpoints have been adequately addressed by a combination of test data and read across from appropriate category chemicals.

Reproductive toxicity studies were not provided for any of the category members. Evaluation of reproductive organs following repeated-dose toxicity studies along with developmental toxicity studies were used to address this endpoint for the purposes of the HPV Challenge Program. Repeated (oral) exposure to two of the category members (CAS Nos. 25973-55-1 and 70321-86-7) resulted in effects on male reproductive organs. The developmental toxicity study on CAS No. 70321-86-7 also revealed fetal effects. Hence, while the data submitted are adequate for performing this screening-level hazard characterization, the results of the reproductive organ evaluation and the developmental toxicity indicate the category members have the potential to cause reproductive toxicity.

1. Physical-Chemical Properties and Environmental Fate

A summary of physical-chemical properties and environmental fate data submitted is provided in Table 1. For the purpose of the screening-level hazard characterization, the review and summary of these data was limited to the octanol-water partition coefficient and biodegradation endpoints as indicators of bioaccumulation and persistence, respectively.

Octanol-Water Partition Coefficient

2-(2'-Hydroxy-5- methylphenyl) benzotriazole (CAS No. 2440-22-4)

Log K_{ow}: 4.2 (measured)

2-(2'-Hydroxy-5'-octylphenyl) benzotriazole (CAS No. 3147-75-9)

Log K_{ow}: 6.2 (estimated)

2-(2'-Hydroxy-3,5-di-tert-amylphenyl) benzotriazole (CAS No. 25973-55-1)

Log K_{ow}: 7.3 (estimated)

2-(2H-Benzotriazol-2-yl)-4,6-bis(1-methyl-1-phenylethyl) phenol (CAS No. 70321-86-7)

Log K_{ow}: 6.5 (measured)

Biodegradation

2-(2'-Hydroxy-5- methylphenyl) benzotriazole (CAS No. 2440-22-4)

The inoculum (fresh sewage treatment plant sample and sewage sludge) was incubated for 28 days with the test chemical at 11 and 20.1 mg/L. An emulsifier, nonylphenol 10E05PO, was added to enhance the solubility of the test chemical. By day 28, 0 and 2% degradation of the test material was observed at 11 and 20.1 mg/L, respectively. The reference substance, aniline, showed an appropriate response (80% degradation after 28 days).

2-(2'-Hydroxy-5- methylphenyl) benzotriazole is not readily biodegradable.

2-(2'-Hydroxy-5'-octylphenyl) benzotriazole (CAS No. 3147-75-9)

The inoculum (bacteria from the sewage treatment plant) was incubated for 28 days with the test chemical at 10.2 and 21.5 mg/L. An emulsifier, nonylphenol 10E05PO, was added to enhance the solubility of the test chemical. By day 28, 0 and 1% degradation of the test material was observed at 10.2 and 21.5 mg/L, respectively. The reference substance, aniline, showed an appropriate response (84.3% degradation after 28 days).

2-(2'-Hydroxy-5'-octylphenyl) benzotriazole is not readily biodegradable.

2-(2'-Hydroxy-3,5-di-tert-amylphenyl) benzotriazole (CAS No. 25973-55-1)

The inoculum (fresh sewage treatment plant sample and sewage sludge) was incubated for 28 days with the test chemical at 10 and 20 mg/L. By day 28, 8 and 2% degradation of the test material was observed at 10 and 20 mg/L, respectively. The reference substance, aniline, showed an appropriate response (94.4% degradation after 28 days).

2-(2'-Hydroxy-3,5-di-tert-amylphenyl) benzotriazole is not readily biodegradable.

2-(2H-Benzotriazol-2-yl)-4,6-bis(1-methyl-1-phenylethyl) phenol (CAS No. 70321-86-7)

The inoculum (bacteria from the sewage treatment plant) was incubated for 28 days with the test chemical at 10 and 20 mg/L. By day 28, 6 and 3% degradation of the test material was observed at 10 and 20 mg/L, respectively. The reference substance, aniline, showed an appropriate response (99% degradation after 28 days).

2-(2H-Benzotriazol-2-yl)-4,6-bis(1-methyl-1-phenylethyl) phenol is not readily biodegradable.

Conclusion: The log K_{ow} of the phenolic benzotriazoles category members indicate that their potential to bioaccumulate is high. Members of this category are not readily biodegradable, indicating they have the potential to persist in the environment.

Table 1. Summary of Physical-Chemical Properties and Environmental Fate Data				
Endpoints	2-(2'-Hydroxy-5-methylphenyl)benzotriazole (2440-22-4)	2-(2'-Hydroxy-5'-octylphenyl)benzotriazole (3147-75-9)	2-(2'-Hydroxy-3,5-di-tert-amylphenyl)benzotriazole (25973-55-1)	2-(2H-benzotriazol-2-yl)-4,6-bis(1-methyl-1-phenylethyl)phenol (70321-86-7)
Melting Point (°C)	131 – 133 (m)	106 – 108 (m)	80 – 83 (m)	139 – 143 (m)
Boiling Point (°C)	225 (m)	N/A	477.8 (e)	599.8 (e)
Vapor Pressure (hPa at 25°C)	10.57×10^{-8} (e)	1.47×10^{-9} (e)	2.57×10^{-10} (e)	2.15×10^{-14} (e)
Log K _{ow}	4.2 (m)	6.2 (e)	7.3 (e)	6.5 (m)
Water Solubility (mg/L at 25°C)	0.173 (m)	< 1 (m) 0.168 (e)	0.015 (e)	0.04 (m) 0.0097 (e)
Direct Photodegradation (cm ³ /molecule-sec)	No Data	No Data	No Data	No Data
Indirect (OH ⁻) Photodegradation t _{1/2} (h)	1.39 (e)	4.02 (e)	8.1 (e)	1.06 (e)
Stability in Water (Hydrolysis) (year)	No Data ¹	No Data ¹	No Data ¹	No Data ¹
Fugacity (Level III Model)				
Water (%)	3.1	4.0×10^{-5}	2.3×10^{-4}	0
Sediment (%)	4.9	3.5	2.2	2.2
Soil (%)	187.3	44.6	40.4	40.1
Air (%)	4.6	51.9	57.5	57.7
Biodegradation at 28 days (%)	0 – 2 (m) Not Readily Biodegradable	0 – 1 (m) Not Readily Biodegradable	2 – 8 (m) Not Readily Biodegradable	3 – 8 (m) Not Readily Biodegradable

(m) = measured data (i.e. derived from testing); (e) = estimated data (i.e., derived from modeling)

¹The low water solubility of these compounds makes it impractical to conduct hydrolysis studies.

2. Environmental Effects – Aquatic Toxicity

Based on EPA's comments on the original submission, a study to evaluate the acute toxicity to fish was conducted on the least hydrophobic category member for which measured solubility data were available (i.e., 2-(2'-hydroxy-5'-methylphenyl) benzotriazole, CAS No. 2440-22-4). EPA indicated that if no effects were seen in this test at the limit of solubility, no further aquatic toxicity testing would be needed. Therefore, no data are available for aquatic invertebrates and aquatic plants for 2-(2'-hydroxy-5'-methylphenyl) benzotriazole or for any aquatic organisms (fish, invertebrates or plants) on the other category members.

Acute Toxicity to Fish

A summary of aquatic toxicity data submitted for SIDS endpoints is provided in Table 2. The table also indicates where data for tested category members are read-across (RA) to untested members of the category.

2-(2'-Hydroxy-5'-methylphenyl) benzotriazole (CAS No. 2440-22-4)

Rainbow trout (*Oncorhynchus mykiss*) were exposed to 2-(2'-hydroxy-5'-methylphenyl) benzotriazole at nominal concentrations of 0.022, 0.037, 0.061, 0.20 and 0.17 mg/L for 96 hours under static conditions. The highest concentration tested approximated the water solubility limit of the compound. The concentrations were measured in the exposure solutions. At test termination, no mortality or adverse effects were observed in the test.

96-h LC₅₀ > 0.17 mg/L

Conclusion: The evaluation of available aquatic toxicity data for fish indicates that the potential acute hazard of phenolic benzotriazoles category to aquatic organisms is low.

Table 2. Summary of Environmental Effects – Aquatic Toxicity Data				
Endpoints	2-(2'-Hydroxy-5-methylphenyl)benzotriazole (2440-22-4)	2-(2'-Hydroxy-5'-octylphenyl)benzotriazole (3147-75-9)	2-(2'-Hydroxy-3,5-di-tert-amylphenyl)benzotriazole (25973-55-1)	2-(2H-benzotriazol-2-yl)-4,6-bis(1-methyl-1-phenylethyl)phenol (70321-86-7)
Fish 96-h LC ₅₀ (mg/L)	> 0.17 (m)	No Data > 0.17 (m) (RA)	No Data > 0.17 (m) (RA)	No Data > 0.17 (m) (RA)
Aquatic Invertebrates 48-h EC ₅₀ (mg/L)	No Data ¹	No Data ¹	No Data ¹	No Data ¹
Aquatic Plants 72-h EC ₅₀ (mg/L)	No Data ¹	No Data ¹	No Data ¹	No Data ¹

(m) = measured data (i.e., derived from testing); ¹ In test plan comments EPA indicated that if no effects were seen in the test with 2-(2'-Hydroxy-5-methylphenyl)benzotriazole at the limit of solubility, no further aquatic toxicity testing would be needed.

3. Human Health Effects

A summary of health effects data submitted for SIDS endpoints is provided in Table 3. The table also indicates where data for tested category members are read-across (RA) to untested members of the category.

Acute Oral Toxicity

2-(2'-Hydroxy-5'-methylphenyl) benzotriazole (CAS No. 2440-22-4)

Tif Ralf (SPF) rats were administered the test substance (suspended in PEG 400) via gavage at 4640, 7750 and 10,000 mg/kg-bw and observed for 14 days. Clinical signs of toxicity in all treated animals were sedation, dyspnea, urved position, and ruffled fur within 2 hours after treatment. All animals recovered within 8-10 days following treatment. No treatment-related effects were observed at necropsy.

LD₅₀ > 10,000 mg/kg-bw

2-(2'-Hydroxy-5'-octylphenyl) benzotriazole (CAS No. 3147-75-9)

Male Wistar rats (20/dose) were administered the an aqueous suspension of test substance at 125, 250, 500 and 1000 mg/kg-bw via gavage at and observed for 14 days. No clinical signs of toxicity or mortality were observed.

LD₅₀ > 1000 mg/kg-bw

2-(2'-Hydroxy-3,5-di-tert-amylphenyl) benzotriazole (CAS No. 25973-55-1)

Tif Ralf (SPF) rats (5/sex/dose) were administered the test substance (30% suspension in PEG 400) via gavage at 1392, 1800 and 2325 mg/kg-bw and observed for 14 days. Clinical signs of toxicity in all treated animals were sedation, dyspnea, curved position, and ruffled fur within 2 hours after treatment. All animals recovered within 8-9 days following treatment. No treatment-related effects were observed at necropsy.

LD₅₀ > 2325 mg/kg-bw

2-(2H-Benzotriazol-2-yl)-4,6-bis(1-methyl-1-phenylethyl) phenol (CAS No. 70321-86-7)

Tif Ralf (SPF) rats (5/sex/dose) were administered the test substance (suspension in PEG 400) via gavage at 1000, 2150, 4640 and 7750 mg/kg-bw and observed for 14 days. Clinical signs of toxicity in all treated animals were sedation, dyspnea, curved position, and ruffled fur within 2 hours after treatment. All animals recovered within 8-10 days following treatment. No treatment-related effects were observed at necropsy.

LD₅₀ > 7750 mg/kg-bw

Acute Inhalation Toxicity

2-(2'-Hydroxy-5'-methylphenyl) benzotriazole (CAS No. 2440-22-4)

Charles Rivers rats (5/sex) were administered the test substance via air-dust mixture at 1420 mg/m³ (1.42 mg/L) for 4 hours and observed for 14 days following treatment. No test substance-related effects were noted.

LC₅₀ > 1420 mg/m³ (1.42 mg/L)

Acute Dermal Toxicity

2-(2H-Benzotriazol-2-yl)-4,6-bis(1-methyl-1-phenylethyl) phenol (CAS No. 70321-86-7)

Albino rats (5/sex/dose) were exposed to the test substance dermally via a semi-occlusive dressing for 24 hours. Following removal of the dressing at 24 hours, the rats were observed for 14 days. Piloerection was seen following test substance application; recovery was noted within 1 day. No mortalities occurred during this study. Necropsy examination did not reveal any gross pathologic alterations.

LD₅₀ > 2000 mg/kg-bw

Repeated-Dose Toxicity

2-(2'-Hydroxy-5'-methylphenyl) benzotriazole (CAS No. 2440-22-4)

Beagle dogs (6/sex/group) were administered the test substance in their diet at 1000, 3000 or 10,000 ppm for 13 weeks. No clinical signs of toxicity were observed. At 10,000 ppm, a decrease in body weight gain and food consumption was seen. Alanine aminotransferase activity was increased in the 3000 and 10,000 ppm groups and gamma glutamyl transpeptidase activity was increased in the 10,000 ppm group. One female animal from the 10,000 ppm group was emaciated. There were no treatment-related gross or histopathological changes reported.

LOAEL = 3000 ppm (approximately 95.25 and 103.8 mg/kg-bw/day for males and females, respectively)

NOAEL = 1000 ppm (approximately 31.8 and 34.6 mg/kg-bw/day for males and females, respectively)

2-(2'-Hydroxy-5'-octylphenyl) benzotriazole (CAS No. 3147-75-9)

Wistar rats (5/sex/group) were administered the test substance via the diet at 0, 1.25, 2.5 and 5% (corresponding to 0, 1286, 2594 and 5658 mg/kg-bw/day) for 30 days. There were no deaths and no effect on body weight or food consumption during the test period. Hydronephrosis was noted in the high dose (four animals) and control (three animals) groups. No lesions were noted that were attributable to ingestion of the test substance.

NOAEL = 5658 mg/kg/day (highest dose tested)

2-(2'-Hydroxy-3',5'-di-*t*-amylphenyl) benzotriazole (CAS No. 25973-55-1)

(1) Beagle dogs (3/sex/group) were administered the test substance via the diet at 0, 15, 30, 60, 120 or 240 mg/kg-bw/day daily for 3 months. Males were more sensitive than females with mortality of one male dog in the highest dose group. Decreases in body weight and food consumption were evident in the high-dose group animals. The liver was the target organ for toxicity. Anemia was noted in animals from the two highest doses groups and changes in blood chemistry parameters included increased serum bilirubin levels and gamma glutamyl transpeptidase (GTP), glutamyl oxalacetic transaminase (GOT) and alkaline phosphatase activity. Increased liver weights associated with severe liver damage including icterus (jaundice) were observed upon gross and histopathological examination in a few dogs in the 120 and 240 mg/kg-bw/day dose groups. Microscopically, fatty degeneration of hepatocytes, presence of protein globules in the cytoplasm, Kupffer cell hyperplasia and centrilobular cholestasis were seen. It was reported that the kidneys also exhibited toxicity, but no details were provided. In higher dose groups, some animals showed atrophy of uterus, abnormal spermiogenesis and atrophy of the prostate.

LOAEL = 15 mg/kg-bw/day (based on body weight, liver and kidney effects)

NOAEL = Not established

(2) Rats (10/sex/group) were administered the test substance via the diet at 0, 100, 200, 400, 800 or 1600 ppm (approximately 5, 10, 20, 40 or 800 mg/kg/day) for 90 days. Signs of anemia (decreased hemoglobin and packed cell volume) were seen in males at dietary concentrations of 200 ppm and above. In females, this effect was less pronounced. An increase in glucose-6-phosphatase activity was noted at all dietary concentrations. Liver, kidney, spleen and testes weights were increased in higher exposure groups with an indication of increased thyroid weights. The liver was the primary target organ and had a greenish-drab discoloration in males and females at higher exposure levels. Microscopic examination revealed foci of necrosis and slight proliferation of bile duct epithelia. Paraenchymal cells were enlarged. In the kidney, tubular necrosis was seen in some males from the higher dose groups. In females, a treatment-related, yellowish-brown pigmentation in the cytoplasm of the proximal tubular cells was noted.

LOAEL = 100 ppm (approximately 20 mg/kg/day; based on blood, liver and kidney effects)

NOAEL = Not established

2-(2H-Benzotriazol-2-yl)-4,6-bis (1-methyl-1-phenylethyl) phenol (CAS No. 70321-86-7)

Rats (10/sex/dose) were administered the test substance via the diet at 0, 50, 300, 2000 or 10,000 ppm (approximately 0, 2.5, 15, 100 or 500 mg/kg-bw/day) for 92-94 days. No treatment-related clinical symptoms or signs of toxicity were observed. There was no effect on mortality; body weight; hematological, blood chemistry and urinalysis parameters; nor macroscopic findings. A statistically significant increase in absolute and relative (to body and brain weight) liver weight was seen in males and females at 2000 ppm and above and in females at 300 ppm. A slight to moderate hypertrophy and/or cytoplasmic vacuolization of hepatocytes were seen in males and females at 2000 ppm and above and in females from 300 ppm and above.

LOAEL = 300 ppm (approximately 15 mg/kg-bw/day; based on liver effects)

NOAEL = 50 ppm (approximately 2.5 mg/kg-bw/day)

Reproductive Toxicity

Reproductive toxicity tests were not submitted to address the reproductive toxicity endpoint for this category. Evaluation of reproductive organs in repeated-dose toxicity and other studies were used to address the reproductive endpoints for the purposes of the HPV Challenge Program. Therefore, NOAEL/LOAELs for fertility and/or reproductive toxicity cannot be determined for this endpoint.

2-(2'-Hydroxy-5'-methylphenyl) benzotriazole (CAS No. 2440-22-4)

(1) In the 90-day toxicity study in dogs described previously, gonads were weighed and examined macroscopically (ovaries or testes and uterus or prostate). Although not statistically significant, ovary weights were decreased in a dose-dependent manner. No gross or histopathological changes in gonads were seen.

(2) In a dietary toxicity study in rats (0, 100, 300, 1000 and 3000 ppm) and mice (0, 5, 50 and 500 ppm), organ weight analysis performed on the gonads of male and female rats sacrificed after 104 weeks of treatment revealed no differences between control and treatment groups. General histopathology of the gonads of rats and mice sacrificed after 104 weeks of treatment showed no abnormalities.

(3) In a Dominant Lethal Assay, male mice were treated with the test chemical by gavage at a dose of 0, 1000 or 3000 mg/kg-bw and were mated with untreated females. The vehicle was aqueous carboxymethyl cellulose. Females were necropsied on day 14 of pregnancy. There were no differences in mating ratio, number of implantations, or embryonic deaths between control and treated groups.

2-(2'-Hydroxy-3',5'-di-t-amylphenyl) benzobenzotriazole (CAS No. 25973-55-1)

(1) In the 90-day repeated-dose toxicity study conducted in Beagle dogs as described previously, effects on gonads were more pronounced in males than females. In males, decreases were seen in testes and epididymal weights at the two highest doses and prostate weights were decreased in a dose-dependent manner. In females, body weight and ovary weight were decreased in the highest dose group and uterus weight was decreased in the three highest dose groups. Microscopic changes included atrophy of the uterus, abnormal spermiogenesis and atrophy of the prostate.

(2) In the 90-day repeated-dose toxicity study conducted in rats as described previously, there was no treatment-related effect on ovary weight (reported as ovary weight per 100 g body weight). Increases in testes weights

(reported as testes weight per 100 g body weight) were statistically significant at 400 ($p < 0.05$), 800 and 1600 ppm ($p < 0.01$). Ovary weights were not affected. Gonads were not evaluated microscopically.

2-(2H-Benzotriazol-2-yl)-4,6-bis(1-methyl-1-phenylethyl) phenol (CAS No. 70321-86-7)

In the 90-day repeated-dose toxicity study conducted in rats as described previously, gonads were weighed and evaluated microscopically. Gonad weights were not affected at the end of the treatment but there was a statistically significant ($p = 0.05$) increase in testes weights after a 4-week recovery period. The summary states that there were histological effects on reproductive organs; however, they were not reported.

Developmental Toxicity

2-(2'-Hydroxy-5'-methylphenyl) benzotriazole (CAS No. 2440-22-4)

(1) Groups of presumed pregnant female rats and mice received the test substance (in 2% aqueous carboxymethyl cellulose) by gavage at 150, 500 or 1000 mg/kg-bw on days 6-15 of gestation. No maternal toxicity was evident and the rates of implantation and embryotoxicity were not affected by treatment.

LOAEL (maternal/developmental toxicity) > 1000 mg/kg-bw (highest dose tested)

NOAEL (maternal/developmental toxicity) = 1000 mg/kg-bw

(2) In a Dominant Lethal Assay, male mice were treated with the test chemical by gavage at a dose of 0, 1000 or 3000 mg/kg-bw and were mated with untreated females. The vehicle was aqueous carboxymethyl cellulose. Females were necropsied on day 14 of pregnancy. There were no differences in mating ratio, number of implantations, or embryonic deaths between controls and treated groups.

2-(2H-Benzotriazol-2-yl)-4,6-bis(1-methyl-1-phenylethyl) phenol (CAS No. 70321-86-7)

Groups of presumed pregnant female rats received the test substance by gavage at 300, 1000 or 3000 mg/kg-bw on days 6 through 15 of gestation. No maternal toxicity was evident at any dose. Fetal data indicated a significant reduction in body weight for the 1000 mg/kg-bw dose group. In addition, an increased delay of skeletal maturation was noted for this group. Fetuses in the high dose group showed omphalocele (failure of ventral closure during last stages of embryonic development).

LOAEL (maternal toxicity) > 3000 mg/kg-bw/day (highest dose tested)

NOAEL (maternal toxicity) = 3000 mg/kg-bw/day

LOAEL (developmental toxicity) = 1000 mg/kg-bw/day (based on body weight and skeletal effects)

NOAEL (developmental toxicity) = 300 mg/kg-bw/day

Genetic Toxicity – Gene Mutation

In vitro

2-(2'-Hydroxy-5'-methylphenyl) benzotriazole (CAS No. 2440-22-4)

An Ames assay was conducted using *Salmonella typhimurium* strains TA 98, TA 100, TA 1535 and TA 1535 with and without metabolic activation at 10, 30, 90, 270 and 810 $\mu\text{g}/0.1 \text{ mL}$.

2-(2'-Hydroxy-5'-methylphenyl) benzotriazole was not mutagenic in this assay.

2-(2'-Hydroxy-5'-octylphenyl) benzotriazole (CAS No. 3147-75-9)

A reverse mutation assay was conducted using *S. typhimurium* strains TA 98, TA100, TA 1535 and TA 1535 and *E. coli* WP2uvrA, with and without metabolic activation and at concentrations of 20.5, 61.7, 185.2, 555.5, 1666.6 and 5000 $\mu\text{g}/\text{plate}$. Appropriate positive and negative controls were used. The results revealed no marked difference in mutagenic activity of the tested concentrations when compared with negative controls; positive controls showed appropriate response.

2-(2'-Hydroxy-5'-octylphenyl) benzotriazole was not mutagenic in this assay.

2-(2'-Hydroxy-3',5'-di-t-amylphenyl) benzotriazole (CAS No. 25973-55-1)

An Ames assay was conducted using *S. typhimurium* strains TA 98, TA 100, TA 1535 and TA 1535 with and without metabolic activation at 25, 75, 225, 675 and 2025 $\mu\text{g}/0.1 \text{ mL}$. The comparison of results between the negative control and test chemical revealed no marked differences. No evidence of point mutation was detected.

2-(2'-Hydroxy-3',5'-di-t-amylphenyl) benzotriazole was not mutagenic in this assay.

2-(2H-Benzotriazol-2-yl)-4,6-bis (1-methyl-1-phenylethyl) phenol (CAS No. 70321-86-7)

In a reverse mutation assay, *S.typhimurium* strains TA 98, TA 100, TA 1535, and TA 1535 were exposed to the test chemical at 25, 75, 225, 675 and 2025 µg/0.1 mL with and without metabolic activation. Appropriate positive and negative controls were used. The results revealed no marked difference in mutagenic activity of the tested concentrations when compared with negative controls; positive controls showed appropriate response.

2-(2H-Benzotriazol-2-yl)-4,6-bis (1-methyl-1-phenylethyl) phenol was not mutagenic in this assay.

Genetic Toxicity – Chromosomal Aberrations

In vivo

2-(2'-Hydroxy-5'-methylphenyl) benzotriazole (CAS No. 2440-22-4)

(1) Chinese hamsters were administered the test chemical by gavage for 2 days; injected intra peritoneally with colcemide 2 hours after administration of the second dose and sacrificed 4 hours later. Bone marrow was harvested and analyzed for chromosomal aberrations. Chromosomes from animals treated with the test chemical showed no aberrations; two metaphase figures were observed in the negative control and there was a significant increase in aberrations in the positive controls.

2-(2'-Hydroxy-5'-methylphenyl) benzotriazole did not induce chromosomal aberrations in this assay.

Genetic Toxicity – Other Effects

In vivo

2-(2'-Hydroxy-5'-methylphenyl) benzotriazole (CAS No. 2440-22-4)

(1) In a Dominant Lethal Assay, male mice were treated with the test chemical by gavage at a dose of 0, 1000 or 3000 mg/kg-bw and were mated with untreated females. The vehicle was aqueous carboxymethyl cellulose. Females were necropsied on day 14 of pregnancy. No evidence of dominant lethal effects was noted.

2-(2'-Hydroxy-5'-methylphenyl) benzotriazole was not mutagenic in this assay.

(2) In a Nucleus Anomaly Test on Somatic Interphase Nuclei, the percentage of treated cells displaying anomalies of nuclei was not significantly different from the negative control. The positive control produced significant anomalies. The test chemical was non-mutagenic in this assay.

2-(2'-Hydroxy-5'-methylphenyl) benzotriazole was not mutagenic in this assay.

2-(2H-Benzotriazol-2-yl)-4,6-bis (1-methyl-1-phenylethyl) phenol (CAS No. 70321-86-7)

(1) In a sister chromatid exchange (SCE) assay, Chinese hamsters were administered the test chemical by gavage at 1250, 2500, or 5000 mg/kg-bw; sacrificed 24 hrs after the exposure and 2 hr after an i.p. injection of colcemide. Bone marrow was harvested and analyzed for the number of sister chromatid exchanges. No significant increase in the number of sister chromatid exchanges was found when compared with the negative control. The positive controls showed a significant increase of SCEs per cell.

2-(2H-Benzotriazol-2-yl)-4,6-bis (1-methyl-1-phenylethyl) phenol was not mutagenic in this assay.

(2) In a Nucleus Anomaly Test on Somatic Interphase Nuclei, the percentage of treated cells displaying anomalies of nuclei was not significantly different from the negative control. The positive control produced significant anomalies.

2-(2H-Benzotriazol-2-yl)-4,6-bis (1-methyl-1-phenylethyl) phenol was not mutagenic in this assay.

Additional Information

Carcinogenicity

2-(2'-Hydroxy-5'-methylphenyl) benzotriazole (CAS No. 2440-22-4)

(1) In a lifetime carcinogenicity study, 50 mice/sex/group were given the test substance in the diet for 24 months at concentrations of 5, 50 or 500 ppm (corresponding to 0.8, 6.5 and 64 and 0.8, 6.7 and 62 mg/kg bw/day for males and females, respectively). There was no effect on mortality, bodyweight, food consumption, and clinical signs of toxicity. No systemic toxicity was observed. The test substance did not produce inflammatory, degenerative, proliferative or neoplastic lesions up to the dose levels tested.

(2) In the long-term feeding study, 50 rats/sex/group were administered the test substance for 104 weeks at concentrations of 0, 100, 300, 1000 or 3000 ppm (corresponding to 0, 4-6, 14-17, 47-58 and 142-169 mg/kg bw/day). Although not statistically significant, marginally lower survival rate was noted in males in the 3000 ppm group. A statistically significant decreased bodyweight gain in males and reduced food consumption in females were noted in the 3000 ppm group. Organ weight analysis revealed slightly increased thyroid/parathyroid weights among treated animals achieving a statistical significance in males at 1000 ppm and in females at 1000 and 3000 ppm when related to bodyweight. The administration of the test substance did not have an effect on the spontaneous tumors. The NOAEL for systemic toxicity was 1000 ppm (47-58 mg/kg-bw/day).

Conclusion: The acute toxicity of phenolic benzotriazoles is low via oral, dermal and inhalation routes. Based on read-across values, the potential for the acute inhalation and dermal toxicity is low for the members of this category. Following repeated exposure to phenolic benzotriazoles via the oral route, the liver is the target organ in rodent and non-rodent studies with histopathological changes including hepatocellular hypertrophy and necrosis. Kidney toxicity was observed at higher dose levels. No reproductive toxicity studies are available. In addition, effects on gonads (increased testes weight in rats; atrophy of testes, epididymis, ovary, prostate and uterus and abnormal spermiogenesis in dogs) were seen following repeated exposures to the category members. The repeated-dose data indicate that the more hindered phenolic structures (CAS No. 25973-55-1 and 70321-86-7) were more toxic than the other two category members. Developmental toxicity data were available for two category members; those with the simplest and most complex structures. The developmental toxicity, a slight increase in fetal body weight effects and delayed skeletal maturation, was seen in rats at high doses; maternal toxicity was not evident up to 3000 mg/kg/day. The category members were not mutagenic at the concentrations tested in bacteria and did not induce chromosomal aberration when tested *in vivo*. Long-term studies available for one category member showed no evidence of carcinogenic potential in rats or mice at the doses tested.

The potential health hazard of the phenolic benzotriazoles category members is moderate based on repeated-dose toxicity. Other available data suggest the category members may have the potential to cause reproductive toxicity.

Table 3. Summary of Human Health Data

Endpoints	2-(2'-Hydroxy-5-methylphenyl)benzotriazole (2440-22-4)	2-(2'-Hydroxy-5'-octylphenyl)benzotriazole (3147-75-9)	2-(2'-Hydroxy-3,5-ditert-amylphenyl)benzotriazole (25973-55-1)	2-(2H-benzotriazol-2-yl)-4,6-bis(1-methyl-1-phenylethyl)phenol (70321-86-7)
Acute Oral Toxicity LD ₅₀ (mg/kg-bw)	> 10,000	> 10,000	> 2325	> 7750
Acute Dermal Toxicity LD ₅₀ (mg/kg-bw)	No Data > 2000 (RA)	No Data > 2000 (RA)	No Data > 2000 (RA)	> 2000
Acute Inhalation Toxicity LC ₅₀ (mg/L/6h/day)	> 1420	No Data > 1420 (RA)	No Data > 1420 (RA)	No Data > 1420 (RA)
Repeated-Dose Toxicity NOAEL/LOAEL (mg/kg-bw/day) (male) (female)	NOAEL = 31.8 LOAEL ~ 95 NOAEL = 34.6 LOAEL = 104	NOAEL > 5,658	NOAEL = Not established LOAEL ~ 22 LOAEL = 15	NOAEL ~ 2.5 LOAEL ~ 15
Reproductive Toxicity	No Data Reproductive organ evaluation: effect on ovary weights	No Data	No Data Reproductive organ evaluation: effect on testes weights; testes, epididymis, prostate, ovary, uterus, spermiogenesis	No Data Reproductive organ evaluation: effect on testes weights during recovery period
Developmental Toxicity NOAEL/LOAEL (mg/kg-bw/day) Maternal Toxicity Developmental Toxicity	NOAEL = 1000 NOAEL = 1000	No Data NOAEL = 1000 (RA) NOAEL = 1000 (RA)	No Data NOAEL = 300 (RA) NOAEL = 1000 (RA)	NOAEL = 300 NOAEL = 1000
Genetic Toxicity – Gene Mutation <i>In vitro</i>	Negative	Negative	Negative	Negative
Genetic Toxicity – Chromosomal Aberrations <i>In vivo</i>	Negative	No Data Negative (RA)	No Data Negative (RA)	No Data Negative (RA)
Genetic Toxicity – Other Effects	Dominant-Lethal Assay: Negative Nucleus Anomaly Test: Negative	–	–	Sister Chromatid Exchange Assay: Negative Nucleus Anomaly Test: Negative
Additional Information – Carcinogenicity	No evidence of carcinogenicity at up to 63 – 64 or 142 – 147 mg/kg-bw/day)	–	–	–

Bold = measured data; (RA) = Read Across

4. Hazard Characterization

The log K_{ow} of the phenolic benzotriazoles category members indicates that their potential to bioaccumulate is high. Members of this category are not readily biodegradable, indicating they have the potential to persist in the environment.

The evaluation of available aquatic toxicity data for fish indicates that the potential acute hazard of phenolic benzotriazoles category members to aquatic organisms is moderate.

The acute toxicity of phenolic benzotriazoles is low via oral, dermal and inhalation routes. Based on read-across values, the potential for the acute inhalation and dermal toxicity is low for the members of this category. Following repeated exposure to phenolic benzotriazoles via the oral route, the liver is the target organ in rodent and non-rodent studies with histopathological changes including hepatocellular hypertrophy and necrosis. Kidney toxicity was observed at higher dose levels. No reproductive toxicity studies were submitted. However, effects on gonads (increased testes weight in rats; atrophy of testes, epididymis, ovary, prostate and uterus and abnormal spermiogenesis in dogs) were seen following repeated exposures to the category members. The repeated-dose data indicate that the more hindered phenolic structures (CAS No. 25973-55-1 and 70321-86-7) were more toxic than the other two category members. Developmental toxicity data were available for two category members, those with the simplest and most complex structures. The developmental toxicity, a slight increase in fetal body weight and delayed skeletal maturation, was seen in rats at high doses; maternal toxicity was not evident up to 3000 mg/kg/day. The category members were not mutagenic at the concentrations tested in bacteria and did not induce chromosomal aberration when tested *in vivo*. Long-term studies available for one category member showed no evidence of carcinogenic potential in rats or mice at the doses tested.

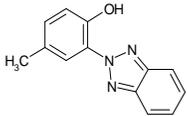
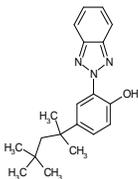
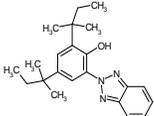
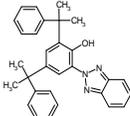
The potential health hazard of the phenolic benzotriazoles category members is moderate based on repeated-dose toxicity. Other available data suggest the category members may have the potential to cause reproductive toxicity.

5. Data Gaps

No data gaps have been identified under the HPV Challenge Program. All HPV Challenge Program endpoints have been adequately addressed by a combination of test data and read across from appropriate category chemicals.

Reproductive toxicity studies were not provided for any of the category members. Evaluation of reproductive organs following repeated-dose toxicity studies along with developmental toxicity studies were used to address this endpoint for the purposes of the HPV Challenge Program. Repeated (oral) exposure to two of the category members (CAS Nos. 25973-55-1 and 70321-86-7) resulted in effects on male reproductive organs. The developmental toxicity study on CAS No. 70321-86-7 also revealed fetal effects. Hence, while the data submitted are adequate for performing this screening-level hazard characterization, the results of the reproductive organ evaluation and the developmental toxicity indicate the category members have the potential to cause reproductive toxicity.

Appendix

Phenolic Benzotriazoles		
CAS No.	Chemical Name	Structure
SPONSORED CHEMICALS		
2440-22-4	2-(2'-Hydroxy-5'-methylphenyl)-benzotriazole	 $C_{13}H_{11}N_3O$
3147-75-9	2-(2'-Hydroxy-5'-octylphenyl)-benzotriazole	 $C_{20}H_{25}N_3O$
25973-55-1	2-(2'-Hydroxy-3', 5'-di-t-amylphenyl)-benzotriazole	 $C_{22}H_{29}N_3O$
70321-86-7	2-(2H-Benzotriazol-2-yl)-4,6-bis(1-methyl-1-phenylethyl)phenol	 $C_{30}H_{29}N_3O$