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CASE STUDY ON THE USE OF AN INTEGRATED APPROACH TO TESTING AND ASSESSMENT FOR THE REPEATED-DOSE TOXICITY OF PHENOLIC BENZOTRIAZOLES

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IOMC
INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS
A cooperative agreement among FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD

Environment Directorate
ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT
Paris 2017

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FOREWORD

OECD member countries have been making efforts to expand the use of alternative methods in assessing chemicals. The OECD has been developing guidance documents and tools for the use of alternative methods such as (Q)SAR, chemical categories and Adverse Outcome Pathways (AOPs) as a part of Integrated Approaches for Testing and Assessment (IATA). There is a need for the investigation of the practical applicability of these methods/tools for different aspects of regulatory decision-making, and to build upon case studies and assessment experience across jurisdictions.

The objective of the IATA Case Studies Project is to increase experience with the use of IATA by developing case studies, which constitute examples of predictions that are fit for regulatory use. The aim is to create common understanding of using novel methodologies and the generation of considerations/guidance stemming from these case studies.

This case study was developed by Japan for illustrating practical use of IATA and submitted to the 2016 review cycle of the IATA Case Studies project. This case study was reviewed by the project team and revised to consider the comments from reviewers. The document was endorsed at the1st meeting of the Working Party on Hazard Assessment in June 2017.

The following four case studies were also reviewed in the project in 2016 and are published with this case study:

1. CASE STUDY ON THE USE OF INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT FOR PESTICIDE CUMULATIVE RISK ASSESSMENT & ASSESSMENT OF LIFESTAGE SUSCEPTIBILITY, ENV/JM/MONO(2017)24, Series on Testing & Assessment No.272.

2. CASE STUDY ON THE USE OF INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT OF 90-DAY RAT ORAL REPEATED-DOSE TOXICITY FOR SELECTED N-ALKANOLS: READ-ACROSS, ENV/JM/MONO(2017)25, Series on Testing & Assessment No. 273.

3. CASE STUDY ON THE USE OF INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT OF 90-DAY RAT ORAL REPEATED-DOSE TOXICITY FOR SELECTED 2-ALKYL-1-ALKANOLS: READ-ACROSS, ENV/JM/MONO(2017)26, Series on Testing & Assessment No. 274.

4. CHEMICAL SAFETY ASSESSMENT WORKFLOW BASED ON EXPOSURE CONSIDERATIONS AND NON-ANIMAL METHODS, ENV/JM/MONO(2017)27, Series on Testing & Assessment No. 275.

In addition, a considerations document summarizing the learnings and lessons of the review experience of the case studies is published with the case studies:

REPORT ON CONSIDERATIONS FROM CASE STUDIES ON INTEGRATED APPROACHES FOR TESTING AND ASSESSMENT (IATA) -Second Review Cycle (2016)- ENV/JM/MONO(2017)22, Series on Testing & Assessment No. 270.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology.

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INTRODUCTION

Repeated-dose toxicity is one of the key regulatory endpoints in the hazard assessment of chemicals. For risk assessment under the Japanese Chemical Substances of Control Law (CSCL), a screening assessment is conducted to select Priority Assessment Chemical Substances. A hazard class is assigned to the repeated-dose toxicity endpoint with data obtained by animal testing. Category assessment is not currently utilized in the screening assessment, but is recommended for chemical assessment under the CSCL (METI *et al*., 2012).

Phenolic benzotriazoles are UV absorbers added to various polymer products to protect against UV degradation. In total, there are around two dozen different phenolic benzotriazoles on the market. Several substances of this group have been described as emerging contaminants with properties of concern for environmental and human health. The phenolic benzotriazole category was previously assessed by the United States Environmental Protection Agency (EPA) High Production Volume (HPV) Challenge Program (U.S. EPA, 2009), National Toxicology Program (NTP) Chemical Information Review (NTP, 2011), and Government of Canada (Environment and Climate Change in Canada and Health Canada, 2016). However, these assessments did not include a detailed examination of the structure−toxicity relationships. A weight-of-evidence approach was used to assess the persistence of certain phenolic benzotriazoles (Brandt *et al*., 2016), but read-across assessment has not yet been attempted for the repeated-dose toxicity endpoint.

This case study focuses on repeated-dose toxicity endpoints for more detailed category assessment of structurally similar but unevaluated phenolic benzotriazoles. Transcriptomic profiles were generated for some category members and then integrated into the assessment. This case study is intended to address how read-across can be applied to screening assessments under the CSCL.

1. PURPOSE

1.1. Purpose of use

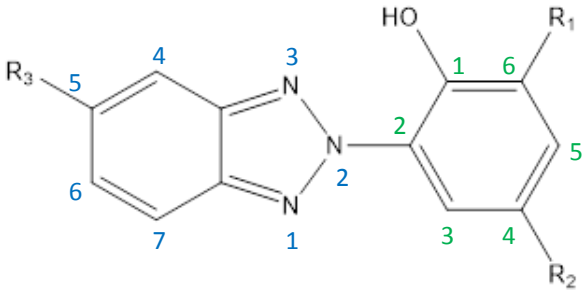
The general purpose of the case study is to improve grouping approaches and OECD guidance on the Integrated Approach to Testing and Assessment (IATA). The specific purpose is to assess repeated-dose toxicity of phenolic benzotriazoles by integrating transcriptomic profiles for the category assessment and then to conduct read-across by considering the toxicity of the nearest tested analog(s). No-observed-(adverse)-effect-level (NO(A)EL) values are derived and finally hazard assessment (D) values are used for hazard classification under the CSCL.

1.2. Target chemicals

Phenolic benzotriazoles are solids with low water solubility and low to negligible vapor pressures. They are expected to have low mobility in soil and bio-accumulative potential. Volatization of phenolic benzotriazoles is considered low based on their Henry's Law constant. Phenolic benzotriazoles have UV absorbing properties and so provide effective light stabilization and prevent degradation of polymers. On this basis, they are used for industrial products, including food packaging as plastic additives, as well as for electrical and electronic products. They are thought to exist as free additives in polymer products. Studies have identified several of these compounds within marine wildlife, soil, and seafood (NTP 2011). Humans may be exposed to phenolic benzotriazoles

from environmental contamination and contaminants in food migrated from packages. Dermal exposure may occur from phenolic benzotriazoles used in cosmetics, but oral exposure may be of greater concern for this category of chemicals. Under the European Union Registration, Evaluation, Authorization, and Restriction of Chemicals guidelines, four phenolic benzotriazoles have been identified as substances of very high concern (SVHCs) (CAS No. 3846-71-7, 25973-55-1, 3864-99-1, and 36437-37-3), as very persistent and very bio-accumulative (vPvB) substances, and in two cases also as persistent, bio-accumulative, and toxic substances (CAS No. 3846-71-7 and 25973-55-1).

Phenolic benzotriazoles have a common phenolic group attached to benzotriazole at the same location, but the substituents (R_1 and R_2) at ortho and para positions to the hydroxyl group of the phenolic ring vary. Some members contain a chlorine atom at the 5 position of the benzotriazole ring. The general structure of phenolic benzotriazoles is presented in Figure 1.



R_1-H, branched alkyl or phenyl alkyl group
R_2-(branched) alkyl or alkanoic acid ester group
R_3-H or chlorine atom

Figure 1. General structure of phenolic benzotriazoles

The Japanese CSCL inventory includes 12 phenolic benzotriazoles. Of these, nine are identified as source chemicals. Inclusion of additional source chemicals is supportive for category assessment. However, reliable repeated-dose toxicity data were not found for these remaining substances. Thus, the remaining three are target chemicals for read-across (Table 1).

Table 1. Structures of target chemicals in this study

Name	CAS	R_1	R_2	R_3
Octyl 3-[3-(benzotriazol-2-yl)-5-tert-butyl-4-hydroxyphenyl]propanoate	127519-17-9	$C(CH_3)_3$	$CH_2CH_2COO(CH_2)_7CH_3$	H
2-(Benzotriazol-2-yl)-6-(2-phenylpropan-2-yl)-4-(2,4,4-trimethylpentan-2-yl)phenol	73936-91-1	$C(CH_3)_2(C_6H_5)$	$C(CH_3)_2CH_2C(CH_3)_3$	H
2-(Benzotriazol-2-yl)-4-tert-butylphenol	3147-76-0	H	$C(CH_3)_3$	H

1.3. Endpoint

Target endpoint is repeated-dose toxicity via the oral route. The primary target organ of phenolic benzotriazoles is the liver. Hepatotoxic effects include histopathological changes such as hypertrophy, degeneration, and necrosis of hepatocytes, and bile duct hyperplasia accompanied by organ weight increase.

2. HYPOTHESIS FOR THE CATEGORY APPROACH

Previous oral repeated-dose toxicity studies showed that hepatotoxicity is the primary adverse effect of these compounds but that toxicity level varies markedly depending on the type and degree of substitution (NTP, 2011). It is expected that structural variety and degree of substitution may influence physicochemical properties, bioavailability, mode of action, and toxicological properties. However, such information is limited. Whole transcriptome analysis can capture global gene expression changes. Toxicogenomic profiling of the liver in association with hepatotoxicity would support subcategorization of phenolic benzotriazoles sharing potential common modes of action and similar toxicological properties.

Some category members also induce nephrotoxicity, but generally at substantially higher doses than required for hepatotoxicity in GLP studies. Hence, hepatotoxic effects are more critical for hazard assessment of the phenolic benzotriazole category.

3. CATEGORY MEMBERS

3.1. Identification and selection of category members

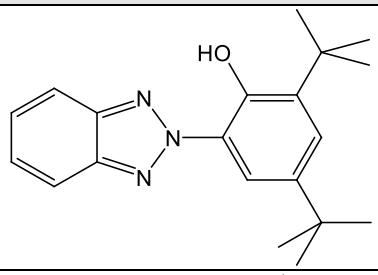
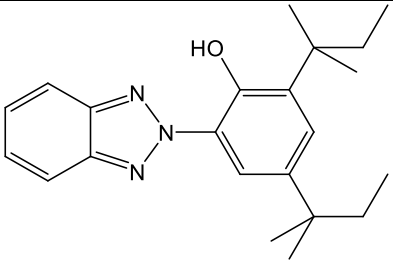
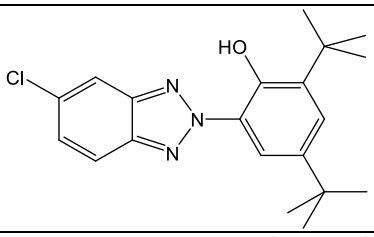
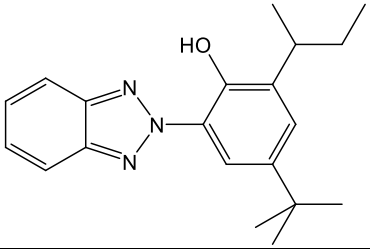
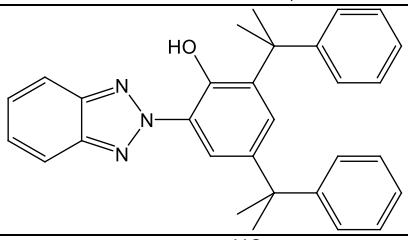
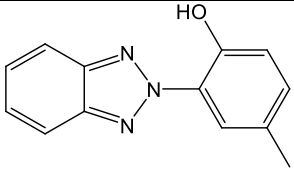
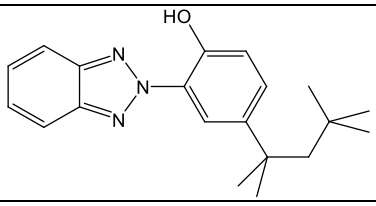
The criterion for the phenolic benzotriazole category is a phenolic group attached to a benzotriazole structure at the same location (Figure 1). Phenolic benzotriazoles have various substituents at 4 and/or 6 positions of the phenolic ring, and some contain a chlorine substituent at the 5 position of the benzotriazole ring.

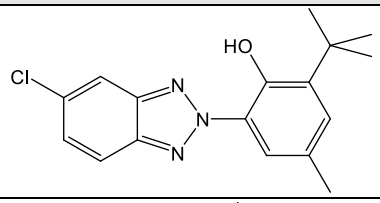
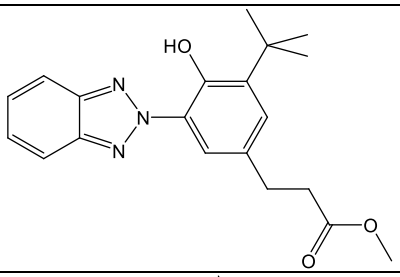
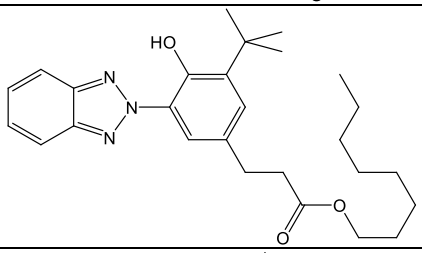
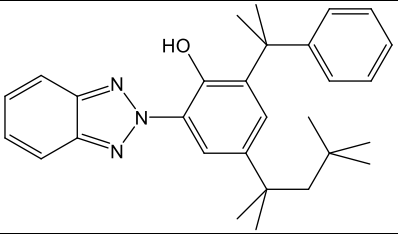
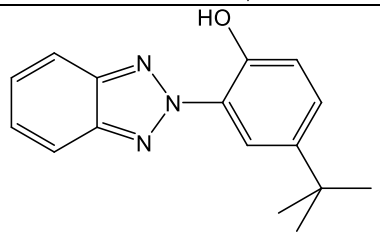
The Chemical Risk Information Platform (CHRIP) was utilized to identify category substances. CHRIP is a web database providing comprehensive information on risk assessments in addition to laws and regulations for chemicals (http://www.nite.go.jp/en/chem/chrip/chrip_search/systemTop). Chemicals of the CSCL inventory can be searched by their number, name, or structure. The database was searched to obtain chemicals with the core phenolic benzotriazole structure. The 12 substances found are listed in Table 2.

3.2. List of category members

List of the selected category members are shown in Table 2.

Table 2. List of phenolic benzotriazole category

No.	CAS No.	Chemical substance Name	Structural formula	Endpoint data
1	3846-71-7	2-(benzotriazol-2-yl)-4,6-bis(2-methylbutan-2-yl)phenol		Yes
2	25973-55-1	2-(2H-benzotriazol-2-yl)-4,6-ditertpentylphenol		Yes
3	3864-99-1	2,4-di-tert-butyl-6-(5-chloro-2H-benzotriazol-2-yl)phenol		Yes
4	36437-37-3	2-(benzotriazol-2-yl)-6-butan-2-yl-4-tert-butylphenol		Yes
5	70321-86-7	2-(benzotriazol-2-yl)-4,6-bis(2-phenylpropan-2-yl)phenol		Yes
6	2440-22-4	2-(benzotriazol-2-yl)-4-methylphenol		Yes
7	3147-75-9	2-(benzotriazol-2-yl)-4-(2,4,4-trimethylpentan-2-yl)phenol		Yes

No.	CAS No.	Chemical substance Name	Structural formula	Endpoint data
8	3896-11-5	2-tert-butyl-6-(5-chlorobenzotriazol-2-yl)-4-methylphenol		Yes
9	84268-33-7	methyl 3-[3-(benzotriazol-2-yl)-5-tert-butyl-4-hydroxyphenyl]propanoate		Yes
10	127519-17-9	octyl 3-[3-(benzotriazol-2-yl)-5-tert-butyl-4-hydroxyphenyl]propanoate		No
11	73936-91-1	2-(benzotriazol-2-yl)-6-(2-phenylpropan-2-yl)-4-(2,4,4-trimethylpentan-2-yl)phenol		No
12	3147-76-0	2-(benzotriazol-2-yl)-4-tert-butylphenol		No

4. JUSTIFICATION FOR DATA GAP FILLING

4.1. Data gathering

4.1.1. Empirical data

Publicly available repeated-dose toxicity data were collected from toxicity databases and literature searches for the 12 category members in the CSCL inventory. The OECD QSAR Toolbox (http://www.oecd.org/env/ehs/risk-assessment/oecd-qsar-toolbox.htm) and Hazard Evaluation Support System Integrated Platform (HESS) (http://www.nite.go.jp/en/chem/qsar/hess-e.html) databases were searched by chemical structure and repeated-dose toxicity data. Toolbox version 3.3 and HESS

version 3.2 were utilized in this study. Then, PubMed (http://www.ncbi.nlm.nih.gov/pubmed), TOXNET (http://toxnet.nlm.nih.gov/), and Google (https://www.google.co.jp/) were searched by chemical name and repeated-dose toxicity data. Using these search tools, toxicity data were obtained for 9 category members (1−9), which were used as source chemicals for read-across. Inclusion of additional source chemicals outside the CSCL inventory is supportive for category assessment. However, reliable data on repeated-dose toxicity were not found for the compounds. In addition, mechanistic information on metabolism and toxicity of category members was collected by literature search as described above.

4.1.2. Transcriptome data

Liver transcriptome data were obtained by the Percellome method, which generates an absolute copy number of mRNAs per cell (Kanno *et al*., 2006). Briefly, a mouse study was designed to monitor time-course and dose-response at the same time. GeneChip analysis was performed using the Mouse Genome 430 2.0 Array (Affymetrix) with "per cell" normalization (Kanno *et al*., 2006). Then, gene expression data were plotted on 3-dimensional surface graphs (Figure 2). In this study, 12-week-old male C57BL/6J mice were administered a single dose of the test substance (member 1, 3, 5, 6, or 7) by oral gavage (100, 300, or 1000 mg/kg bw), and the liver was sampled at 2, 4, 8, and 24 h post-administration. All animal experiments were approved by the Experimental Animal Use Committee of the National Institute of Health Sciences, Japan. Data analysis focused on upregulated probe sets as described previously (Kanno *et al*., 2013). Briefly, 3D-surface expression of Percellome data was generated with the *in-house* software RSort (Roughness Sort) for automatic selection of significantly induced probe sets (Figure 2). Automatically selected upregulated probe sets were then visually checked for their 3D-surface shapes to eliminate noisy data.

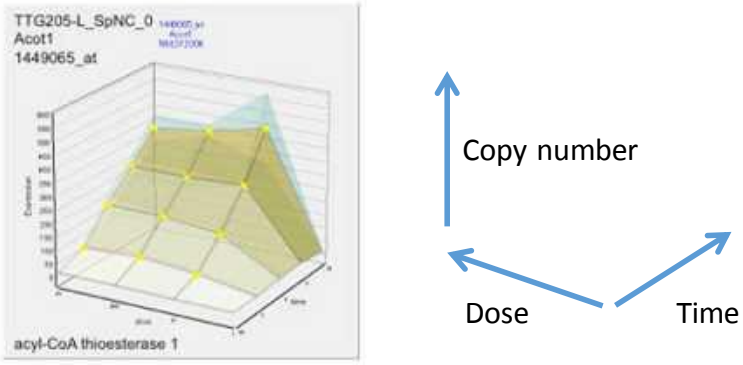


Figure 2. Three-dimensional surface expression of Percellome data

4.1.3. Predicted data

Models used for category justification are as follows. The KOWWIN model (ver.1.68) of EPI Suite was utilized for estimating logKow of category members. OECD QSAR Toolbox was applied to calculate structural similarity and to find the possible toxicological category for systemic effects based on the HESS profiler.

4.2. Data matrix

Chemical structures, toxicity data (observed effects and NO(A)EL or lowest-observed-(adverse)-effect level (LO(A)EL)), estimated logKow, and transcriptomic profiles for the phenolic benzotriazoles were compiled in a data matrix.

4.3. Justification

4.3.1 Absorption, distribution, metabolism, and excretion (ADME)

Member 6, 2-(benzotriazol-2-yl)-4-methylphenol (CAS: 2440-22-4), is considered rapidly and well absorbed from the gut as evidenced by recovery of almost 91% of radioactivity from urine and feces within 48 h following oral administration of the labeled compound to rats. The liver is the main target organ of this substance, and tissue distribution data indicated that the highest radioactivity remained in the liver. Additionally, urine was the predominant excretion route, suggesting a significant renal pathway. Furthermore, evidence that the substance causes enhancement of microsomal UDP-glucuronosyltransferase activity was obtained in rats after repeated oral administration (Schmid *et al*., 1980; Cosmetic Ingredient Review Panel, 2008).

In summary, member 6 has the lowest logKow among the category members, and is well absorbed from the gut, distributed to the liver as the primary target organ, and excreted mainly via the renal pathway. It can also be partially reabsorbed in the kidney. These ADME characteristics are consistent with the hepatotoxicity and relatively weaker nephrotoxicity of member 6.

Member 1, 2-(benzotriazol-2-yl)-4,6-bis(2-methylbutan-2-yl)phenol (CAS: 3846-71-7), was studied under both in vivo and in vitro experimental conditions (See Annex 1). Briefly, male and female rats were administered member 1 by gavage at 0.5, 2.5, or 12.5 mg/kg bw for 28 days, and the plasma levels measured. After the first administration, the substance was rapidly absorbed and eliminated from plasma in both sexes. After 28 days' administration, similar plasma profiles were observed. The calculated values of C_{max}, T_{max}, and $AUC_{0–24h}$ in plasma are given in Annex 2. Moreover, in all dose groups, metabolites of member 1 were not detected in plasma of either sex. In hepatic microsomes of male and female rats 28 days post-administration, the total CYP content was significantly increased in males of the 2.5 and 12.5 mg/kg bw groups. Lauric acid 12-hydroxylase activity was significantly increased at and above 0.5 mg/kg bw in males and at 12.5 mg/kg bw in females (Hirata-Koizumi *et al*., 2009).

In summary, Member 1 was shown to be absorbed rapidly but not metabolized. It is likely that structurally related members with two bulky substitutions (members 2, 3, 4, 5, and 11) are also well absorbed from the gut due to higher hydrophobicity and mainly distributed to the liver. Like member 1, these members are likely not metabolized because of low reactivity of the substituted groups on the phenolic ring.

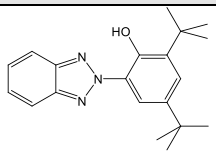
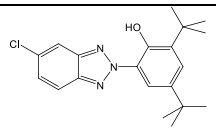
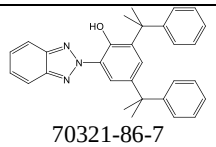
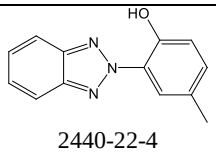
Member 9, methyl 3-[3-(benzotriazol-2-yl)-5-tert-butyl-4-hydroxyphenyl]propanoate (CAS: 84268-33-7), has also been investigated both in vitro and in vivo. Member 9 was shown to be hydrolyzed in rat serum (apparent K_m = 0.13 mM, apparent V_{max} = 1.13 μmol min^{-1} ml^{-1}) and rat liver homogenates (apparent K_m = 0.15 mM, apparent V_{max} = 0.59 μmol min^{-1} ml^{-1}). Metabolism in rat small intestine homogenates was less efficient (apparent K_m = 0.49 mM, apparent V_{max} = 2.15×10^{-4} μmol min^{-1} ml^{-1}) than in liver homogenates. In male rats (n = 2) orally administered 10 mg/kg bw, the maximum blood concentration (1.675 μg/g) reached between 1 and 2 h post-administration. The apparent half-life was less than 12 h and minimal amounts, equaling about 3% of the blood levels at T_{max}, remained 48 h after dosing. The carboxylic acid produced from hydrolysis of the parent substance was the major metabolite (Thomas *et al*., 1995).

The rat liver S9 metabolism simulator of OECD QSAR Toolbox predicts formation of a common carboxylic acid metabolite from members 9 and 10. It is generally established that aliphatic esters with short to medium chains are readily hydrolyzed in intestine, blood, and/or liver. Thus, it is logical to presume that a common carboxylic acid metabolite is generated from members 9 and 10.

4.3.2. Transcriptomic profiles

Members 1, 3, 5, 6, and 7 were monitored for transcriptome responses in mouse liver at 2, 4, 8, and 24 h after single oral administration at 100, 300, or 1000 mg/kg bw. These 5 tested substances were chosen as representatives of category structural diversity. The selection criterion was based on differences in structure and position of substituent(s) compared to member 1, the most hepatotoxic compound in this category. Member 2 differs by having a chlorine atom at the 5 position of the benzotriazole ring. Member 5 has the bulkiest substituents at 4 and 6 positions of the phenolic ring. Member 6 contains a methyl and member 7 branched alkyl chains at the 4 position, and neither has a substitution at the 6 position (Table 2). Members 5 and 6 are structurally most different in bulkiness of substituent on phenolic ring among the members. The number of upregulated probe sets 24 h post-treatment was 5480 for member 1, 3230 for member 3, 370 for member 5, 150 for member 6, and 250 for member 7. Members sharing similar upregulated probe sets were selected from the Percellome project data (168 datasets for liver samples) using the cross-referencing program Percellome Explorer (Kanno *et al*., 2013). These 5 members activated the constitutive androstane receptor (CAR), the pregnane X receptor (PXR), the peroxisome proliferator-activated receptor (PPAR), and/or nuclear factor (erythroid-derived-2)-like 2 (Nrf2) signaling pathways (Table 3). Recent studies revealed that activation of nuclear receptors CAR, PXR, and PPAR contribute to many physiological processes involving Phase I and II drug metabolizing enzymes. These receptors are also molecular targets of exogenous chemicals and are thought to be responsible for inducing hepatocyte hypertrophy (reviewed by Hall *et al*. 2012). Nrf2 is a master regulator of cellular responses against environmental stresses. Nrf2 is regulated by Keap1 (Kelch-like ECH-associated protein 1), which is a sensor for oxidative stress (reviewed by Tang *et al*., 2014; Suzuki and Yamamoto, 2015). It is believed that oxidative stress in hepatocytes induces liver injury (reviewed by Li *et al*., 2015). Based on the transcriptomic results, relative induction levels of the following gene sets were estimated at 24 h post-administration at the highest doses: Cyp1, Cyp2, Cyp3, Cyp4 (Phase I enzymes and biomarkers reflecting signaling of aryl hydrocarbon receptor (AhR), CAR, PXR, and PPAR, respectively), Nrf2-mediated phase II enzymes, and Nrf2/Keap1 (biomarkers of oxidative stress). A summary of the transcriptomic profiles is shown in Table 4.

Table 3. List of chemicals showing similar transcriptomic profiles to phenolic benzotriazoles

No.	Substance	Similar chemicals				
		1	**2**	**3**	**4**	**5**
1	 3846-71-7	Di(2-ethylhexyl) phthalate (DEHP) (CAR + PXR)	Chlofibrate (PPAR)	Mono(2-ethylhexyl) phthalate (MEHP) (CAR + PXR)	Penta chlorophenol (Nrf2)	Member 5
3	 3864-99-1	DEHP (CAR + PXR)	Chlofibrate (PPAR)	Member 1	MEHP (CAR + PXR)	Penta chlorophenol (Nrf2)
5	 70321-86-7	Chlofibrate (PPAR)	DEHP (CAR + PXR)	MEHP (CAR + PXR)	Estragol (PPAR)	Member 6
6	 2440-22-4	1,2-Dichloro-3-nitro benzene	PCB153	Member 5	1,2,3-Triazole	1,2,4-Triazole

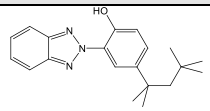
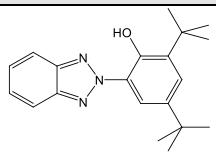
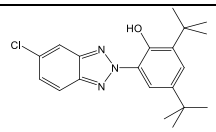
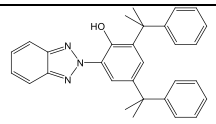
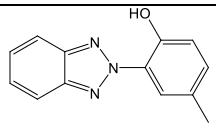
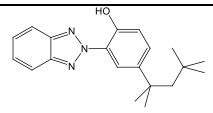
No.	Substance	Similar chemicals				
		1	2	3	4	5
7	 3147-75-9	DEHP (CAR + PXR)	Red No. 225	Member 5	PCB153	1,2,3-Triazole

Table 4. Summary of mouse liver transcriptomic profiles following single oral treatment with phenolic benzotriazoles

No.	Substance	Upregulated probe sets	Gene induction*					
			Phase I				Phase II	
			AhR-Cyp1	CAR-Cyp2	PXR-Cyp3	PPAR-Cyp4	Nrf2-phase II enzymes	Nrf2 + Keap1
1	 3846-71-7	5480	0	100	100	100	100	100
3	 3864-99-1	3230	0	50	80	100	50	80
5	 70321-86-7	370	0	0	0	30	5	0
6	 2440-22-4	150	0	0	0	0	10	0
7	 3147-75-9	250	0	40	0	40	0	0

*Semiquantitative relative degree of induction compared to that of member 1
#Detailed report in preparation

Member 1 induced transcription of CAR- and PXR-dependent CYP genes, Nrf2-dependent Phase II enzymes, and Keap1/Nrf2-dependent phase II enzymes. The transcriptional profile of member 3 was similar to that of member 1, although the induction levels were lower (except for PPAR-mediated Cyp4). Neither agent induced AhR-dependent Cyp1.

Member 1 strongly induced transcription of Cyp4, suggesting PPAR activation. This result was expected, since it was previously demonstrated that lauric acid 12-hydroxylase activity was significantly increased in the rat liver microsomal fraction after repeated administration of member 1 (Hirata-Koizumi *et al*., 2009). In addition to enhanced transcription of CAR- and PXR-dependent CYP genes as well as Nrf2/Keap1- and Nrf2-dependent phase II enzymes, member 1 induced genes related to mitochondrial uncoupling and the ubiquitin pathway. These results suggest that the severe

hepatotoxicity induced by member 1 is caused by perturbation of several major hepatic signaling networks. The induction levels of upregulated probe sets were actually very low at 2 and 4 h post-administration, consistent with the toxicokinetic profile of member 1, where maximum plasma levels were not reached until 6−7 h after dosing (Hirata-Koizumi *et al*., 2009).

Members 5 and 7 both induced Cyp4 genes. However, member 5 increased transcription of genes encoding phase II enzymes while member 7 enhanced transcription of phase I Cyp2 genes. Overall, members 5 and 7 exhibited distinct transcriptomic profiles and the induction levels were relatively low for both.

Member 6 weakly induced transcription of genes encoding Nrf2-dependent phase II enzymes. Chemical activation of Nrf2 in mice resulted in induction of genes encoding UDP-glucuronosyltransferases in the liver (Buckley and Klaassen, 2009). This result is consistent with a previous study demonstrating that repeated administration of member 6 enhances hepatic microsomal UDP-glucuronosyltransferase activity in rats (Schmid *et al*., 1980; Cosmetic Ingredient Review Panel, 2008).

4.3.3. Endpoint data

Inhalation:

No information is available for repeated inhalation exposure toxicity of any category substance.

Dermal:

No information is available for repeated dermal exposure toxicity of any category substance.

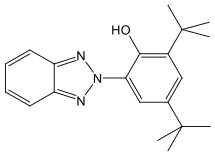
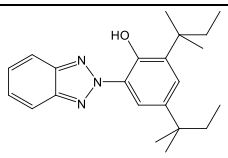
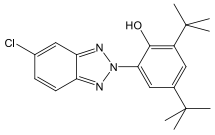
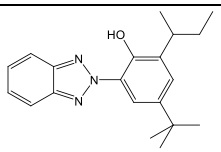
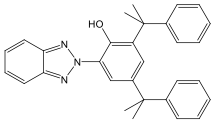
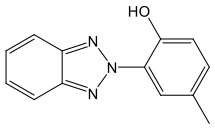
Oral:

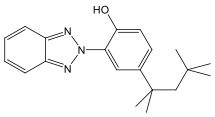
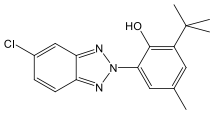
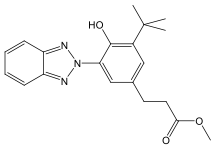
Among the 12 category substances, reliable information on repeated oral dose toxicity was obtained for 9 (members 1−9). A summary of oral repeated-dose toxicity studies in rats is presented in Table 5. In the case of substances for which multiple GLP studies are available, the study showing the lowest NOAEL was selected. Available information obtained for each substance is summarized individually in Annex 3. The target organ of all 9 phenolic benzotriazole category members was the liver. Among the 9 substances, the lowest NOAELs varied from 0.1 mg/kg bw/day (member 1) to beyond 5000 mg/kg bw/day (member 7). Thus, although the target organ was the same, the toxicity levels among these category chemicals varied markedly.

Nephrotoxic effects were also observed in one of the two GLP studies of members 1 and 4, and in one GLP study of member 6. Additionally, anemia-like changes were found in both GLP studies of member 1 and one of the two GLP studies of member 4. However, all these nephrotoxic effects appeared at the same or higher dose at which hepatotoxicity appeared. Other members showed only hepatotoxic effects.

Taken together, these studies indicate that liver is a common target organ of phenolic benzotriazoles. Members 5 and 9 used for read-across later in this document showed exclusive hepatotoxicity. Member 6 also caused primarily liver toxicity, with nephrotoxicity observed only at higher doses in female rats following pregnancy.

Table 5. Summary of repeated oral dose toxicity of phenolic benzotriazoles in rats

No.	Substance	Period	Dose	Toxic effects	NO(A)EL	Reference
1	 (CAS: 3846-71-7)	52 weeks	0, 0.1, 0.5, 2.5 mg/kg bw/d (male), 0, 0.5, 2.5, 12.5 mg/kg bw/day (female)	Liver: weight increase, hypertrophy of hepatocytes (0.5 mg/kg bw/day in male, 12.5 mg/kg bw/d in female), altered hepatocellular foci (0.5 mg/kg bw/day in male), cystic degeneration, and lipofuscin deposition in hepatocytes (2.5 mg/kg bw/day in male) Hematological effects (0.5 mg/kg bw/day in male),	NOAEL: 0.1 mg/kg bw/day (0.0003 mmol/kg bw/day)	Hirata-Koizumi *et al*., 2008, cited in NTP, 2011 (GLP study)
2	 (CAS: 25973-55-1)	90 days	0, 100, 200, 400, 800, 1600 ppm	Liver: foci of necrosis, bile duct proliferation Parenchymal cells enlarged (200 ppm) Kidney: tubular necrosis (200 ppm in male) Hematological effects (200 ppm in male)	NOAEL: 100 ppm (ca. 20 mg/kg bw/day, 0.057 mmol/kg bw/day)	Til *et al*., 1968, cited in EPA, 2009 (non-GLP study)
3	 (CAS:3864-99-1)	56−57 days (male), 55−69 days (female)	0, 2.5, 25, 250 mg/kg bw/day	Liver: weight increase (25 mg/kg bw/day in male)	NOEL: 2.5 mg/kg bw/day (0.007 mmol/kg bw/day)	Ema *et al*., 2008, cited in NTP, 2011 (GLP study)
4	 (CAS: 36437-37-3)	42-day (male), 44–56-day (female)	0, 0.5, 2.5, 12.5, mg/kg bw/day	Liver: weight increase (12.5 mg/kg bw/day)	NOEL: 2.5 mg/kg bw/day (0.008 mmol/kg bw/day)	METI, 2011, collected in this study (GLP study)
5	 (CAS: 70321-86-7)	90 days	0, 50, 300, 2000, 10000 ppm	Liver: weight increase, hypertrophy, and/or cytoplasmic vacuolation of hepatocytes (2000 ppm in male, 300 ppm in female)	NOAEL: 50 ppm (ca. 2.5 mg/kg bw/day, 0.0056 mmol/kg bw/day)	Basler *et al*., 1987. cited in EPA, 2009 and NTP, 2011 (GLP study)
6	 (CAS: 2440-22-4)	42 days (male), 42−53 days (female)	0, 30, 100, 300 mg/kg bw/day	Liver: weight increase, hypertrophy of hepatocytes (30 mg/kg bw/d in male, 100 mg/kg bw/day in female) Kidney: degeneration and regeneration in proximal tubules (100 mg/kg bw/day in female)	NOAEL: <30 mg/kg bw/day (<0.133 mmol/kg bw/day)	METI, 2007, cited in NTP 2011 (GLP study)

No.	Substance	Period	Dose	Toxic effects	NO(A)EL	Reference
7	 (CAS: 3147-75-9)	30 days	0, 12500, 25000, 50000 ppm	No effects seen up to the highest dose	NOAEL: 50000 ppm (5658 mg/kg bw/day) (17.49 mmol/kg bw/day)	American Cyanamid Company, 1968. cited in EPA, 2009 and NTP, 2011 (non-GLP study)
8	 (CAS: 3896-11-5)	42 days (male), 44−56 days (female)	0, 62.5, 250, 1000 mg/kg bw/day	No effects seen up to the highest dose	NOAEL: 1000 mg/kg bw/day (3.167 mmol/kg bw/day)	MHLW, Japan, 2007, collected in this study (GLP study)
9	 (CAS: 84268-33-7)	28 days	0, 50, 200, 1000 mg/kg bw/day	Liver: necrosis (50 mg/kg bw/day in male), diffuse hypertrophy of hepatocytes (50 mg/kg bw/day in female)	NOAEL: < 50 mg/kg bw/day (<0.141 mmol/kg bw/day)	Ciba-Geigy Corporation, 1986, cited in NTP, 2011 (GLP study)

4.3.4. Observed trends between chemical structure and endpoint data

Member 1 has two tert-butyl substituents at 4 and 6 positions of the phenolic ring and no substituent at the benzotriazole ring. Member 1 showed hepatotoxicity at a significantly lower dose than the other members.

Member 2 is quite similar to member 1 in terms of overall molecular structure, position and structure of substituents, physicochemical properties, and chemical reactivity. No metabolism and transcriptomic data are available for this chemical. However, it is unlikely that the single methylene group extension of the branched alkyl chain (compared to member 1) confers a different mode of action. When member 2 was administered to rats via their food for 13 weeks, similar hepatotoxic findings of focal necrosis and bile duct hyperplasia were identified at doses ≥ 200 ppm (ca. 40 mg/kg bw/day) (Til *et al*., 1968).

Member 3 contains one chlorine atom substitution on the benzotriazole ring. The hepatotoxic potential is apparently lower than that of member 1 since member 3 did not induce degenerative or necrotic changes in the liver even at 250 mg/kg bw/day following administration for over 50 days. However, the transcriptomic profile of member 3 was similar to that of member 1, suggesting a similar mode of action. It is logical to presume that member 3 will produce similar hepatotoxic effects at a dose over 250 mg/kg bw/day.

Member 4 has one different branched alkyl substituent at the 6 position of the phenolic ring compared to member 1. Member 4 is very similar to members 1 and 2 in structure and physicochemical properties, and appears to have hepatic effects equivalent to member 3. Taken together, members 1−4 can be further grouped into a subcategory based on a possibly similar mode of action (subcategory 1), although the degree of hepatotoxicity appears sensitive to small structural changes at the 6 position of the phenolic ring.

Members 5 and 6 both induced histopathological changes in the liver despite markedly different physicochemical properties. The liver transcriptomic profiles of members 5 and 6 appear distinct from members 1 and 3. Members 7 and 8 had no adverse effects at over 1000 mg/kg bw/day despite structural similarities to members 1–3. However, like members 1 and 3, member 7 increased

transcription of PPAR-dependent Cyp4 genes (Table 4). It is thus possible that hepatotoxic effects, such as hepatocyte hypertrophy, were not identified in the review of member 7 toxicity data. Member 9 has two substituents, one of which is a straight chain alkanoate substituent at position 4 of the phenolic ring not shared by other members. This substance therefore cannot be classified into any subcategory since it is difficult to exclude the possibility of different modes of action due to the straight chain alkanoate. Further evidence is needed for subcategorization.

Taken together, members 1−4 were grouped into subcategory 1. Members 5, 6, and 7 may belong to different subcategories based on distinct transcriptomic profiles. Members 8 and 9 were not assigned to any subcategory. Additional mechanistic information may support subcategorization of these members. Moreover, subcategorization was also attempted for the untested category members 10-12 below for read-across.

5. STRATEGY FOR DATA GAP FILLING AND INTEGRATED CONCLUSIONS

5.1. Uncertainty

Factor	Uncertainty (low, medium, high)	Comment
Structural boundary of the category and subcategories	High	The category substances have a common basic structure of phenolic benzotriazole with structurally variable substituents. Their toxicity levels may be dependent on the number and structure of the substituents. Forming subcategories could reduce uncertainty of the overall category assessment. However, it is difficult to define structural boundaries of subcategories because small structural changes in source chemicals result in markedly different toxicity levels.
Mode of action/AOP for forming subcategories	Medium	Liver transcriptomic profiles were generated for 5 source substances selected as representative of the structural diversity of phenolic benzotriazoles. Results revealed activation of nuclear receptors and Phase I/II enzymes as well as induction of oxidative stress, responses that may underlie the observed hepatotoxicity. Several members have similar transcriptomic profiles, similar toxicological properties, and possibly common modes of action that support subcategorization. However, involvement of other pathways in the hepatotoxic effects by category members cannot be ruled out. Transcriptomic testing of other members and more detailed comprehensive analysis are needed to strengthen the selection of closest analogs.
Similarity of source chemicals for read-across	Low	For member 10, it is likely that the selected analog (member 9) is suitable for read-across because hydrolysis produces the same carboxylic acid metabolite linked to hepatotoxicity.
	High	For member 11, there is uncertainty as to the similarity of the selected analog. Member 5 was chosen based on structural similarity, having only one different substituent at position 4 of the phenolic ring. The uncertainty could be reduced by obtaining mechanistic information on member 11 for comparison with member 5 in order to elucidate the influence of phenyl substituents at the phenolic ring. Moreover, ADME data demonstrating similar absorption/distribution properties and metabolic pathways will be needed.

Factor	Uncertainty (low, medium, high)	Comment
	High	For member 12, there is also uncertainty in the similarity of the selected analog. Members 7 and 6 differ only in the bulky alkyl chain length at the 4 position of the phenolic ring. Member 12 is structurally intermediate between the two source substances. However, transcriptomic data is not available for member 12. The uncertainty could be reduced by mechanistic information on member 12 for comparison with members 6 and 7.
Quality of the target endpoint data used for the read-across	Low	For read-across of member 10, one test study of member 9 was used. Quality of the test data is considered high. It is a GLP study showing that the toxic effects are dose-dependent.
	Low	For read-across of member 11, one test study of member 5 was used. Quality of the test data is considered high, with a reliability code of 1 (reliable without restriction) by the US Challenge Program.
	Low	For read-across of member 12, test data of member 6 hepatotoxicity was used. That is the only test study available for member 6. Quality of the test data is considered high because it was performed in compliance with OECD TG 422 and GLP. The study revealed nephrotoxic effects in rats after pregnancy. Pregnant rats appeared more sensitive to kidney toxicity than normal female rats. Effects in the liver are more relevant for read-across.
Use of read-across	Low	Member 10 likely undergoes metabolic hydrolysis to form a carboxylic acid metabolite identical to that of member 9. Given that the metabolite may be responsible for hepatotoxicity, it is appropriate to conduct read-across using toxicity data of member 9.
	High	For member 11, read-across was performed using toxicity data of member 5. Availability of toxicity data for only one analog increases uncertainty of read-across assessment. Use of data for a couple of analogs could decrease the uncertainty of read-across.
	High	Members 7 and 6 are structural analogs of member 12 (target substance) among the category substances. However, NOAEL differs substantially between the two source substances. It is over 5000 mg/kg bw/day for member 7 (non-GLP study) and less than 30 mg/kg bw/day for member 6 based on hepatotoxicity (GLP study). Source chemicals have distinct transcriptomic profiles, and member 12 has not been tested for transcriptional effects. Moreover, this case study is for screening assessment of CSCL. Thus, read-across is performed using member 6 as a more conservative choice based on higher quality study data.

5.2. Integrated conclusion

Data gap filling by read-across was carried out for three substances, members 10, 11, and 12, as follows.

Member 10 is octyl 3-[3-(benzotriazol-2-yl)-5-tert-butyl-4-hydroxyphenyl]propanoate (CAS: 127519-17-9). The nearest structural analog is member 9, methyl 3-[3-(benzotriazol-2-yl)-5-tert-butyl-4-hydroxyphenyl]propanoate (CAS: 84268-33-7). The two substances differ only in the length of the straight alkyl ester chain substituent at the 4 position of the phenolic ring. It was shown that member 9 was readily hydrolyzed to generate the carboxylic acid metabolite 3-(2H-benzotriazol-2-yl)-5-(1,1-dimethylethyl)-4-hydroxy-benzenepropanoic acid (Thomas *et al*., 1995). No empirical metabolism data is available for member 10. However, the rat liver S9 metabolism simulator of

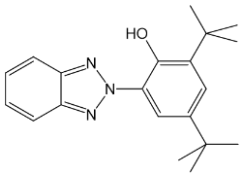
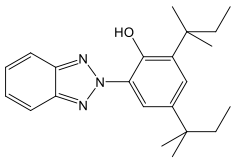
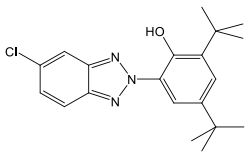
OECD QSAR Toolbox predicted that this same carboxylic acid is a metabolite of member 10 (See Appendix). Given the broad substrate specificity of lipases and carboxylesterases, it is plausible that member 10 is hydrolyzed to the same carboxylic acid metabolite. If the carboxylic acid metabolite is an active form responsible for hepatotoxicity of member 9, it is assumed that member 10 will produce hepatotoxicity by the same mode of action. A previous rat study showed that the liver damage resulting from member 9 and that from the carboxylic acid metabolite were clearly related to the induction of peroxisomal proliferation. These similar effects suggest that the carboxylic acid metabolite is responsible for member 9 hepatotoxicity (Thomas *et al*., 1995). The NOAEL and LOAEL of member 10 are estimated to be less than 0.141 mmol/kg bw/day (64 mg/kg bw/day) and 0.141 mmol/kg bw/day (64 mg/kg bw/day), respectively, by read-across using the hepatotoxicity data of member 9. There may be ADME differences between members 9 and 10 even if the mode of action is the same. However, pancreatic lipases exhibit a higher hydrolytic rate for esters of C6 alcohols than esters of C1 alcohols (Mattson and Volpenhein, 1969). It is possible that production of carboxylic acid metabolite at higher rates in the gut may decrease the rate of absorption by the gut. Thus, ADME data of member 10 is critical for precise estimation of toxicity levels. Nephrotoxicity is not expected for member 10 because such effects were not observed in the study of member 9 used for read-across.

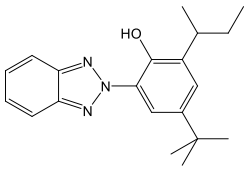
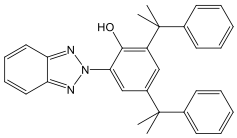
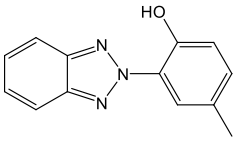
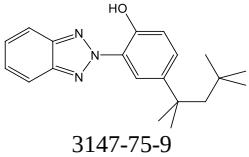
Member 11 is 2-(benzotriazol-2-yl)-6-(2-phenylpropan-2-yl)-4-(2,4,4-trimethylpentan-2-yl)phenol (CAS: 73936-91-1). Member 5 (2-(benzotriazol-2-yl)-4,6-bis(2-phenylpropan-2-yl)phenol) is considered an analog due to structurally similar substituents at 4 and 6 positions of the phenolic ring. Liver is the primary target organ of member 5. NOAEL of member 11 is estimated to be 0.0056 mmol/kg bw/day (2.5 mg/kg bw/day) using the value of member 5. Possible nephrotoxicity cannot be excluded due to the structural differences in the substituent group at the 4 position of member 11. To reduce uncertainty in selecting an analog of member 11, it is necessary to generate the profile of transcripts responsible for activation of the nuclear receptor pathways and induction of oxidative stress in hepatocytes by member 11 for comparison with the effects of member 5. Moreover, ADME data demonstrating similar absorption/distribution properties and metabolic pathways will be needed.

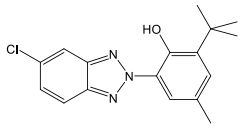
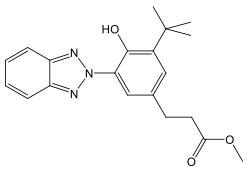
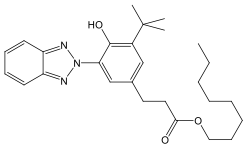
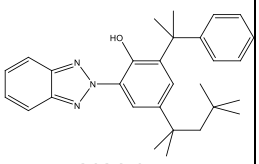
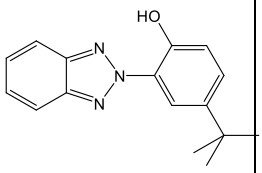
Member 12 is 2-(benzotriazol-2-yl)-4-tert-butylphenol (CAS: 3147-76-0). Members 7 and 6 are closest structural analogs of member 12 among the category substances. They differ in the length of the branched alkyl substituent at the 4 position of the phenolic ring. Member 12 is intermediate between members 7 and 6 in terms of structural complexity of the substituted group. However, NOAEL differs substantially between the two source substances. It is over 5000 mg/kg bw/day for member 7 (non-GLP study) and less than 30 mg/kg bw/day for member 6 based on hepatotoxicity (GLP study). The higher NOAEL of member 7 may reflect the study authors not considering hepatocyte hypertrophy as having toxicological significance. This notion is supported by the transcriptomic profile of member 7 showing activation of CAR and PPAR pathways. On the other hand, high-quality study results are available for member 6. Members 7 and 6 have different transcriptomic profiles and member 12 has not been tested for effects on gene transcription. Moreover, this case study is for screening assessment of CSCL. Thus, read-across is performed based on member 6 data as a more conservative choice based on higher quality. Hepatotoxicity is expected and NOAEL is estimated to be less than 0.133 mmol/kg bw/day (35 mg/kg bw/day) for member 12.

It is unclear whether member 12 is associated with kidney toxicity. Phenolic benzotriazoles tend not to have toxic effects on kidney, or such effects only appear at higher dose than needed to induce hepatotoxicity. For members 1 and 4, one of two GLP studies showed nephrotoxicity. For member 6, one GLP study revealed nephrotoxic effects in rats after pregnancy (See data matrix). That study suggested that pregnant rats are more sensitive to nephrotoxicity because such effects were not observed in a satellite female group at the end of the recovery period (METI, 2007). Hence, it appears that effects in kidney are not generated by a specific mode of action. It is possible that member 12 may show effects in kidney, but it is not likely that kidney is a primary target organ.

Table 6. Summary of integrated conclusions

No.	Chemical structure Chemical name CAS No.	Sub Category	Trans criptomic profile	Repeated-dose toxicity		
				Experimental results (GLP/non-GLP)	Integrated conclusion (read-across)	D value (hazard assessment value)*
1	 3846-71-7	1	CAR PXR PPAR Nrf2	Species: rats Dosing: 0, 0.1, 0.5, 2.5 mg/kg bw/day (male), 0, 0.5, 2.5, 12.5 mg/kg bw/day (female), 52 weeks by gavage NO(A)EL: 0.1 mg/kg bw/day (0.0003 mmol/kg bw/day) Liver effects: weight increase, hypertrophy of hepatocytes (0.5 mg/kg bw/day in male 12.5 mg/kg bw/day in female), altered hepatocellular foci (0.5 mg/kg bw/day in male) (Hirata-Koizumi *et al*., 2008) GLP study		0.001 mg/kg bw/day
2	 25973-55-1	1	Not tested	Species: rats Dosing: 0, 100, 200, 400, 800, 1600 ppm, 90-day feeding NO(A)EL: 100 ppm (ca. 20 mg/kg bw/day, 0.057 mmol/kg bw/day) Liver effects: focal necrosis, bile duct proliferation, parenchymal cells enlarged (200 ppm in male and female) Kidney effect: tubular necrosis (200 ppm in male), Hematological effects (200 ppm in male), (Til *et al*., 1968) Non-GLP study		0.010 mg bw/kg/day
3	 3864-99-1	1	CAR PXR PPAR Nrf2	Species: rats Dosing: 0, 2.5, 25, 250 mg/kg bw/day, 56−57 days for male, 55−69 days for female by gavage NOEL: 2.5 mg/kg bw/day (0.007 mmol/kg bw/day) Liver effect: weight increase (25 mg/kg bw/day in male) non-adverse (Ema *et al*., 2008) GLP study		0.004 mg/kg bw/day

No.	Chemical structure Chemical name CAS No.	Sub Category	Transcriptomic profile	Repeated-dose toxicity		
				Experimental results (GLP/non-GLP)	Integrated conclusion (read-across)	D value (hazard assessment value)*
4	 36437-37-3	1	Not tested	Species: rats Dosing: 0, 0.5, 2.5, 12.5 mg/kg bw/days, 42 days (male), 41−55 days (female) by gavage NO(A)EL: 2.5 mg/kg bw/day (0.008 mmol/kg bw/day) Liver effect: weight increase (12.5 mg/kg bw/day) Kidney effect: weight increase (12.5 mg/kg bw/day) (METI, 2011) GLP study		0.004 mg/kg bw/day
5	 70321-86-7	(2)	PPAR	Species: rats Dosing: 0, 50, 300, 2000, 10000 ppm, 90 days by feeding NO(A)EL: 50 ppm (ca. 2.5 mg/kg bw/day (0.0056 mmol/kg bw/day) Liver effects: weight increase, hypertrophy, and/or cytoplasmic vacuolation of hepatocytes (2000 ppm in male, 300 ppm in female), (Basler, Phil II W. and Gfeller, W., 1987) GLP study		0.0125 mg/kg bw/day
6	 2440-22-4	(3)	Nrf2	Species: rats, Dosing: 0, 30, 100, 300 mg/kg bw/day, 42 days (male), 42−53 days (female) by gavage NOEL: < 30 mg/kg bw/day (< 0.133 mmol/kg bw/day) Liver effects: weight increase (30 mg/kg bw/day in male, 100 mg/kg bw/day in female), hypertrophy of hepatocytes (300 mg/kg bw/day in male, 100 mg/kg bw/day in female), Kidney effects: degeneration and regeneration in proximal tubules (100 mg/kg bw/day in female), (METI, 2007) GLP study		0.005 mg/kg bw/day
7	 3147-75-9	(4)	CAR PPAR	Species: rats Dosing: 0, 12500, 25000, 50000 ppm, 30 days of feeding NO(A)EL: 5658 mg/kg bw/day (17.49 mmol/kg bw/day) No effects, (American Cyanamid Company, 1968) Non-GLP study		9.43 mg/kg bw/day

No.	Chemical structure Chemical name CAS No.	Sub Category	Trans criptomic profile	Repeated-dose toxicity		
				Experimental results (GLP/non-GLP)	Integrated conclusion (read-across)	D value (hazard assessment value)*
8	 3896-11-5	Not defined	Not tested	Species: rats Dosing: 0, 62.5, 250, 1000 mg/kg bw/day, 42 days for male, 44−56 days for female by gavage NO(A)EL: 1000 mg/kg bw/day (3.167 mmol/kg bw/day) No effects (MHLW., 2007) GLP study		5 mg/kg bw/day
9	 84268-33-7	Not defined	Not tested	Species: rats Dosing: 0, 50, 200, 1000 mg/kg bw/day, 28 days by gavage NO(A)EL: < 50 mg/kg bw/day (< 0.141 mmol/kg bw/day) Liver effects: weight increase, necrosis, hypertrophy of hepatocytes (50 mg/kg bw/day in male), diffuse hypertrophy of hepatocytes (50 mg/kg bw/day in female) (Ciba-Gaigy, 1986) GLP study		0.008 mg/kg bw/day
10	 127519-17-9	Not defined	Not tested		NO(A)EL: <64 mg/kg bw/day) (< 0.141 mmol/kg bw/day Hepatotoxic effects	0.011 mg/kg bw/day
11	 73936-91-1	(2)	Not tested		NO(A)EL: 2.5 mg/kg bw/day (0.0056 mmol/kg bw/day) Hepatotoxic effects	0.004 mg/kg bw/day
12	 3147-76-0	Not defined	Not tested		NO(A)EL: < 35 mg/kg bw/day (< 0.133 mmol/kg bw/day) Hepatotoxic effects	0.006 mg/kg bw/day

*D (mg/kg bw/day) = NOEL (mg/kg bw/day) /uncertainty factor.
If NOEL was not available, D = LOEL (mg/kg bw/day)/uncertainty factor.

Screening assessment under CSCL is conducted for human health and/or ecological concerns to select priority assessment chemical substances. The human health endpoints include repeated-dose toxicity, reproductive and developmental toxicity, genotoxicity, and carcinogenicity. Hazard classes

are assigned based on the hazard assessment value (D value). Then, the most severe class among the endpoints is applied to the target chemical.

The D value of repeated-dose toxicity is described by the following equation: D = NOEL/uncertainty factor. The uncertainty factor is calculated by multiplying the species difference (10), the individual difference (10), the study duration factor (1: if the NOEL is derived from a 1-year study or longer, 2: 13-week to 1-year study, 6: 13-week or shorter), and severity of toxicity (1: no severe toxicity was observed). If the NOEL is not available, LOEL is used to calculate the D value with uncertainty factor 10. Hazard classification of the toxicity endpoint is based on the following criteria: Class 2, $D \leq 0.005$; Class 3, $0.005 < D \leq 0.05$; Class 4, $0.05 < D \leq 0.5$; Out of Class, $D > 0.5$.

Consequently, D values for repeated-dose toxicity of category members were estimated to be 0.001 to 9.43 mg/kg bw/day (Table 6). Hazard classification of phenolic benzotriazole category is as follows. Member 1, 3, 4, 6, and 11, Class 2; members 2, 5, 9, 10, and 12, Class 3; members 7 and 8; Out of Class.

In this case study, category assessment of phenolic benzotriazoles was performed for repeated-dose hepatotoxicity. As indicated, the category members are structurally related but toxicologically distinct. Thus, transcriptomic profiles were integrated into the assessment. Liver transcriptomic profiles strongly suggest activation of nuclear receptor pathways and/or induction of oxidative stress, which are likely associated with the observed liver effects. Moreover, it was revealed that even minor structural differences affect transcriptomic profiles. The results demonstrate that transcriptomic data could be supportive for subcategory formation of phenolic benzotriazoles based on possible mode of actions. It may be possible to apply the category/subcategories defined in this study for screening level hazard classification under CSCL. However, transcriptomic data were not available for all members, and mode of action/AOP was not well described due to currently limited resources for testing and data analysis. Gene expression profiling of the data-poor members will help to strengthen the selection of closest analogs for read-across and reveal mode of actions/AOPs for phenolic benzotriazoles.

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ANNEX 1. SUMMARY OF PHENOLIC BENZOTRIAZOLE ADME AND TOXICOKINETIC DATA

In vitro studies:

Reliable study reports are available for member 1, 2-(benzotriazol-2-yl)-4,6-bis(2-methylbutan-2-yl)phenol (CAS: 3846-71-7) and member 9, methyl 3-[3-(benzotriazol-2-yl)-5-tert-butyl-4-hydroxyphenyl]propanoate.

Member 1: 2-(benzotriazol-2-yl)-4,6-bis(2-methylbutan-2-yl)phenol (CAS: 3846-71-7)

The enzymatic transformation of member 1 was investigated using hepatic S9 fractions and microsomes prepared from male and female rats, and using microsomes containing cDNA-expressed individual rat cytochrome P450 (CYP) enzymes. These in vitro studies was performed in combination with an in vivo rat study of changes in member 1 plasma concentrations after repeated oral administration designed to investigate gender difference in repeated-dose toxicity. The in vitro study using hepatic microsomes from male and female rats found that the substance was slightly metabolized, but no sex differences were found in the residual substance ratio after a 60-minute incubation with an NADPH-generation system. In the experiments using microsomes containing cDNA-expressed individual rat CYP enzymes, CYP1A1 exhibited the greatest metabolic activity, and CYP1A2, 2A2, 2B1, 2C6, 2C11, and 2D2 also partially metabolized member 1 (Hirata-Koizumi *et al*., 2009).

Member 9: methyl 3-[3-(benzotriazol-2-yl)-5-tert-butyl-4-hydroxyphenyl]propanoate

Enzymatic hydrolysis of member 9 was examined in rat serum, liver, and small intestine. The substance was readily hydrolyzed by rat serum as well as by liver homogenate, while a homogenate of rat small intestine was three orders of magnitude less effective than liver per gram of tissue. Hydrolysis was determined in 50 mM Tris-phosphate buffer, pH 7.5, containing either 1% (v/v) rat serum, 1.25% (w/v) rat liver homogenate, or 10% (w/v) small intestine homogenate. The kinetic parameters were as follows: in rat serum, apparent K_m = 0.13 mM and apparent V_{max} = 1.13 μmol min^{-1} ml^{-1}; in rat liver homogenate, apparent K_m = 0.15 mM and apparent V_{max} = 0.59 μmol min^{-1} ml^{-1}; in rat small intestine homogenate, apparent K_m = 0.49 mM and apparent V_{max} = 2.15×10^{-4} μmol min^{-1} ml^{-1} (Thomas *et al*., 1995).

In vivo studies:

One study was conducted for member 1 in which the plasma level of the unaltered substance was traced in rats after the first and last of 28 daily oral administrations. Furthermore, two reliable reports are available on the basic toxicokinetics of member 6, 2-(benzotriazol-2-yl)-4-methylphenol (CAS: 2440-22-4). One is a typical study on distribution and excretion using radiolabeled compound, and the other is related to the effects on enzyme induction in the liver of rats treated with repeated doses. One report is available for member 9, methyl 3-[3-(benzotriazol-2-yl)-5-tert-butyl-4-hydroxyphenyl]propanoate (CAS: 84268-33-7), in which the plasma levels of the unaltered substance and the carboxylic acid metabolite were measured in rats for 168 h after a single oral administration of radiolabeled compound.

No information is available for the basic toxicokinetics of the other 9 members.

The results of these studies are summarized as follows.

Member 1: 2-(benzotriazol-2-yl)-4,6-bis(2-methylbutan-2-yl)phenol (CAS: 3846-71-7)

In order to explain the gender difference in hepatotoxicity observed following repeated oral doses, the time course of plasma levels were analyzed in male and female SD rats (4 animals/sex/dose) given the

substance orally by gavage at 0, 0.5, 2.5, or 12.5 mg/kg bw/day for 28 days. Plasma profiles were examined on both the first and the last day of administration. The substance was rapidly absorbed and eliminated from the plasma in both sexes. No clear gender differences were found in the plasma profiles at any dose. After 28 days of repeated administration, similar plasma profiles were observed, and again there were no gender differences (Hirata-Koizumi *et al*., 2009).

Member 6: 2-(benzotriazol-2-yl)-4-methylphenol (CAS: 2440-22-4)

Distribution and excretion were examined in male rats treated with member 6 ^{14}C-labeled in the benzene ring and the 4-methyl group. A dose of 10 mg/kg bw in PEG 400 solution was given by single oral administration to a group of 4 rats, and urine and feces samples were collected at 24-h periods until 168 h after dosing, when the animals were sacrificed for harvesting of organs and tissues. The radioactivity remaining in the urine, feces, organs, and tissues were then measured. Within 48 h after administration, ca. 91% of the total radioactivity was eliminated from the body, and radioactivity was almost completely eliminated from the body within 168 h, with about 73% having been recovered from the urine and 23% from the feces. The highest level of radioactivity was present in the liver, which contained 0.1% of the initial dose (Schmid *et al*., 1980; Cosmetic Ingredient Review Panel, 2008).

In another test, male rats (10 animals/group) were administered member 6 orally at 300 mg/kg bw/day for 14 or 28 days. Following the administration period, livers were removed and weighed. Then, activities of several drug-metabolizing enzymes were measured in liver microsomal fractions. Repeated administration of the substance caused an increase in relative liver weight, which persisted to some extent 28 day after the last administration. Microsomal UDP glucuronosyltransferase was enhanced in the treatment groups, whereas activities of drug-metabolizing enzymes, glucose-6-phosphate, and lysosomal acid hydrolases were only marginally stimulated or remained essentially unchanged (Schmid *et al*., 1980; Cosmetic Ingredient Review Panel, 2008).

Member 9: methyl 3-[3-(benzotriazol-2-yl)-5-tert-butyl-4-hydroxyphenyl]propanoate (CAS: 84268-33-7)

When given to rats (n = 2) orally at 10 mg/kg, ^{14}C-phenyl-labeled member 9 was readily absorbed from the gastrointestinal tract. Blood radioactivity reached a maximum in 1 to 2 h and was subsequently eliminated with an apparent half-life of 10−11.8 h. After 48 h, only minute amounts of radioactivity, equaling about 3% of blood levels at T_{max}, were detectable. Analysis of the metabolites in blood showed that hydrolysis was the major metabolic pathway as evidenced by the high concentration of the carboxylic acid (34%−77%) and an unidentified metabolite (17%−36%) presumed to derive from the carboxylic acid metabolite through an additional metabolic step. Pharmacokinetic parameters (Thomas *et al*., 1995) were as follows: apparent T_{max} (h), 1.0/2.0, blood C_{max} (μg/g) 1.866/1.675; apparent $T_{1/2}$ (h), 11.8/10.0; Total $AUC_{0–168\ hr}$ (area under the curve in μg hr/g_{blood}) 12.316/13.827; ester (member 9) $AUC_{0–168\ hr}$ (μg hr/g_{blood}) 7.364/7.022; free acid $AUC_{0–168\ hr}$ (μg hr/g_{blood}) 3.048/3.611; unidentified metabolite $AUC_{0–168\ hr}$ (μg hr/g_{blood}), 1.904/3.144.

In vitro studies in human

No human tokixicokinetic data are available for any of the 12 category substances.

In vivo studies in human

No human tokixicokinetic data are available for any of the 12 category substances.

ANNEX 2. TOXICOKINETIC PARAMETERS OF MEMBER 1: 2-(BENZOTRIAZOL-2-YL)-4,6-BIS(2-METHYLBUTAN-2-YL)PHENOL (CAS: 3846-71-7).

Doses	**Sex**	**C_{max} (μg/ml)**	**T_{max} (h)**	**$AUC_{0–24hr}$ (μg·h/ml)**
After the first administration (Day 1)				
0.5 mg/kg/day	Male	0.145 ± 0.031	5.75 ± 1.50	1.59 ± 0.32
	Female	0.116 ± 0.036	5.75 ± 1.50	1.25 ± 0.10
2.5 mg/kg/day	Male	0.484 ± 0.276	5.75 ± 1.50	4.99 ± 1.45
	Female	0.573 ± 0.165	7.25 ± 1.50	6.65 ± 1.61
12.5 mg/kg/day	Male	2.85 ± 0.64	6.50 ± 1.73	34.4 ± 7.1
	Female	3.84 ± 1.71	7.25 ± 1.50	47.1 ± 15.7
After the last administration (Day 28)				
0.5 mg/kg/day	Male	0.214 ± 0.054	6.50 ± 1.73	2.49 ± 0.62
	Female	0.154 ± 0.009	8.00 ± 0.00	1.98 ± 0.15
2.5 mg/kg/day	Male	1.14 ± 0.42	5.75 ± 1.50	13.6 ± 5.0
	Female	0.636 ± 0.221	7.25 ± 1.50	8.89 ± 3.25
12.5 mg/kg/day	Male	4.27 ± 0.96	5.75 ± 1.50	54.0 ± 11.4
	Female	3.80 ± 0.89	8.00 ± 0.00	50.1 ± 9.8

Referred from Hirata-Koizumi *et al*., 2009

ANNEX 3. REPEATED-DOSE TOXICITY DATA ON BENZOTRIAZOLES USED IN THIS STUDY

Among 12 category substances, reliable information on repeated oral dose toxicity was obtained for 9 (members 1−9). Available information obtained for each substance is summarized individually below.

Member 1: 2-(benzotriazol-2-yl)-4,6-bis(2-methylbutan-2-yl)phenol (CAS: 3846-71-7)

Three reliable studies are available.

The first report is a one-year chronic gavage study in rats. This is the key study, as it was performed in accordance with OECD TG452 under GLP (Hirata-Koizumi *et al*., 2008).

Male and female rats were administered member 1 orally by gavage 7 days per week for 52 weeks. The dose given was 0, 0.1, 0.5, or 2.5 mg/kg bw/day in males and 0, 0.5, 2.5, or 12.5 mg/kg bw/day in females. Decreases in body weight gain continued from week 5 to the end of the 52-week administration period at 2.5 mg/kg bw/day in males. At the end of the dosing period, a decrease in red blood cells (RBCs) at 0.5 mg/kg bw/day and higher and in hematocrit at 2.5 mg/kg bw/day was observed in males. Blood biochemical changes, including increases in the levels of alkaline phosphatease (ALP), glucose, and the A/G ratio were also found at 0.5 mg/kg bw/day and higher in males and at 12.5 mg/kg bw/day in females. At necropsy, absolute and relative liver weights were increased at 0.5 mg/kg bw/day and higher in males and at 12.5 mg/kg bw/day in females. Histopathological changes observed in the liver included centrilobular hypertrophy of hepatocytes at 0.5 mg/kg bw/day and higher in males and at 12.5 mg/kg bw/day in females, altered hepatocellular foci at 0.5 mg/kg bw/day and higher in males, and cystic degeneration and lipofuscin deposition in hepatocytes at 2.5 mg/kg in males. NOAEL in this study was 0.1 mg/kg bw/day in males and 2.5 mg/kg bw/day in females.

The second report is a 28-day gavage study in rats. This study is also key as it was performed in accordance with OECD TG407 under GLP (Hirata-Koizumi *et al*., 2007).

Male and female rats (5 animals/sex/dose) were administered member 1 by gavage at a dose of 0, 0.5, 2.5, 12.5, or 62.5 mg/kg bw/day for 28 days. At the end of the administration period, decreases in RBCs, hemoglobin, and hematocrit were noted only in males at 2.5 mg/kg bw/day and higher. Blood biochemical changes were noted at 0.5 mg/kg bw/day and higher in males and at 62.5 mg/kg bw/day in females. Histopathologic changes were observed principally in the liver (vacuolar degeneration and hypertrophy of hepatocytes, bile duct proliferation) and in the heart (degeneration and hypertrophy of myocardium and cell infiltration). These changes were noted at 0.5 mg/kg bw/day and higher in males and at 12.5 mg/kg bw/day and higher in females. At higher doses, hypertrophy of tubular epithelium in the kidneys and diffuse follicular cell hyperplasia of the thyroid were observed in both sexes. Increased severity of basophilic tubules in the kidneys and extramedullary hematopoiesis in the spleen of males were also detected. Thus, anemia-like effects (males only) and degenerative changes in both liver and heart were found at the lowest doses in males. NOAEL in this study was determined to be less than 0.5 mg/kg bw/day in males and 2.5 mg/kg bw/day in females.

The third report is 90-day feeding study in rats (Ciba-Geigy Corporation, 1988).

Male and female rats were fed the substance in diet at 0, 100, 200, 400, 800, or 1600 ppm for 90 days. Decreased body weight gain, food consumption, and food efficiency occurred at 800 ppm and higher, and body weights of males at 100 to 400 ppm were slightly lower than the control. Hemoglobin content, PCV, and RBC count were decreased in all treated animals. Glucose-6-phosphatase activity in liver was increased at 100 ppm and higher. Relative weights of liver and kidney were increased in all treated animals. Gross examination after 90 days revealed enlargement

and discoloration of liver and kidney in all treated males. In females, liver abnormalities occurred only at 800 ppm and higher and kidney abnormalities at 400 ppm and above. Under microscopy, hepatic damage (necrosis of individual cells and hypertrophic parenchymal cells) was observed in all treated males and at 400 ppm and higher in females. Signs of toxic tubular nephrosis were present in kidneys of males at 200 ppm and higher and in females at 800 ppm and higher. Due to hepatic histopathology even at the lowest dose, NOAEL was not determined in this study.

Among the three studies described above, the lowest determined NOAEL was 0.1 mg/kg bw/day for males in the one-year gavage study. In that study, LOAEL in males was 0.5 mg/kg bw/day based on anemia-like changes and liver histopathology (altered hepatocellular foci).

Member 2: 2-(2H-benzotriazol-2-yl)-4,6-ditertpentylphenol (CAS: 25973-55-1)

Two reliable study reports are available.

The first is a 90-day feeding study in rats (Til *et al*., 1968). This is the key study.

Male and female rats (10 animals/sex/dose) were fed a diet containing the substance at 0, 100, 200, 400, 800, or 1600 ppm for 90 days. The main target organs affected were the liver and kidney. Microscopic examination of the liver revealed occasional necrotic foci, slight proliferation of bile duct epithelia, and enlarged parenchymal cells. In the kidney, tubular necrosis was observed in some males from the higher dose groups. In females, a treatment-related yellowish-brown pigmentation in the cytoplasm of proximal tubular cells was noted. Hematological effects were also observed. Opinion on the Committee for Risk Assessment on the target organ toxicity of this substance suggests that the hepatic damage occurred to a lesser extent also at 100 ppm level (ECHA, 2013). However, NOAEL in this study was 100 ppm (ca. 20 mg/kg bw/day) according to the U.S. EPA (2009).

The other report is a 90-day feeding study in dogs (Insitut fur Industrielle und Biologische Forschung, 1970).

Male and female beagle dogs (3 dogs/sex/dose; 5/sex for control) were given the substance orally in feed at 0, 15, 30, 60, 120, or 240 mg/kg bw/day for 90 days. The main target organs were liver and kidney. Pathological changes in liver at the two highest doses included fatty degeneration of hepatocytes, protein globules in the cytoplasm, Kupffer cell hyperplasia, and centrilobular choleostasis. The kidneys also exhibited toxicity. In some animals of the higher dose groups, atrophy of the uterus, abnormal spermiogenesis, and atrophy of the prostate were also observed. According to the U.S. EPA (2004), NOEL in this study was < 15 mg/kg bw/day.

As described above, toxic effects of member 2 were observed mainly in the liver and kidney. NOAEL was determined in rat study as ca. 22 mg/kg bw/day. In the dog feeding study, organ toxicity was observed at the lowest dose so a NOAEL was not determined.

Member 3: 2,4-di-tert-butyl-6-(5-chloro-2H-benzotriazol-2-yl)phenol (CAS: 3864-99-1)

Three reliable studies are available, which can be summarized as follows.

The first is a key study combining repeated-dose and reproductive/screening toxicity in rats (Ema *et al*., 2008).

Male and female rats (10 rats/sex/dose) were given the substance by gavage at 0, 2.5, 25, or 250 mg/kg bw/day. Males were dosed for a total of 56–57 days and females for a total of 55–69 days, including mating, pregnancy, and up to day 3 of lactation. Significant increases in serum albumin and albumin/globulin ratio at 25 mg/kg bw/day and higher and ALP levels at 250 mg/kg bw/day were noted in males. The absolute and relative weights of the liver were significantly increased in males at 25 mg/kg bw/day and higher. Significantly increased serum albumin, absolute liver weight, and relative liver weight were also found in males at 250 mg/kg bw/day after the recovery period. No changes in these parameters were observed in females of any dose group. No significant changes in

organ histopathology were found in males or females. These findings indicate a sex difference in the toxicity of this substance in rats. The changes observed in this study were not accompanied by significant histopathological anomalies. Thus, these are considered rather as non-toxic effects. NOAEL and NOEL of this study were determined as 250 mg/kg bw/day in both sexes and 2.5 mg/kg bw/day in male, respectively.

The second report is 90-day feeding study in rats (Til *et al*., 1992).

The rats were fed a diet containing the substance at 0, 5, 15, or 45 ppm for 90 days. At the highest dose, increased weight of the liver was found in males only. Glucose 6-phosphatase activity in liver of male rats was increased at 15 ppm or higher. Histopathology revealed slightly enlarged hepatocytes containing homogeneous cytoplasm in males fed 15 ppm or higher. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and ALP levels were unaltered. According to the author, NOEL in males was 5 ppm based on the effects in liver. However, rats fed up to 45 ppm did not show obvious hepatotoxicity or any other organ toxicity. NOAEL of the substance in this study was considered to be over 45 ppm.

The third report is a 90-day feeding study in dogs (Ciba-Geigy Corporation, 1992).

Beagle dogs (3/sex/dose) received the test substance for 90 days in the diet at 15, 30, 60, 120, or 240 mg/kg bw/day. In addition, five male and five female beagle dogs were fed a test substance-free diet (control group). Increased activity of serum ALP was seen in several beagle dogs at 60 mg/kg bw/day or higher. Pathological examinations showed increased liver weight at 60 mg/kg bw/day or higher. No distinct dose-related changes were seen in liver slides. Remarkable dose-related changes in kidney consisted of adhesions and homogeneous inclusions in the glomeruli at 60 mg/kg bw/day or higher. NOAEL in this study was determined as 30 mg/kg bw/day and LOAEL as 60 mg/kg bw/day based on renal pathology.

The lowest NOAEL among these three reliable studies was 30 mg/kg bw/day in the 90-day feeding study of dogs. LOAEL in the study was 60 mg/kg bw/day based on renal histological changes (adhesions and homogenous inclusion in the glomeruli).

Member 4: 2-(benzotriazol-2-yl)-6-butan-2-yl-4-tert-butylphenol (CAS: 36437-37-3)

Two reliable study reports are available.

The first report is the key study, combining repeated-dose and reproductive/screening toxicity in rats (METI, 2011).

Male and female rats were administered member 4 by gavage at 0, 0.5, 2.5, or 12.5 mg/kg bw/day. Males were dosed for 42 days and females for 41 to 55 days through the pre-mating, mating, and pregnancy periods until day 4 of lactation. Changes in liver and kidney weights and some clinical chemistry parameters were observed at 12.5 mg/kg bw/day. No histopathological changes were detected in any organ of either sex up to the highest dose. Therefore, NOAEL and NOEL in this study were determined as 12.5 mg/kg bw/day and 2.5 mg/kg bw/day, respectively.

The second report is also a key study combining repeated-dose and reproductive/screening toxicity in rats (MHLW, 2011).

Male and female rats were administered the substance by gavage at 0, 0.8, 4, 20, or 100 mg/kg bw/day. Males were dosed for 42 days and females for 42 to 56 days through the pre-mating, mating and pregnancy period until day 4 of lactation. Increased ALP and relative liver weight as well as centrilobular hypertrophy of hepatocytes were observed in males at 20 mg/kg bw/day. No change was found in females at 20 mg/kg bw/day. At 100 mg/kg bw/day, the effects on hematology (anemia-like changes) and on the liver were observed in both sexes. Based on these results, NOAEL values of males and females were determined as 4 mg/kg bw/day and 20 mg/kg bw/day, respectively.

From the above two studies, the lowest NOAEL was determined to be 4 mg/kg bw/day and the lowest NOEL 2.5 mg/kg bw/day.

Member 5: 2-(benzotriazol-2-yl)-4,6-bis(2-phenylpropan-2-yl)phenol (CAS: 70321-86-7)

One reliable study is available, a 90-day feeding study in rats (Basler *et al*., 1987). Male and female rats (20 animals/sex/dose) were given the substance in feed at 0, 50, 300, 2000, or 10000 ppm for 90 days. Treatment-related effects were observed only in the liver. Increases in absolute and relative liver weights were observed in males at 2000 ppm or higher and in females at 300 ppm or higher. Hypertrophy and/or cytoplasmic vacuolation of hepatocytes were also noted in males at 2000 ppm or higher and in females at 300 ppm or higher. Considering that the toxic change in liver was limited to cytoplasmic vacuolation of hepatocytes, NOAEL in this study was determined as 50 ppm (equivalent to ca. 2.5 mg/kg bw/day). LOAEL was 300 ppm based on microscopic changes in the liver (cytoplasmic vacuolation of hepatocytes).

Member 6: 2-(benzotriazol-2-yl)-4-methylphenol (CAS: 2440-22-4)

Four reliable study reports are available, briefly summarized as follows.

The first was the combined repeated-dose and reproductive/developmental toxicity screening test (METI, 2007). The study was conducted according to OECD TG 422 in compliance with GLP. Male and female SD rats were administered 0, 30, 100, or 300 mg/kg bw/day by gavage, once daily for 42 days in males and for 42 to 53 days until day 4 of lactation in females. Effects were mainly found in liver and kidney. Increased relative liver weight was observed in males at 30 mg/kg bw/day or higher and in females at 100 mg/kg bw/day or higher. Hypertrophy of hepatocytes was found in males at 300 mg/kg bw/day and in females at 100 mg/kg bw/day or higher. The kidneys exhibited increased absolute and relative weights in females at the highest dose. Histopathological changes included eosinophilic bodies in the proximal tubules of males at the highest dose and hydropic-like degeneration as well as regeneration in proximal tubules of females at 100 mg/kg bw/day or higher. Eosinophilic bodies were generally recognized as a male-specific phenomenon and not relevant to assessment for human health effects. On the contrary, the changes observed in female kidneys were considered adverse. The study suggests that pregnant rats were more sensitive to kidney damage because the changes were not observed in a satellite group at the end of the recovery period. In conclusion, toxic effects were observed in male rats up to 300 mg/kg bw/day, while in females histopathological changes in hepatocytes and renal tubules were found at 100 mg/kg bw/day or higher. NOAEL and NOEL in this study were determined as 30 mg/kg bw/day and less than 30 mg/kg bw/day, respectively.

The second report was a 90-day feeding study in rats (Feron *et al*., 1992). Male and female Wistar rats were given the substance in feed at 0, 2000, 10000, or 50000 ppm for 13 weeks. In the 10000 ppm or higher groups, the main effects observed included transient growth retardation, alterations in the numbers of erythrocytes and leucocytes, and distinct histopathological changes in the kidneys. The liver showed hypertrophic changes, but these were considered non-adverse. The kidneys exhibited distinct nephropathy plus hyperplastic renal tubules. In females, however, only a single nephrotic tubule was observed. Thus, 4000 ppm was considered the LOAEL based on renal toxicity. NOAEL in this study was determined as 2000 ppm (equivalent to 100 mg/kg bw/day).

The third report was a 90-day feeding study in dogs (Ciba-Geigy Corporation, 1981a). Four groups of male and female Beagles (6 animals/sex/dose) were given the substance in feed at 0, 1000, 3000, or 10000 ppm for 13 weeks. Increased serum ALT activity was observed in the 3000 and 10000 ppm groups while increased serum G-GT activity was noted in the 10000 ppm group. One female of the highest dose group became emaciated. No other gross or histopathological changes related to the treatments were noted. The authors stated that NOEL was 1000 ppm (corresponding to 31.75 mg/kg bw/day for males and 34.6 mg/kg bw/day for females).

The forth report was a two-year feeding study in rats (Ciba-Geigy Corporation, 1975). Male and female CFY rats were fed diets containing the test substance at 0, 100, 300, 1000, or 3000 ppm for

104 weeks, equivalent to 0, 4−6, 14−17, 47−58, or 142−169 mg/kg bw/day, respectively. At 3000 ppm, slight decreases in male body weight gain and female food intake were observed. No treatment-related abnormalities were found in any other examinations. NOEL in this study was determined as 1000 ppm (equivalent to 47 to 58 mg/kg bw/day) based on decreased body weight and food intake observed at 3000 ppm.

Member 7: 2-(benzotriazol-2-yl)-4-(2,4,4-trimethylpentan-2-yl)phenol (CAS: 3147-75-9)

One report on a 30-day feeding study in rats is available. Male and female rats were fed a diet containing 0, 12500, 25000, or 50000 ppm member 7 for 30 days, equivalent to 0, 1286, 2594, or 5658 mg/kg bw/day, respectively. No indices of toxicity were found by macroscopic and microscopic examinations up to the highest dose (American Cyanamid Company, 1968). NOAEL in this study was determined to be 5658 mg/kg bw/day. This study was conducted prior to GLP/OECD Guidelines. According to the High Production Volume Information System by the US EPA, Reliability of the study is assigned as valid with restrictions.

Member 8: 2-tert-butyl-6-(5-chlorobenzotriazol-2-yl)-4-methylphenol (CAS: 3896-11-5)

Four reliable study reports are available.

The first is the key study combining repeated-dose and reproductive/screening toxicity in rats (MHLW, 2007).

Male and female rats were given the substance by gavage at 0, 62.5, 250, or 1000 mg/kg bw/day for 42 days in males and 44−56 days up to day 6 of lactation in females. No effects were seen in any parameter up to the highest dose tested. NOAEL in this study was determined as 1000 mg/kg bw/day.

The second report is a 90-day feeding study in dogs (Ciba-Geigy Corporation, 1981b).

Male and female beagle dogs (4 or 5 dogs/sex/dose) were fed a diet containing 0, 200, or 1000 ppm for 90 days. No toxic effects were seen up to the highest dose. NOAEL in this study was 1000 ppm (equivalent to 29.6 mg/kg bw/day for males and 32.2 mg/kg bw/day for females).

The third report is a 2-year feeding study in rats (Ciba-Geigy Corporation, 1978).

Male and female rats (50 rats/sex/dose) were fed 0, 1000, 3000, or 10000 ppm in the diet for 104 weeks. A marginally lower rate of body weight gain was recorded during the first year of the study among males receiving 10000 ppm. A lower food intake was recorded between weeks 6 and 78 for males receiving 10000 ppm. Increases in liver weight were observed, but no macroscopic or microscopic changes were noted. NOEL was determined as 3000 ppm (equivalent to 113.2 mg/kg bw/day in males and 147.7 mg/kg bw/day in females) and NOAEL as over 10000 ppm (equivalent to 500 mg/kg bw/day) based on the lack of obvious toxic effects in any organs.

The fourth report is a 2-year feeding study in mice (Ciba-Geigy Corporation, 1981c).

Male and female mice (50 mice/sex/dose) were given a diet containing 0, 5, 50, or 500 ppm for 104 weeks. No toxic effects were seen up to the highest dose. NOAEL in this study was over 500 ppm (equivalent to 62 mg/kg bw/day in males and 59 mg/kg bw/day in females).

LOAEL was not determined in any of the 4 studies. Therefore, NOAEL of this substance was determined to be 1000 mg/kg bw/day according to the highest NOAEL in the first study.

Member 9: methyl 3-[3-(benzotriazol-2-yl)-5-tert-butyl-4-hydroxyphenyl]propanoate (CAS: 84268-33-7)

One reliable study is available, a 28-day gavage study in rats (Ciba-Geigy Corporation, 1986).

Male and female rats (5 animals/sex/dose) were administered member 9 by gavage for 28 days at 0, 50, 200, or 1000 mg/kg bw/day. Hepatotropic effects were recognized as evidenced by increased liver weight and liver enzyme activities as well as by both macroscopic and microscopic findings. Histopathological effects on the liver, particularly recent necrosis, were found in 2/5 males at 50 mg/kg bw/day. In females, no necrotic changes were found, but diffuse hypertrophy of hepatocytes was observed in 1/5 females at 50 mg/kg bw/day. Since some of these effects were found even at the lowest dose (50 mg/kg bw/day), NOAEL was determined to be below 50 mg/kg bw/day.

Member 10: octyl 3-[3-(benzotriazol-2-yl)-5-tert-butyl-4-hydroxyphenyl]propanoate (CAS: 127519-17-9)

No information is available.

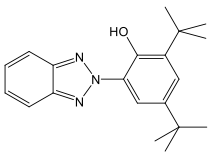
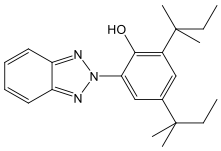
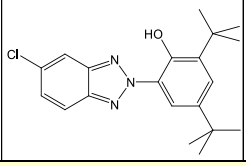
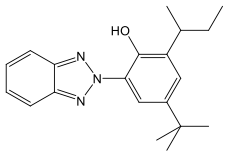
Member 11: 2-(benzotriazol-2-yl)-6-(2-phenylpropan-2-yl)-4-(2,4,4-trimethylpentan-2-yl)phenol (CAS: 73936-91-1)

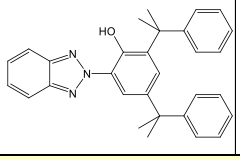
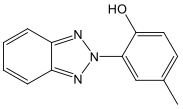
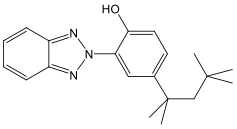
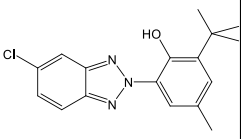
No information is available.

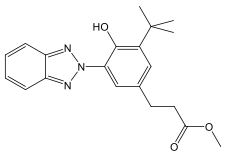
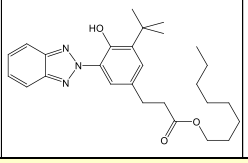
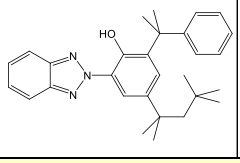
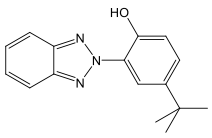
Member 12: 2-(benzotriazol-2-yl)-4-tert-butylphenol (CAS: 3147-76-0)

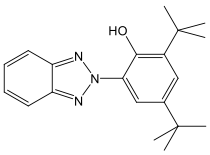
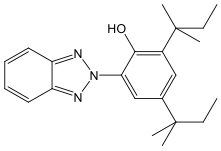
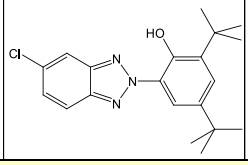
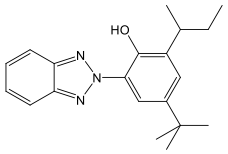
No information is available.

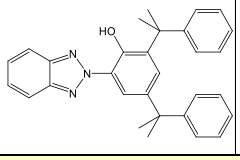
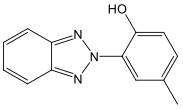
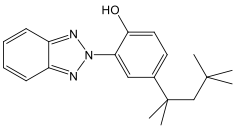
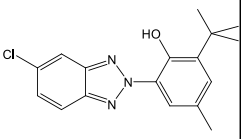
APPENDIX - DATA MATRIX

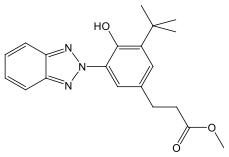
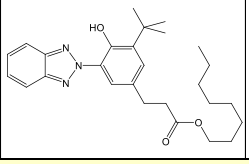
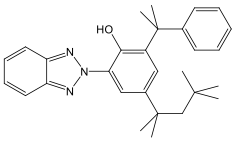
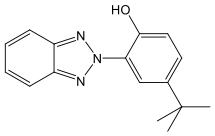
Chemical ID					
		Member 1	Member 2	Member 3	Member 4
CAS		3846-71-7	25973-55-1	3864-99-1	36437-37-3
Name		2-(benzotriazol-2-yl)-4,6-bis(2-methylbutan-2-yl)phenol	2-(2H-benzotriazol-2-yl)-4,6-ditertpentylphenol	2,4-di-tert-butyl-6-(5-chloro-2H-benzotriazol-2-yl)phenol	2-(benzotriazol-2-yl)-6-butan-2-yl-4-tert-butylphenol
Structure					
Summary of data gap filling					
		Subcategory 1	Subcategory 1	Subcategory 1	Subcategory 1
		Member 1	Member 2	Member 3	Member 4
Repeated-dose toxicity	Experimental result (GLP)	NOAEL=0.1 mg/kg bw/day (0.0003 mmol/kg/day) Liver; weight increase, hypertrophy of hepatocyte (0.5 mg/kg bw/day in male 12.5 mg/kg bw/day in female), altered hepatocellular foci (0.5 mg/kg bw/day in male), cystic degeneration and lipofuscin deposition in hepatocyte (2.5 mg/kg bw/day in male) Hematological effects (0.5 mg/kg bw/day in male), Rats, 0, 0.1, 0.5, 2.5 mg/kg bw/day (male), 0, 0.5, 2.5, 12.5 mg/kg bw/day (female) 52-week, Gavage (Hirata-Koizumi et al., 2008) [key study]	No data	NOEL=2.5 mg/kg bw/day (0.007 mmol/kg/day) Alb, A/G ratio increase (25 mg/kg bw/day in male), Liver; weight increase (25 mg/kg bw/day in male), Rats, 0, 2.5, 25, 250 mg/kg bw/day, 56-57-day for male, 55-69-day for female, Gavage (Ema et al., 2008) [key study]	NOEL=2.5 mg/kg bw/day (0.008 mmol/kg bw/day) Decrease in TG, PL (0.5 mg/kg bw/day) Increase in Alb, A/G, ALP (12.5 mg/kg bw/day) Liver; weight increase (12.5 mg/kg bw/day) Kidney; weight increase (12.5 mg/kg bw/day) Rats, 0, 0.5, 2.5, 12.5 mg/kg bw/day, 42-day (male), 41-55-day (female), Gavage (METI, 2011) [key study]
	Experimental result (GLP)	NOAEL=<0.5 mg/kg bw/day (0.0015 mmol/kg/day) Liver; vacuolar degeneration, hypertrophy of hepatocyte, bile duct proliferation (0.5 mg/kg bw/day in male), focal necrosis (2.5 mg/kg bw/day in male) Hematological effects (2.5 mg/kg bw/day in male), Heart; degeneration and hypertrophy of myocardium and cell infiltration (0.5 mg/kg bw/day in male and 12.5 mg/kg bw/day in female) Kidney; hypertrophy of tubular epithelium (62.5 mg/kg bw/day in male and female), Thyroid; follicular cell hyperplasia (62.5 mg/kg bw/day in male and female) Rats, 0, 0.5, 2.5, 12.5, 62.5 mg/kg bw/day, 28-day, Gavage (Hirata-Koizumi et al., 2007) [key study]	No data	No data	NOAEL=4 mg/kg bw/day (0.012 mmol/kg bw/day) Liver; weight increase, centrilobular hypertrophy of hepatocyte (20 mg/kg bw/day in male, 100 mg/kg bw/day in female) Hematological effects (100 mg/kg bw/day in male and female) Rats, 0, 0.8, 4, 20, 100 mg/kg bw/day, 42-day (male), 42-56-day (female), Gavage (MHLW, 2011) [key study]
	Experimental result (non-GLP)	NOAEL=<100 ppm Liver; hepatic damage (400 ppm in female) Kidney; tubular nephrosis (200 ppm in male) Hematological effects (100 ppm) Rats, 0, 100, 200, 400, 800, 1600 ppm, 90 -day, Feeding (Ciba-Gaigy, 1988)	NOAEL=100 ppm (ca. 20 mg/kg bw/day, 0.057 mmol/kg/day) Liver; focal necrosis, bile duct proliferation, parenchymal cells enlarged (200 ppm in male and female) Kidney; tubular necrosis (200 ppm in male), Hematological effects (200 ppm in male), Rats, 0, 100, 200, 400, 800, 1600 ppm, 90-day, Feeding (Til et al., 1968) [key study]	NOEL=5 ppm Liver; enlarged hepatocyte (15 ppm), weight increase (45 ppm), Rats, 0, 5, 15, 45 ppm, 90-day, Feeding (Til et al., 1992)	No data
	Experimental result (non-GLP)	No data	NOAEL=<15 mg/kg bw/day (0.043 mmol/kg/day) Liver; fatty degeneration, protein globules in cytoplasm, Kupffre cell hyperplasia, centrilobular cholestatis (120 mg/kg bw/day) Kidney; toxic effects (60 mg/kg bw/day), Hematological effects (120 mg/kg bw/day in male and female), Beagle dogs, 0, 15, 30, 60, 120, 240 mg/kg bw/day, 90-day, Feeding (Insitut for Indusstrielle und Biologische Foreschung, 1970)	NO(A)EL=30 mg/kg bw/d (0.084 mmol/kg bw/day) Liver; weight increase (60 mg/kg bw/day), Kidney; adhesion, homogeneous inclusions in glomeruli (60 mg/kg bw/day), Beagle dogs, 0, 15, 30, 60, 120, 240 mg/kg bw/day, 90-day, Feeding (Ciba-Geigy, 1992)	No data
	Experimental result (non-GLP)	No data	No data	No data	No data
	Integrated conclusion (read-across)				
	D value				

Chemical ID					
		Member 5	Member 6	Member 7	Member 8
CAS		70321-86-7	2440-22-4	3147-75-9	3896-11-5
Name		2-(benzotriazol-2-yl)-4,6-bis(2-phenylpropan-2-yl)phenol	2-(benzotriazol-2-yl)-4-methylphenol	2-(benzotriazol-2-yl)-4-(2,4,4-trimethylpentan-2-yl)phenol	2-tert-butyl-6-(5-chlorobenzotriazol-2-yl)-4-methylphenol
Structure					
Summary of data gap filling					
		subcategory 2	subcategory 3	subcategory 4	not defined
		Member 5	Member 6	Member 7	Member 8
Repeated-dose toxicity	Experimental result (GLP)	NOAEL=50 ppm (ca. 2.5 mg/kg bw/day (0.0056 mmol/kg bw/day), female), 300 ppm (ca. 15 mg/kg bw/day (0.0335 mmol/kg bw/day), male) Liver; weight increase, hypertrophy and/or cytoplasmic vacuolation of hepatocyte (2000 ppm in male, 300 ppm in female), Rats, 0, 50, 300, 2000, 10000 ppm, 90-day, Feeding (Basler et al., 1987)	NOEL= <30 mg/kg bw/day (<0.133 mmol/kg bw/day) Liver; weight increase, hypertrophy of hepatocyte (30 mg/kg bw/day in male, 100 mg/kg bw/day in female), Kidney: degeneration and regeneration in proximal tubules (100 mg/kg bw/day in female), Rats, 0, 30, 100, 300 mg/kg bw/day, 42-day (male), 42-53-day (female), Gavage (METI., 2007) [key study]	No data	NOAEL=1000 mg/kg bw/d (3.167 mmol/kg bw/day) No effects, Rats, 0, 62.5, 250, 1000 mg/kg bw/day, 42-day for male, 44-56-day for female, Gavage (MHLW., 2007) [key study]
	Experimental result (GLP)	No data	No data	No data	No data
	Experimental result (non-GLP)	No data	NO(A)EL=2000 ppm (100 mg/kg bw/day (0.444 mmol/kg bw/day)) Liver; hypertrophy of hepatocyte (10000 ppm) Kidney; nephropathy and hyperplastic renal tubules (10000 ppm), Rats, 0, 2000, 10000, 50000 ppm 13-week, Feeding (Feron et al., 1992)	NOAEL=5658 mg/kg bw/day (17.49 mmol/kg bw/day) No effects Rats 0, 12500, 25000, 50000 ppm 30-day, Feeding (American Cyanamid Company, 1968)	NOAEL=1000 ppm (29.6 mg/kg bw/day (0.094 mmol/kg bw/day) in male, 32.2 mg/kg bw/day (0.102 mmol/kg bw/day) in female) No effects Beagle dogs, 0, 200, 1000 ppm, 90-day, Feeding (Ciba-Gaigy, 1981b)
	Experimental result (non-GLP)	No data	NOEL=1000 ppm (31.75 mg/kg bw/day (0.141 mmol/kg bw/day) for male and 34.6 mg/kg bw/day (0.154 mmol/kg bw/day) for female) Increase in ALT activity (3000 ppm), Increase in G-GT (10000 ppm) Beagle dogs, 0, 1000, 3000, 10000 ppm 13-week, Feeding (Ciba-Geigy, 1981a)	No data	NOEL=3000 ppm (113.2 mg/kg bw/day (0.358 mmol/kg bw/day) in male, 147.7 mg/kg bw/day (0.468 mmol/kg bw/day) in female) Liver: weight increase (10000 ppm) Rats, 0, 1000, 3000, 10000 ppm, 104-week, Feeding (Ciba-Gaigy, 1978)
	Experimental result (non-GLP)	No data	NOEL=1000 ppm (47 mg/kg bw/day (0.209 mmol/kg bw/day) for male, 58 mg/kg bw/day (0.257 mmol/kg bw/d) for female) Decrease in body weight gain (3000 ppm in male) Reduction in food intake (3000 ppm in female) Rats, 0, 100, 300, 1000, 3000 ppm, 104-weeks, Feeding (Ciba-Geigy, 1975)	No data	NOAEL=500 ppm (62 mg/kg bw/day (0.196 mmol/kg bw/day) for male, 59 mg/kg bw/day (0.189 mmol/kg bw/day) for female) No effects Mice, 0, 5, 50, 500 ppm, 104-week, Feeding (Ciba-Gaigy, 1981c)
	Integrated conclusion (read-across)				
	D value				

Chemical ID					
		Member 9	Member 10	Member 11	Member 12
CAS		84268-33-7	127519-17-9	73936-91-1	3147-76-0
Name		methyl 3-[3-(benzotriazol-2-yl)-5-tert-butyl-4-hydroxyphenyl]propanoate	octyl 3-[3-(benzotriazol-2-yl)-5-tert-butyl-4-hydroxyphenyl]propanoate	2-(benzotriazol-2-yl)-6-(2-phenylpropan-2-yl)-4-(2,4,4-trimethylpentan-2-yl)phenol	2-(benzotriazol-2-yl)-4-tert-butylphenol
Structure					
Summary of data gap filling					
		not defined	not defined	subcategory 2	Not defined
		Member 9	Member 10	Member 11	Member 12
Repeated-dose toxicity	Experimental result (GLP)	NOAEL= <50 mg/kg bw/day (<0.141 mmol/kg bw/day) Liver; weight increase, necrosis, hypertrophy of hepatocyte (50 mg/kg bw/day in male), diffuse hypertrophy of hepatocyte (50 mg/kg bw/day in female) Rats, 0, 50, 200, 1000 mg/kg bw/day, 28-day, Gavage (Ciba-Gaigy, 1986)	No data	No data	No data
	Experimental result (GLP)	No data	No data	No data	No data
	Experimental result (non-GLP)	No data	No data	No data	No data
	Experimental result (non-GLP)	No data	No data	No data	No data
	Experimental result (non-GLP)	No data	No data	No data	No data
	Integrated conclusion (read-across)		NOAEL= <64 mg/kg bw/day (<0.141 mg/kg bw/day) LOAEL=64 mg/kg bw/day (0.141 mg/kg bw/day) Hepatotoxic effects	NOAEL=2.5 mg/kg bw/day (0.0056 mmol/kg bw/day) Hepatotoxic effects	NOAEL= <35 mg/kg bw/day (<0.133 mmol/kg bw/day) LOAEL=35 mg/kg bw/day (0.133 mmol/kg bw/day) Hepatotoxic effects
	D value				

Chemical ID					
		Member 1	Member 2	Member 3	Member 4
CAS		3846-71-7	25973-55-1	3864-99-1	36437-37-3
Name		2-(benzotriazol-2-yl)-4,6-bis(2-methylbutan-2-yl)phenol	2-(2H-benzotriazol-2-yl)-4,6-ditertpentylphenol	2,4-di-tert-butyl-6-(5-chloro-2H-benzotriazol-2-yl)phenol	2-(benzotriazol-2-yl)-6-butan-2-yl-4-tert-butylphenol
Structure					
Molecular profiling related to the category hypothesis					
Parent chemical	Toxicological category (HESS ver. 3.0)	None	None	None	None
	Structural alert (Derek Nexus)	None	None	None	None
	Similarity (%) (QSAR Toolbox)	100%	80-90%	80-90%	70-80%
Physical-chemical data					
logKow (calculated value)		6.27	7.25	6.91	6.31
MW		323.44	351.49	357.88	323.43
Kinetics					
Absorption		Rapidly absorbed, no gender differences (Hirata-Koizumi et al., 2009)	No data	No data	No data
Distribution		No data	No data	No data	No data
Metabolism		in vitro: slightly metabolized in rat hepatic microsomes in NADPH-generation system in vivo: no metabolites in the plasma, no gender differences (Hirata-Koizumi et al., 2009)	No data	No data	No data
Excretion		Rapidly eliminated from the plasma, no gender differences (Hirata-Koizumi et al., 2009)	No data	No data	No data
Supporting data related to the target endpoint(s)					
		Member 1	Member 2	Member 3	Member 4
Transcriptomic profile (*semiquantitative relative degree of induction)	Up probesets	5480	No data	3230	No data
	AhR-Cyp1*	0	No data	0	No data
	CAR-Cyp2*	100	No data	50	No data
	SXR/PXR-Cyp3*	100	No data	80	No data
	PPAR-Cyp4*	100	No data	100	No data
	Nrf2-phase II enzymes*	100	No data	50	No data
	Nrf2/Keap1*	100	No data	80	No data

Chemical ID					
		Member 5	Member 6	Member 7	Member 8
CAS		70321-86-7	2440-22-4	3147-75-9	3896-11-5
Name		2-(benzotriazol-2-yl)-4,6-bis(2-phenylpropan-2-yl)phenol	2-(benzotriazol-2-yl)-4-methylphenol	2-(benzotriazol-2-yl)-4-(2,4,4-trimethylpentan-2-yl)phenol	2-tert-butyl-6-(5-chlorobenzotriazol-2-yl)-4-methylphenol
Structure					
Molecular profiling related to the category hypothesis					
Parent chemical	Toxicological category (HESS ver. 3.0)	None	None	None	None
	Structural alert (Derek Nexus)	None	None	None	None
	Similarity (%) (QSAR Toolbox)	60-70%	60-70%	70-80%	70-80%
Physical-chemical data					
logKow (calculated value)		7.67	3.00	6.21	5.55
MW		447.57	225.25	323.43	315.8
Kinetics					
Absorption		No data	No data	No data	No data
Distribution		No data	Highest in the liver (Schmid et al., 1990)	No data	No data
Metabolism		No data	No data	No data	No data
Excretion		No data	More than 70 % radioactivity in urine, about 20 % raduioactivity in feces (Schmid et al., 1990)	No data	No data
Supporting data related to the target endpoint(s)					
		Member 5	Member 6	Member 7	Member 8
Transcriptomic profile (*semiquantitative relative degree of induction)	Up probesets	370	150	250	No data
	AhR-Cyp1*	0	0	0	No data
	CAR-Cyp2*	0	0	40	No data
	SXR/PXR-Cyp3*	0	0	0	No data
	PPAR-Cyp4*	30	0	40	No data
	Nrf2-phase II enzymes*	5	10	0	No data
	Nrf2/Keap1*	0	0	0	No data

Chemical ID					
		Member 9	Member 10	Member 11	Member 12
CAS		84268-33-7	127519-17-9	73936-91-1	3147-76-0
Name		methyl 3-[3-(benzotriazol-2-yl)-5-tert-butyl-4-hydroxyphenyl]propanoate	octyl 3-[3-(benzotriazol-2-yl)-5-tert-butyl-4-hydroxyphenyl]propanoate	2-(benzotriazol-2-yl)-6-(2-phenylpropan-2-yl)-4-(2,4,4-trimethylpentan-2-yl)phenol	2-(benzotriazol-2-yl)-4-tert-butylphenol
Structure					
Molecular profiling related to the category hypothesis					
Parent chemical	Toxicological category (HESS ver. 3.0)	None	None	None	None
	Structural alert (Derek Nexus)	None	None	None	None
	Similarity (%) (QSAR Toolbox)	70-80%	60-70%	60-70%	70-80%
Physical-chemical data					
logKow (calculated value)		4.94	8.38	8.82	4.36
MW		353.42	451.61	441.61	267.33
Kinetics					
Absorption		Readily absorbed from gastrointestinal tract	No data	No data	No data
Distribution		No data	No data	No data	No data
Metabolism		High concentration of the carboxylic acid (34-77%)	No data	No data	No data
Excretion		Maximum radioactivity: 1-2 hr Half-life: 10-12 hr (Thomas et al., 1995)	No data	No data	No data
Supporting data related to the target endpoint(s)					
		Member 9	Member 10	Member 11	Member 12
Transcriptomic profile (*semiquantitative relative degree of induction)	Up probesets	No data	No data	No data	No data
	AhR-Cyp1*	No data	No data	No data	No data
	CAR-Cyp2*	No data	No data	No data	No data
	SXR/PXR-Cyp3*	No data	No data	No data	No data
	PPAR-Cyp4*	No data	No data	No data	No data
	Nrf2-phase II enzymes*	No data	No data	No data	No data
	Nrf2/Keap1*	No data	No data	No data	No data