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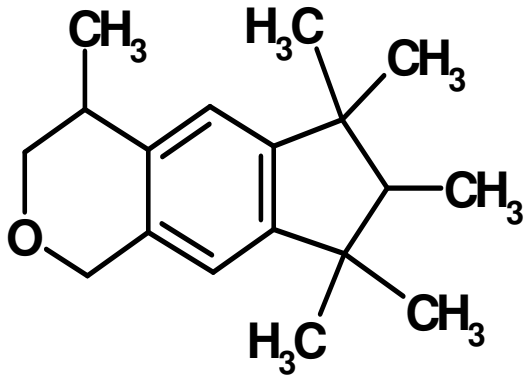
Office of Chemical Safety and Pollution Prevention

TSCA Work Plan Chemical Risk Assessment

HHCB

1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-γ-2-benzopyran

CASRN: 1222-05-5



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This report was developed by the United States Environmental Protection Agency (US EPA), Office of Chemical Safety and Pollution Prevention (OCSPP), Office of Pollution Prevention and Toxics (OPPT). The Workplan Chemical Risk Assessment for 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-γ-2-benzopyran (HHCB) was prepared based on currently available data and any additional information received during the public comment period and peer review process. Mention of trade names does not constitute endorsement by the EPA.

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External Peer Review

EPA/OPPT released the peer review plan in August of 2012 and draft risk assessment and charge questions for peer review for public comment in January 2013. EPA/OPPT contracted with The Scientific Consulting Group, Inc. (SCG) to convene a panel of *ad hoc* reviewers to conduct an independent external peer review for the EPA's draft work plan risk assessment for HHCB. As an influential scientific product, the draft risk assessment was peer reviewed in accordance with EPA's peer review guidance. The peer review panel performed its functions by web conference and teleconference between December 4, 2013 and February 6, 2014. The panel consisted of the following individuals:

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Please visit the EPA/OPPT's Work Plan Chemicals web page for additional information on the peer review process (http://www.epa.gov/oppt/existingchemicals/pubs/riskassess.html), and the public docket (Docket: EPA-HQ-OPPT-2012-0722) for the independent external peer review report and the response to comments document.

GLOSSARY OF TERMS AND ABBREVIATIONS

BB	Benzyl benzoate
BAF	Bioaccumulation factor
BCF	Bioconcentration factor
CASRN	Chemical Abstract Service Registry Number
CBI	Confidential Business Information
CDR	Chemical Data Reporting
ChV	Chronic value
COC	Concentration of concern
DEP	Diethyl phthalate
dw	Dry weight
E_2	Estradiol
EC	European Commission
ECHA	European Chemicals Agency
EPA	Environmental Protection Agency
EPCRA	Emergency Planning and Community Right-to-Know
EPI	Estimation Programs Interface
ESD	Emission Scenario Document
EU	European Union
EUSES	European Union System for the Evaluation of Substances
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
GC/MS	Gas chromatography/mass spectrometry
GD	Gestation day
GLP	Good Laboratory Practice
HERA	Human and Environmental Risk Assessment Project
HHCB	1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-γ-2-benzopyran
HPLC	High performance liquid chromatography
HPV	High production volume
IFRA	International Fragrance Association
IPM	Isopropyl myristate
IUR	Inventory Update Reporting Rule
k_1	Uptake rate constant
k_2	Elimination rate constant
K_d	Sorption coefficient
kg	Kilogram(s)
K_{OC}	Organic carbon-normalized sorption coefficient
K_{OW}	Octanol:water partition coefficient
lbs	Pounds
LDR	Larval development ratio
LOAEC	Lowest-observed-adverse-effect concentration
LOAEL	Lowest-observed-adverse-effect level
LOEC	Lowest-observed-effect concentration

Log K_{OC}	Logarithmic organic carbon partition coefficient
Log K_{OW}	Logarithmic octanol:water partition coefficient
LRL	Laboratory reporting level
LT-MDL	Long-term method detection level
lw	Lipid weight
MATC	Maximum Acceptable Toxicant Concentration
MOS	Margin of safety
NAICS	North American Industry Classification System
ND	Not detected
NHANES	National Health and Nutrition Examination Survey
NOAEC	No-observed-adverse-effect concentration
NOAEL	No-observed-adverse-effect level
NOEC	No-observed-effect concentration
NWIS	National Water Information System
NWQL	National Water Quality Laboratory
OCSPP	Office of Chemical Safety and Pollution Prevention
OECD	Organisation for Economic Co-operation and Development
OPPT	Office of Pollution Prevention and Toxics
OSPAR	Oslo Paris
PNEC	Predicted no-effect concentration
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
$RHO_{earthworm}$	Bulk density of earthworm
RAR	Risk assessment report
RQ	Risk quotient
SCAS	Semi-continuous activated sludge
SIAM	SIDS Initial Assessment Meeting
SIAR	SIDS Initial Assessment Report
SIDS	Screening Information Data Set
SSO	Sanitary sewer overflow
TG	Test Guideline
TLC	Thin-layer chromatography
TSCA	Toxic Substances Control Act
UF	Uncertainty factor
UF_{total}	Total uncertainty factor
US	United States
USGS	United States Geological Survey
wt%	Weight percent
ww	Wet weight
WWTP	Wastewater treatment plant
yr(s)	Year

EXECUTIVE SUMMARY

As a part of EPA's comprehensive approach to enhance the Agency's existing chemicals management, in March 2012 EPA identified a work plan of chemicals for further assessment under the Toxic Substances Control Act (TSCA).[1] The Agency is performing risk assessments on chemicals in the work plan. If an assessment identifies unacceptable risks to humans or the environment, EPA will pursue risk management. 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-γ-2-benzopyran (HHCB) was assessed as part of the work plan.

HHCB is one of the most widely used polycyclic musk fragrance ingredients in a range of consumer products including perfumes, cosmetics, shampoos, lotions, detergents, fabric softeners, and household cleaners. HHCB is not produced in the US, but is imported. The volume of HHCB imported into the US has been reported in the range of 1 to 10 million pounds (lbs) per year from 1990 to 2012. Within this range, the volume of use in the US has increased steadily since 2000, suggesting that polycyclic musks are not being replaced with other synthetic musks, as may be occurring in Europe.

In the US, imported HHCB is compounded into fragrance oils, which are blended into end-use products and sold for both commercial and consumer use. An estimated 90 to 94 percent of the HHCB use-volume is released to municipal wastewater. HHCB is moderately persistent in soil and sediment, has low mobility in soil, suboptimal removal in wastewater treatment plants (WWTP) (with removal primarily through sorption to sludge), and bioconcentration and bioaccumulation into aquatic organisms is low to moderate, depending on the species in which it is measured.

Problem formulation resulted in the assessment focusing on environmental risk to the aquatic environments from the use of HHCB as a fragrance ingredient in consumer and commercial products. Exposure pathways of interest included discharge of wastewater to surface water or application of biosolids to land. The assessment endpoints of interest were acute and chronic toxicity to aquatic organisms, chronic toxicity to sediment organisms, and chronic toxicity to terrestrial invertebrates and plants. In addition, EPA reviewed the available human health data, including a risk assessment performed by the European Union, evaluated the weight of the evidence and determined that further assessment of human health risk was not currently needed. Appendix A summarizes the human health information for HHCB.

US environmental monitoring data from the literature and from the US Geological Survey (USGS) National Water Information System (NWIS) database were used in the risk assessment. Levels of HHCB measured in wastewater, surface water, sediment, and soil were identified from these sources. More than 6800 data points for effluent, surface water and sediment were available for HHCB in the NWIS database. Soil monitoring data was limited to two published studies.

[1] http://www.epa.gov/oppt/existingchemicals/pubs/workplans.html

Ecotoxicity studies for HHCB have been conducted in fish, aquatic invertebrates, aquatic plants, sediment invertebrates, soil invertebrates, and terrestrial plants. Concentrations of concern (COCs) were derived from these studies according to established EPA/OPPT methods (EPA 2012f; 2013). This assessment considered only direct exposure to aquatic and sediment-dwelling organisms via contact with contaminated water or sediment. Based on half-lives in water, sediment and continuous wastewater inputs, exposure to organisms was considered continuous.

Potential risks were calculated using the risk quotient (RQ) approach. In this deterministic approach, the RQ is calculated by dividing a point estimate of exposure concentration by a point estimate of the effects concentration of concern (the COC). A RQ greater than one indicates there may be risk of concern. To incorporate the range of measured environmental concentrations, the RQ was calculated using the mean, maximum, and 95th percentile of measured environmental concentrations for surface water and sediment. Available soil concentrations were inadequate for calculating an RQ for soil organisms. RQs were calculated for aquatic and sediment dwelling organisms for acute and chronic exposures to water and sediments. RQs were not calculated for terrestrial invertebrates and plants due to insufficient exposure data.

Maximum measured surface water concentrations were approximately 25 to >150 times lower than acute COCs, and 4 to 25 times lower than the chronic COC for aquatic organisms (i.e., RQs ranged from 0.006 to 0.04 and 0.04 to 0.23, respectively). The chronic COC for sediment-dwelling organisms was also not exceeded at the upper range of measured environmental concentrations for the maximum published values or the more than 600 sediment measurements from the USGS. RQs for chronic risk to sediment dwelling organisms ranged from 0.001 to 0.36. RQs were not calculated for terrestrial invertebrates and plants *via* contact with contaminated water, sediment, or soil.

In conclusion, under the exposure scenarios assessed in this this risk assessment, current environmental exposure concentrations are one to two orders of magnitude below hazard concentrations (RQs < 1) of concern for aquatic or sediment-dwelling organisms. The inability to assess potential risks to terrestrial invertebrates and plants is a major uncertainty associated with this assessment.

1 BACKGROUND AND SCOPE

1.1 INTRODUCTION

As a part of EPA's comprehensive approach to enhance the Agency's existing chemicals management, in March 2012 EPA identified a work plan of chemicals for further assessment under the Toxic Substances Control Act (TSCA).[2] After gathering input from stakeholders, EPA developed criteria used for identifying chemicals for further assessment.[3] The criteria focused on chemicals that meet one or more of the following factors: (1) potentially of concern to children's health (for example, because of reproductive or developmental effects); (2) neurotoxic effects; (3) persistent, bioaccumulative, and toxic (PBT); (3) probable or known carcinogens; (4) used in children's products; or (5) detected in biomonitoring programs. Using this methodology, EPA identified a TSCA Work Plan of chemicals as candidates for risk assessment in the next several years. In the prioritization process, 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-γ-2-benzopyran (HHCB) was identified for assessment based on high production volume, high estimated releases to the environment, moderate environmental persistence, moderate bioaccumulation, moderate toxicity in rodents, and high potential for human exposure.

The Agency is performing risk assessments on chemicals in the work plan. If an assessment identifies unacceptable risks to humans or the environment, EPA will pursue risk management. The target audience for this risk assessment is primarily EPA risk managers; however, it may also be of interest to the broader risk assessment community as well as US stakeholders that are interested in HHCB. The information presented in the risk assessment may be of assistance to other Federal, State, and Local agencies as well as to members of the general public who are interested in the risks of HHCB.

The initial step in EPA's risk assessment development process, which is distinct from the initial prioritization exercise, includes scoping and problem formulation. During these steps EPA reviews currently available data and information, including but not limited to, assessments conducted by others (e.g., authorities in other countries), published or readily available reports, and published scientific literature. During scoping and problem formulation the more robust review of the factors influencing initial prioritization may result in refinement – either addition/expansion or removal/contraction – of specific hazard or exposure concerns previously identified in the prioritization methodology.

HHCB (CASRN 1222-05-5) is a high production volume fragrance ingredient in consumer and commercial products. HHCB is one of the most common synthetic polycyclic musks and is used as an ingredient in consumer products including perfumes, cosmetics, shampoos, lotions, detergents, fabric softeners, air fresheners, and cleaning agents.

[2] http://www.epa.gov/oppt/existingchemicals/pubs/workplans.html
[3] http://www.epa.gov/oppt/existingchemicals/pubs/wpmethods.pdf

Previous assessments in Europe, Sweden, and Australia have evaluated the human and/or environmental risk from HHCB (Balk and Ford, 1999a; 1999b; Balk et al., 2004; HERA, 2004; OSPAR, 2004; EC, 2008; SWECO, 2008; Langdon et al., 2010). The 2008 EU Risk Assessment Report (RAR) for HHCB (EC, 2008), concluded: "There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already." No potential risk concerns were identified for surface water and sediment in Sweden (SWECO, 2008) or for the marine environment (OSPAR, 2004). Potential risk concerns were identified for HHCB in certain highly contaminated sediments in Europe (HERA, 2004) and in an initial screening study of pharmaceuticals, personal care products, and endocrine disrupting compounds in surface runoff from biosolids-amended land in Australia (Langdon et al., 2010).

A screening-level risk assessment for chemicals of emerging concern in California receiving waters recently identified a risk concern for HHCB in effluent dominated inland waterways and coastal embayments and recommended monitoring in inland waterways (Anderson et al., 2012). Further, California lists HHCB as a designated chemical for biomonitoring, however, California is not yet biomonitoring for these chemicals (CA EPA OEHHA, 2014b). In addition, the Oregon Department of Environmental Quality lists HHCB as a priority persistent pollutant because it has a documented effect on humans, fish, wildlife, and plants (Oregon DEQ, 2010a; Oregon DEQ, 2011). Oregon also posts use, exposure pathways and release data for HHCB under this program (Oregon DEQ, 2010b). In addition, Minnesota classifies HHCB as a chemical of high concern (MDH, 2013).

HHCB has not been the subject of specific regulatory activity in the US, Canada, or the European Union (EU). HHCB is listed on the TSCA Inventory, is a High Production Volume (HPV) chemical substance, and is reported for Chemical Data Reporting (CDR), but is not otherwise subject to specific TSCA regulations. It is not listed on the Emergency Planning and Community Right-to-Know (EPCRA) Section 313 List of Toxic Chemicals. HHCB is approved as an inert ingredient for fragrance and non-food use in pesticide products under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA).[4] In Canada, HHCB is listed on the Domestic Substances List and is characterized as "inherently toxic to aquatics," but did not meet Canada's full categorization criteria and was not recommended for further assessment. In Europe, HHCB is registered under the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation.

[4] HHCB is commonly diluted in diethyl phthalate (DEP), benzyl benzoate (BB), or isopropyl myristate (IPM) before being added to fragrance formulations Rimkus (1999). However, EPA recently proposed a ban for DEP as an inert ingredient in pesticide products (EPA, 2012c).

1.2 PROBLEM FORMULATION

EPA/OPPT relied on Agency guidance to develop this the risk assessment (EPA, 1998 and EPA 2014a). EPA followed the risk assessment paradigm beginning with planning and scoping and problem formulation.

EPA/OPPT approached problem formulation by reviewing other recently performed assessments and searching published literature from 2005, the date of the last literature review indicated in the EU RAR (EC, 2008), to May, 2012. The literature review included HHCB chemistry, uses, sources including industrial releases, fate, exposure, and hazard to humans and ecological receptors. Other sources of information reviewed were unpublished reports provided by the Research Institute for Fragrance Materials (RIFM), the publicly available EPA Inventory Update Reporting (IUR) and Chemical Data Reporting (CDR) databases, and the US Geological Survey National Water Information Service (NWIS). Data was acceptable if it met standard quality criteria which varies according to the type of information (data quality criteria are described in each later section of this report).

During problem formulation, EPA/OPPT reviewed and summarized the following information:

- HHCB has an aggregate annual import volume of 3,126,728 lbs. HHCB was reported as imported to the US by three companies in 2012. Three additional companies that reported to CDR are also assumed to import.
- TSCA uses for HHCB are as an ingredient in detergents, fabric softeners, dishwashing detergents, and commercial and consumer general purpose cleaners. Non-TSCA uses include cosmetics and personal care products, which are regulated under the Federal Food, Drug, and Cosmetic Act.
- HHCB is not included on the list of substances for which information on environmental releases is collected as part of the Toxics Release Inventory (TRI) Program.
- Due to use in a wide variety of commercial and consumer products, HHCB is continuously released to the environment in municipal wastewater and sludge. Releases to the environment from industrial sites are also expected.
- HHCB is not readily biodegradable and is expected partition to organic-rich particles in sediment, soil and sludge, based on the physical-chemical properties.
- HHCB is moderately persistent in the environment and bioconcentration has been measured as low to moderate, depending on the species tested. HHCB has been found to have low bioaccumulation potential and to be biotransformed to more polar, less toxic, metabolites in some organisms.
- HHCB is highly toxic to aquatic organisms under both acute and chronic exposure conditions. Chronic effects of HHCB in aquatic organisms may include effects on adult and embryo survival, behavior, respiration, and larval development. Mechanistic studies (described in Appendix F) show that HHCB may interfere with steroid hormone

receptors, metabolism, and membrane transporters in fish and aquatic invertebrates, but not in mammals.

- Acute and chronic toxicity of HHCB has been studied in benthic and terrestrial invertebrates and in crop plants.
- Monitoring data for the US are available from the US Geological Survey (USGS) National Water Information System (NWIS) database and in the published scientific literature.
- Human health hazards and risks of HHCB have been previously assessed by other organizations. Appendix A summarizes the conclusions of the 2008 EU RAR.
- Human dermal and inhalation exposures occur intentionally as a result of use of cosmetics and personal care products and may also occur during use of detergents and cleaners. HHCB is frequently measured in human blood and breast milk (see Appendix A for a summary of human biomonitoring data).
- Developmental effects occur at relatively high oral exposures (yielding a conservative LOAEL of 500 mg/kg/day) and no developmental toxicity occurs at levels several times greater than levels detected in human breast milk (in rodent studies, pups in the high-dose group were estimated to be exposed to levels approximately 100 to 360 times the maximum level found in human milk samples (1,316 µg/kg fat)). A 2-year cancer study is not available for HHCB.
- Based on review of available human health data, including a risk assessment performed by the European Union, EPA/OPPT determined that further assessment of human health risk was not currently needed.

1.3 CONCEPTUAL MODEL FOR ENVIRONMENTAL ASSESSMENT

The exposure scenarios within the scope of EPA/OPPT's HHCB assessment are depicted in the Conceptual Model (Figure 1-1).

As mentioned previously, HHCB is one of the most widely used polycyclic musk fragrance ingredients in a range of consumer products including perfumes, cosmetics, shampoos, lotions, detergents, fabric softeners and household cleaners. Because exposure to consumers occurs intentionally for a majority of these uses, EPA/OPPT reviewed the available human health data and subsequently determined that further assessment of human health risks was not currently needed.

After commercial or residential use of consumer products containing HHCB, the majority of HHCB enters the wastewater stream. Releases to the environment from industrial sites were estimated to be at most < 10% of the HHCB production/import volume (see Appendix E: Release Estimates). Due to the ubiquity of fragranced products, consumer uses are a continuing source of HHCB to wastewater. When HHCB enters wastewater treatment plants (WWTPs), the chemical is negligibly volatile and expected to partition to organic-rich particles during wastewater treatment based on the physical-chemical properties. However, HHCB removal

during treatment in WWTPs is suboptimal (see Chapter 2, Fate in Wastewater) and HHCB is present in both wastewater effluent and sludge (WERF, 2007).

During the wastewater treatment process, influent is treated, producing sludge and effluent that is released to surface water at outfall sites, which may be located in freshwater, estuarine, or marine environments. Under this scenario, HHCB enters the environment via the effluent and is diluted to varying degrees in streams, lakes, estuaries, and oceans where aquatic organisms may be exposed. In addition, thousands of sanitary sewer overflows (SSOs) occur each year in the US, resulting in releases of billions of gallons of untreated wastewater directly to surface water. When HHCB has not been removed during the treatment process and is present in effluent, sorption to suspended solids in the surface water and partitioning to sediment is expected. Under these conditions, HHCB may be available for uptake by fish, aquatic invertebrates, aquatic plants, and benthic organisms, leading to potential exposure. The route of exposure is likely dermal and branchial in aquatic organisms and can also be by oral ingestion in sediment and soil organisms. As HHCB is negligibly volatile, it is likely to enter the environment during the wastewater treatment process or subsequently, from receiving waters.

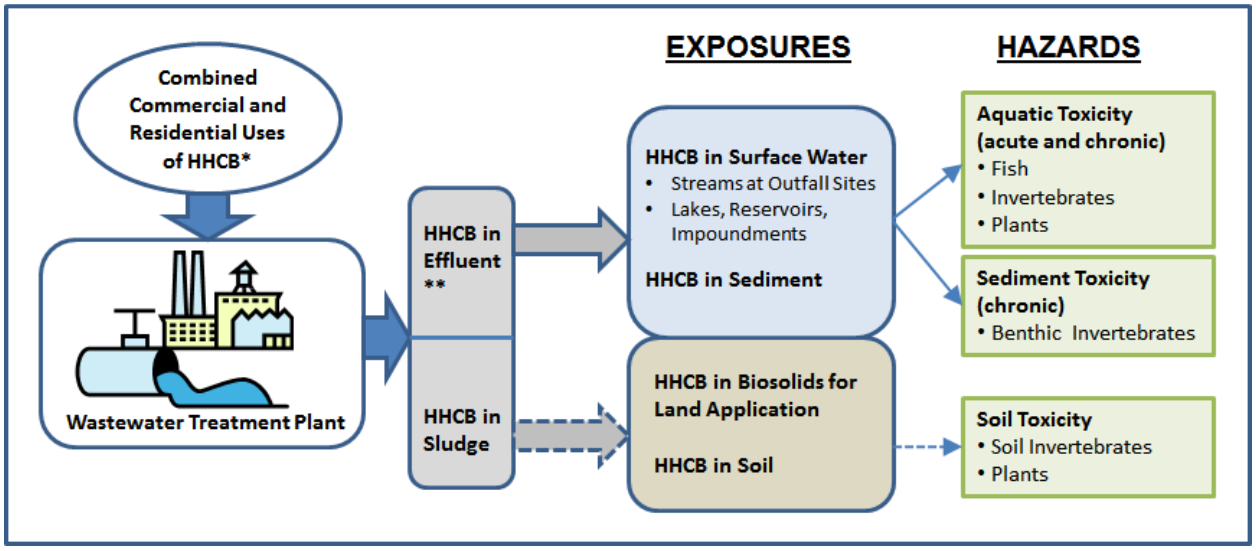

Figure 1-1. Conceptual Model for HHCB Assessment

* Includes >90% of releases to the environment from all fragranced products such as soaps, detergents, fabric softeners, shampoos, cosmetics, and cleaners. Industrial releases are <10% of releases and are not indicated in the diagram.
**EPA estimates that between 23,000 and 75,000 sanitary sewer overflows (SSOs) occur each year in the US, resulting in releases of untreated wastewater between 3 billion and 10 billion gallons.
Dotted line indicates the pathway was not assessed.

Because of its properties, HHCB also partitions to solid phases in the wastewater treatment process (sludge), and with further treatment can become concentrated in biosolids. This organic carbon-rich material is disposed of by landfill or incineration, or may be utilized for land application to enhance physical soil properties as well as plant yield (Correa et al., 2005). WWTPs need to dispose of waste sludge continuously, and about half of all sludge is applied to

agricultural land each year as biosolids.[5] Once biosolids containing HHCB are applied to land, HHCB has a long half-life in soil and may be available for uptake by plants and soil invertebrates, leading to potential exposure.

1.4 ANALYSIS PLAN FOR ENVIRONMENTAL ASSESSMENT

Based on the Conceptual Model described above, the scenarios evaluated in this assessment are release of HHCB in wastewater to surface water and sediment. The exposure assessment is based on available monitoring data for the US. The ecological assessment endpoints evaluated for HHCB are acute and chronic toxicity to aquatic organisms and chronic toxicity to sediment organisms. Chronic toxicity to terrestrial invertebrates and plants was not evaluated due to insufficient data for HHCB concentrations in soil.

EPA/OPPT reviewed other recently performed assessments and searched published literature from 2005, the date of the last literature review indicated in the EU RAR (EC, 2008), to May, 2012. The literature review included HHCB chemistry, uses, sources including industrial releases, fate, exposure, and hazard to humans and the environment. Other sources of information reviewed were unpublished reports provided by the Research Institute for Fragrance Materials (RIFM), the publicly available EPA Inventory Update Reporting and Chemical Data Reporting databases, and the US Geological Survey National Water Information Service (NWIS).

Key ecological hazard reports were obtained for critical review and data quality assessment. Details on the data quality criteria for ecological hazard assessment are included in Chapter 3.

Due to the availability of a sufficient quantity of monitoring data for the US, EPA/OPPT analyzed measured levels of HHCB and data from ecotoxicological studies of acceptable quality to assess the risks of HHCB to the aquatic environment by calculating conservative acute and chronic concentrations of concern (COCs). The potential risks to aquatic and benthic organisms were characterized by calculating the risk quotient (RQ) for each route of environmental exposure (*i.e.*, water and sediment) based on measured media concentrations.

[5] About 7.2 dry US tons of biosolids were beneficially used or disposed in 50 states in 2004. Of that total, approximately 55 percent was applied to soils for agronomic, silvicultural, and/or land restoration purposes, or was likely stored for such uses. The remaining 45 percent was disposed of in municipal solid waste landfills, surface disposal units, and or incineration facilities. (NEBRA, 2007).

2 SOURCES AND ENVIRONMENTAL FATE

2.1 INTRODUCTION

As an initial step in assessing the environmental risks of HHCB, physical and chemical properties of HHCB, sources of HHCB related to production and uses, and fate of HHCB in the environment are described.

2.1.1 Physical and Chemical Properties

HHCB is an almost colorless viscous liquid with a musk-like odor. The polycyclic chemical structure for HHCB is shown in Figure 2-1.

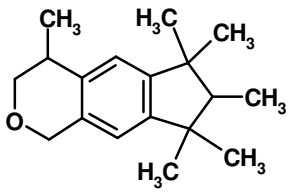

Figure 2-1. Chemical Structure of HHCB (HSDB, 2007)

Synonyms for HHCB include:

- 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamehyl-cyclopenta-gamma-2-benzopyran
- 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta-[γ]-2-benzopyran
- 6-oxa-1,1,2,3,3,8-hexamethyl-2,3,5,6,7,8-hexahydro-1H-benz[f]indene
- Galaxolide
- Galaxolide 50
- Galaxolide 50BB
- Galaxolide 50IPM
- Galaxolide White
- Abbalide
- Pearlide

Technical HHCB consists of a mixture of isomers that are unspecified. Four diastereoisomers (*i.e.*, an isomer that differs in the spatial arrangement of atoms in the molecule, but is not a mirror image) of HHCB are known to exist: two (-)/4S isomers (4S, 7R & 4S, 7S) have the characteristic odor, and the other two (+)/4R isomers (4R, 7R & 4R, 7S) have little to no odor

(DrugLead, 2009; http://www.druglead.com/cds/hhcb.html). There are many other structural isomers or analogs related to HHCB; a complete list is provided in Appendix C.

The physical and chemical properties of HHCB are shown in Table 2-1.

Table 2-1. Physical-Chemical Properties of HHCB[a]

Molecular formula	$C_{18}H_{26}O$
Molecular weight	258.44 $g \cdot mol^{-1}$
Physical form	Colorless liquid; highly viscous liquid at 20 °C and 1,013 hPa with a musk odor
Melting point	-10 to 0 °C (determined by cooling viscous liquid to –30 °C and gradual warm up)
Boiling point	320 °C at 760 mmHg (converted from 160 °C at 4 hPa)
Vapor pressure	0.0727 Pa (5.47×10^{-4} mmHg) @ 25 °C (measured; OECD[b] Test Guideline 104)
Logarithmic octanol:water partition coefficient (log K_{OW})	5.3 ("slow stirring" method)[b]; 5.9 @ 25 °C (measured; OECD Test Guideline 117)
Water solubility	1.65 mg/L at pH 7 (at 25 °C)[c]; 2.3 mg/L at 20 °C (measured; OECD Test Guideline 105)
Flash point	144 °C
Henry's Law constant	1.13×10^{-4} $atm \cdot m^3/mol$ (at 25 °C, calculated using measured vapor pressure and water solubility); 1.32×10^{-4} $atm \cdot m^3/mol$ (at 25 °C, estimated using HENRYWIN program in EPI Suite v4.11); 3.65×10^{-4} $atm \cdot m^3/mol$ (at 25 °C, measured)[d]

[a] Source: (HSDB, 2007) except as noted.
[b] OECD – Organization for Economic Cooperation and Development
[c] pH is not expected to effect HHCB solubility as it is non-ionizable
[d] Sources: EC, 2008; Artola-Garicano, 2002

2.2 PRODUCTION AND USES

This section discusses the US production volumes and uses specifically for HHCB and more generally for fragrances. The manufacturing process for HHCB is described in Appendix B. The

HHCB commercial product is diluted (65 percent wet weight [ww]) in diethyl phthalate (DEP)[6], benzyl benzoate (BB), or isopropyl myristate (IPM) prior to compounding and formulation into products (HERA, 2004b). HHCB is a High Production Volume chemical that is widely used in cleaning and personal care products. Data on production volume and uses are amenable for determining release estimates to the environment (See Appendix E). These data are also useful for understanding potential exposure routes and pathways through which HHCB may enter the environment through industrial and consumer applications.

2.2.1 Production

HHCB is one of the most widely used and consumed polycyclic musks that represents 90 percent of the total US polycyclic musk market (EC, 2008; HERA, 2004a, respectively). Five US firms reported some non-confidential business information (CBI) CDR data for HHCB in 2012 (EPA, 2014)[7] and a sixth reported only CBI information[7]. One of the five firms reported production at two sites. A list of the US producers of HHCB is provided in Table 2-2. Of the five companies listed, three reported importing HHCB, two did not indicate whether they are a manufacturer or importer. EPA/OPPT assumes that these companies and the sixth company that reported only CBI information are also importers because HHCB is not manufactured in the US (IFRA, 2012a). HHCB is imported in liquid forms at a maximum concentration of ≥90 percent (EPA, 2014b).

Table 2-2. Major US Manufacturers or Importers of HHCB

Chemical	Company[a]	Reported CDR Data in 2012	Manufacturer or Importer
HHCB, Galaxolide, Musk GX, Abbalide	Berje, Inc.	Yes	Importer
	International Flavors & Fragrances, Inc.	Yes	Importer
	Symrise, Inc.	Yes	Importer
	S C Johnson & Son, Inc.	Yes	No data[b]
	Firmenich, Inc.	Yes	No data[b]

[a] One company reported as a producer of HHCB in 2012, however all data were CBI so the company is not included in this table.
[b] 'No data' indicates that data are not available on whether the company is a manufacturer or importer.
Source: EPA (2014b).

Between 1996 and 2000, the US consumption of synthetic musk fragrances increased by 25 percent, from about 5,200 to 6,500 tons (10.4 to 13 million lbs), while the consumption of fragrance chemicals only grew by 15 percent (Somogyi and Kishi, 2001; as cited in Peck et al., 2006). Global musk production increased by 12.5 percent between 1987 and 1996 and during

[6] EPA plans to no longer approve DEP as an inert ingredient in pesticide products (US EPA, 2012c).
[7] These six producers may be an underestimate because a production site is only required to submit a Form U (the CDR reporting instrument) if it produces or imports more than 25,000 pounds of a chemical during the reporting year.

this time period, production shifted from nitro musks, such as musk xylene, to polycyclic musks (Rimkus, 1999; as cited in Luckenbach and Epel, 2005). This shift, reflected in decreasing production rates of nitro musks and increasing production rates of polycyclic musks (Hutter et al., 2005), is expected to continue for several reasons. In June 2009, the European Chemicals Agency (ECHA) recommended xylene musk for authorization under REACH (ECHA, 2009a). It was added to the authorization list in February 2011, which means that musk xylene can be used only in cases where an authorization has been granted for a specific use (European Union, 2011). Additionally, in June 2009, the International Fragrance Association (IFRA) voluntarily phased out musk xylene through the IFRA Standards, part of the fragrance industry's global self-regulatory program contained in the IFRA Code of Practice. The Code of Practice is mandatory for IFRA members, and membership accounts for approximately 90 percent of the global volume of fragrance materials (IFRA, 2011).

Data from IFRA have shown an increase in the volume of HHCB (point estimate opposed to range estimate) used in the US from the years (yrs) 2000 to 2011. IFRA estimated US HHCB use volume to be approximately 1,275 tons (2.8 million lbs) per yr in 2000 and slightly under 1,400 metric tons (3.1 million lbs) per yr in 2004 (IFRA, 2012a). More recently, IFRA provided additional use volume estimates in the US for 2008 and 2011 of approximately 1,600 and 1,700 metric tons (3.52 and 3.74 million lbs) per yr, respectively (IFRA, 2012b).[8] According to IFRA, the increase in HHCB use in the US is not a reflection of increased use of this particular musk over others, but a reflection of increased market demand for fragranced consumer products, which has expanded the market for HHCB and other musk chemicals (IFRA, 2012c).

It is unclear whether the North American market for synthetic musks will eventually experience the same shift, as in the EU, from nitro musks to polycyclic musks (Gatermann et al., 1999; as cited in Peck et al., 2006). At present, it appears as though the US production volume (which includes import) trends for HHCB and musk xylene in the US are consistent for both chemicals. For example, the annual (non-CBI) production volume of HHCB has ranged between 1 and 10 million lbs since 1990 (Table 2-3). In addition, with the exception of 2002, the non-CBI IUR production volume for musk xylene has been consistently reported to be <500,000 lbs (EPA, 2012d; 2012e). Therefore, and in contrast to the EU, the steady range of production volumes for both HHCB and musk xylene suggests that there has not been a significant shift away from nitro musks to polycyclic musks in the US.

[8] IFRA's letter to EPA dated March 30, 2012 indicated that the 2008 use volume of HHCB in the US was approximately 1,300 tons per year. This estimate was revised by IFRA in an email dated June 29, 2012 to 1,600 tons per year in 2008.

Table 2-3. US Production/Import Volume of HHCB

Chemical	Production/Import Volume (in Thousands, K, or Millions, M, of lbs)						
	Year						
	1986	1990	1994	1998	2002	2006	2012
HHCB, Galaxolide, Musk GX, Abbalide	500K-1M	1M-10M	1M-10M	1M-10M	1M-10M	1M-10M	3.1M
Musk Xylene	500K-1M	10K-500K	10K-500K	10K-500K	10K-500K	<500K	NR

NR- No volume reported
Sources: EPA (2012d; 2012e; 2014b)

2.2.2 Uses

Musks are considered important compounds to the fragrance industry because of their unique odor properties, ability to improve the fixation of fragrance compounds, and ability to bind fragrances to fabrics (Sommer, 2004). The function of HHCB in fragrance formulations is as both a fragrance and a fragrance enhancer (ECHA, 2009b).

HHCB is used as a fragrance ingredient in cleaning and personal care products because it is alkali-stable and does not discolor in light (Ash et al., 2009; OSPAR, 2004; IFF, 2012; Fahlbusch et al., 2012). HHCB and other musks provide a unique, dominant scent in products. Because HHCB binds fragrances to fabric and skin, the scent is balanced and longer lasting (OSPAR, 2004). HHCB is often used in laundry detergent fragrances because it is one of the few chemicals that can leave a small residual fragrance on cloth after washing and can cover up odors from the detergent itself as well as from dirt in the wash solution (Schreiber, 2004). Synthetic musks, including HHCB, also may be used to mask chemical odors and can be found in products labeled "unscented," but do not seem to be added to products labeled "fragrance free" (Potera, 2007).

The EU RAR (EC, 2008) estimated that in the EU, approximately 86 percent of manufactured HHCB is used as part of a fragrance mixture, and 14 percent is used directly in the bulk formulation of products. HHCB is, however, generally estimated to make up only two to four percent of fragrance compounds because fragrance mixtures can contain from 50 to 300 ingredients (HERA, 2004a; Bickers et al., 2003). As shown in Table 2-4, the majority of fragrance oils (25 percent) are used in detergents, followed by fabric softeners (14 percent) and personal care products (13 percent), whereas on a weight percent (wt%) basis, perfume extracts have the highest level of fragrance incorporation at 20 wt% (Table 2-5). Given the importance of HHCB as a fragrance ingredient, EPA/OPPT expects that the use of HHCB has a similar distribution pattern as fragrance oils in general with the largest percentage of volume used in detergents, fabric softeners, and personal care products.

Table 2-4. Estimated Distribution of Fragrance Oils by Use

Use	Fragrance Oils (percent)
Detergent	25
Fabric softeners	14
Personal care	13
Bath and shower	10
Hair care	10
Soaps	9
Industrial and household cleaner	8
Other	6
Fine fragrances	5
Source: Balk et al. (2001); as cited in OSPAR (2004).	

Table 2-5. Cosmetic Product Types and Upper Levels of Fragrance Incorporation

Cosmetic Product Type	Fragrance Level (wt%)[a,b]
Perfume extracts	20.00
Toilet waters	8.00
Fragranced cream	4.00
Bath products	2.00
Toilet soap	1.50
Shower gel	1.20
Antiperspirant/deodorant	1.00
Hair spray	0.50
Shampoo	0.50
Body lotion	0.40
Face cream	0.30

[a] Industry survey on typical quantities used per application of different cosmetics (COLIPA, 1987; as cited in Cadby et al., 2002).
[b] Estimates of typical fragrance levels in different products and maximum likely proportion of fragrance remaining on skin after normal product use (RIFM, 1996; as cited in Cadby et al., 2002).
Source: Cadby et al., 2002

Several recent studies have measured the levels of HHCB in a variety of consumer products. One study found HHCB in 55% of the personal care products tested, including 100% of

deodorants tested (Roosens et. al., 2007). A 2002 survey found that HHCB may be found at levels between 0.02 and 0.90% in household cleaning products (IFRA, 2002 as reported in HERA, 2004b). Another recent study by Reiner and Kannan (2006) analyzed products for concentrations of HHCB and a degradant of HHCB, HHCB-lactone. In this study, HHCB concentrations were from <5 to 646,000 mg/kg sample and HHCB-lactone was <5 mg/kg in the same products. This study also showed that products within a single class might contain differing levels of HHCB. For example, it was noted that HHCB concentrations in two different laundry detergents were 79 and 84,900 mg/kg sample.

A wide range of consumer products contain HHCB. However, these studies indicate that levels varied significantly across and even within product types. This variation is not unexpected given the complexity of fragrance mixtures which often contain hundreds of constituents.

2.2.3 Conclusions of Production and Use

Use volume of HHCB has increased gradually during 2000-2011 due to increased demand for fragranced products. HHCB is used as part of fragrance mixtures in products such as detergents and fabric softeners, and personal care products. The concentration of HHCB in any of these products ranges widely across product types as well as within single product categories (*e.g.*, laundry detergent).

2.3 ENVIRONMENTAL FATE

HHCB enters domestic wastewater treatment and is subjected to removal by a variety of processes. HHCB and any degradation products generated in wastewater treatment are mainly discharged to receiving waters or disposed of in biosolids. This section summarizes current knowledge of the fate and degradation of HHCB in waste water and the persistence, bioconcentration and bioaccumulation of HHCB in the environment. Biological and abiotic reactions of HHCB as they relate to environmental fate and distribution are summarized below and in Table 2-6.

2.3.1 Environmental Persistence

2.3.1.1 Fate in Wastewater Treatment

The half-life of HHCB in activated sludge at concentrations of 5, 17.4, 25, 25 µg/L has been reported as 69, 10-15, 21, 33 hours, respectively (Federle et al., 2002; Schaefer, 2005; Langworthy et al., 2000, as cited in EC, 2008). HHCB disappearance with subsequent appearance of more polar entities was observed (Langworthy et al., 2000; as cited in EC, 2008). The geometric mean from these studies for activated sludge half-disappearance time was is 22.5 hours. This corresponds to “moderate-to-slow” biodegradation in activated sludge; see

guidance in the Estimation Programs Interface (EPI) Suite v4.11[9] (EPA, 2011). The principal degradation product of HHCB is HHCB-lactone, which can be present at levels as high as 50% of HHCB concentrations (Bester, 2005; Horii et al., 2007). Formation of the lactone appears to occur via aerobic biodegradation. However, it has also been reported to be formed from treatment with hypochlorite (Kulich et al., 2010) and present in products containing HHCB, which the authors suggest may be as an impurity in technical HHCB (Reiner and Kannan, 2006).

HHCB is expected to partition strongly to solid phases based on its high measured log K_{OW} of 5.9 (see Rimkus, 1999 for a summary of values for musks) and the soil/sediment organic carbon partition coefficient (log Koc = 3.6-3.9; EC, 2008) which is supported by the estimated K_{OC} (log basis) of 4.1-4.3 (KOCWIN™ program v2.00; in EPI Suite™ v4.11[9], (EPA, 2011)). Values for both K_d (sorption coefficient) and K_{OC} (organic carbon-normalized sorption coefficient) are generally in the range of 3 to 4 on a logarithmic scale. This means that HHCB will be substantially removed by sorption to sludge in WWTPs; will have low mobility in soil; and will bind strongly to benthic and suspended sediment. The Human and Environmental Risk Assessment Project (HERA, 2004a) summarized both theoretical and measured percent removal values (up to 2004) for European and US activated sludge-based treatment systems. Observed removal was generally >50 percent (usually >80 percent), and removal percentage correlated well with removal of total suspended solids (Simonich et al., 2002). Kupper et al. (2006) reported overall HHCB removal of 81% for a full-scale Swiss treatment plant, in line with the earlier results; and Horii et al. (2007) reported similar overall HHCB removal for wastewater treatment plants in Kentucky and Georgia.

Waste (settled) activated sludge in WWTPs is generally sent to anaerobic digesters to reduce sludge volume and organic load before disposal of the concentrated material. Removal of HHCB under anaerobic treatment conditions has been addressed in several studies. Xue et al. (2010) reported poor overall removal of HHCB in a full-scale anaerobic/anoxic/aerobic system combined with membrane bioreactor for municipal wastewater reclamation, and little removal was attributed to biodegradation. In contrast Carballa et al. (2007) reported good removal of HHCB (60-80%) in mesophilic and thermophilic anaerobic sludge digestion. Kupper et al. (2006) observed apparent anaerobic degradation of HHCB in the sludge line of a full-scale treatment system, but the mechanism was not confirmed. Therefore, no direct evidence of anaerobic (methanogenic) biodegradation of HHCB exists.

2.3.1.2 Fate in Water

HHCB will bind strongly to solid phases in the environment; therefore to benthic and suspended sediment. Though HHCB is negligibly volatile in WWTP, volatilization is an important process for HHCB in water. After 12.5 days, about 40 percent of the radioactivity was lost by volatilization. In a more recent presentation reporting on the same or extended experiments, with 0.5 µg/L of HHCB in river water, the half-life for disappearance of the

[9] Available for download from http://www.epa.gov/opptintr/exposure/pubs/episuite.htm and http://www.epa.gov/oppt/exposure/pubs/halflife.htm

parent substance was 43 hours (Federle et al., 2002). In a series of die-away tests, an overall half-life of 100 hours was derived for disappearance of HHCB in river water (Schaefer, 2005; as reported in EC, 2008). A significant fraction (up to 16 percent) was volatilized after 28 days. These studies show that specific test conditions impact observed volatilization behavior, but that in any case volatilization is significant and half-lives are on the order of days to weeks.

HHCB was not readily biodegradable (0 percent in 28 days) in an OECD 301B test (Jenkins, 1991; as cited in EC, 2008) and a sealed vessel headspace test (OECD 310), the latter with adapted inoculum (King, 1994; as cited in EC, 2008). HHCB appears not to undergo direct photolysis in water at a significant rate (Buerge et al., 2003).

In the modified river die-away tests of Langworthy et al. (2000), Federle et al. (2002), and Schaefer (2005) (all as cited in EC, 2008), which were designed to simulate river water conditions immediately below discharge of treated sewage from activated sludge treatment plants, half-lives for HHCB disappearance of 10-69 hours were observed (see Table 2-6). Degradation is expected to be slower in streams that do not receive effluents from WWTPs or that are less impacted by such effluents as it can be expected that below WWTP discharge points, acclimated microbial populations will have developed.

In batch experiments (Langworthy et al., 2000; Federle et al., 2002; as cited in EC, 2008), effluent diluted in river water was spiked with radiolabeled HHCB at concentrations of 1.0 or 0.5 µg/L. The observed degradation half-life of HHCB was 33 to 43 hours, depending on the starting concentration. Following 29 days, polar metabolites made up 60% of the original radioactivity and the mass balance accounted for 92% of the radioactivity.

In a series of die-away tests, a variety of metabolites were formed at different rates and with increasing polarity over time (Schaefer et al., 2005; as reported in EC, 2008). In the river die-away test the total radioactivity of metabolites rose from 25 to 56 percent between days 2 and 7 and to nearly 62 percent at 28 days. When the results were corrected for the amount volatilized and the non-recovered fraction as well as for abiotic transformation, the primary biodegradation extent on day 28 was approximately 60 percent. About 40 percent of the radioactivity was lost by volatilization at 300 hours, but some of the volatilized radioactivity could have been in the form of degradation products, since degradation in water was also happening during the test.

2.3.1.3 Fate in Soil and Sediment

HHCB has low mobility in soil. The fate of HHCB was studied using microcosms containing oak forest soil and agricultural soils, river sediment, and agricultural soil that routinely receives applications of biosolids from a domestic WWTP (sludge amended soil) (Envirogen, 1998; as cited in EC, 2008). In the study, sealed flasks with HHCB-spiked soil at 10 µg/g soil (10 mg/kg) were incubated at laboratory ambient temperature for one year. Periodically the headspace was flushed for oxygen replenishment and volatile organics and carbon dioxide were captured.

After incubation, and exhaustive extraction with ethyl acetate and/or acetone/hexane, an aliquot of the solvent was used for TLC. After one year, significant amounts of polar metabolites were found, and HHCB was present at 4, 7, 9 and 35 percent of the initial concentration in the river sediment, forest soil, sludge-amended soil, and agricultural soil, respectively. After one year, the average remaining HHCB for the four soil types was 14 percent of the HHCB initially present. The TLC results showed that HHCB was degraded to various more polar metabolites. It was hypothesized that the majority of unrecovered radiolabel became covalently bound to soil organic compounds (*i.e.*, was immobilized by humification).

DiFrancesco et al. (2004) studied the dissipation of fragrance materials in biosolids-amended soils in a one year die-away experiment with four different soils, with and without spiking of the test materials. The four soils included sandy agricultural soil; silty agricultural soil; clayey soil; and highly weathered oxide-rich soil. The anaerobically digested and dewatered sludge was obtained from activated sludge WWTPs in Georgetown, Delaware (100 percent domestic sewage; 10 percent solids) and Wilmington, Delaware (70 percent domestic sewage; 17 percent solids). The unamended concentration of HHCB in the digested sludge from Georgetown, Delaware was 86 and 38 mg/kg dw in the years 2000 and 2002, respectively and from Wilmington was 43 and 22 mg/kg dw, respectively. The initial HHCB concentrations in spiked soil were 6 and 13 mg/kg soil, whereas the levels in unspiked soil were 0.1 to 0.27 mg/kg. In all soils, the concentrations rapidly decreased. After one month, the concentrations of HHCB in the four soils were 30 to 90 percent of the initial concentration, and after 90 days, they ranged from 8 to 60 percent of the initial concentration. During the three months when the soil was frozen, concentrations of HHCB were stable. After one year, the residues ranged from below 10 to 14 percent of the initial concentration. The rate of dissipation was higher in the soils with lower content of organic material. Loss processes could have included volatilization and leaching as well as biotransformation. The EC (EC, 2008) calculated half-disappearance times based on these data and reported values of 141 and 144 days for the spiked and unspiked biosolids-amended soils, respectively. In this study, leachates were also collected over 3 to 5 months, and the HHCB found amounted to 0.03 to 0.18 percent of the initial amount in the spiked soils. HHCB was not detected in leachate from unspiked soils (EC, 2008). The low percentage of HHCB initially in the sludge and present in leachate was probably a result of both degradation and sorption.

Observed soil and sediment half-lives consistently exceeded 60 days (Table 2-6). Field measurements on biosolids-amended soil indicated that HHCB disappeared almost completely from soil within one year. The half-life based on unfrozen conditions in biosolids-amended soil studies was around 140 to 144 days (DiFrancesco et al., 2004). The residues in soil after one year ranged from below 10 to 14 percent of the initial concentrations. In the EU RAR (EC, 2008), a half-life of 105 days in the biosolids-amended soil was deemed most relevant for modeling the fate of HHCB in soil using the European Union System for the Evaluation of Substances (EUSES) model, while 79 days was noted for the sediment (Envirogen, 1998; as cited in EC, 2008). EPA/OPPT agrees that these values are reasonable for modeling and assessment purposes.

Table 2-6. HHCB Degradation Half-Lives and Half-Disappearance Times for Environmental Media

Degradation Half-Life or Half-Disappearance Time[a]					Reference
Water	**Sediment**	**Soil**	**Sludge**	**Biosolids-Amended Soil**	
33 hours			21 hours (DT50)		Langworthy et al. (2000) (as cited in EC, 2008)
43 hours			33-69 hours (DT50)		Federle et al. (2002) (as cited in EC, 2008)
100 hours[b] (DT50)			10-15 hours (DT50)		Schaefer (2005) (as cited in EC, 2008)
	79 days	95 days (forest soil)		105 days	Envirogen (1998) (as cited in EC, 2008)
		239 days (agricultural soil)			Envirogen (1998) (as cited in EC, 2008)
				141-144 days (DT50)	DiFrancesco et al. (2004)

[a] DT50 is the time required for disappearance of 50% of the starting material. Half-disappearance time (DT50) differs from degradation half-life in that it includes all mechanisms contributing to disappearance (*e.g.*, volatilization in addition to degradation). Numbers that are DT50 values are indicated in the table; otherwise the data are degradation half-lives.
[b] Water with 10 mg/L suspended solids.

2.3.1.4 Fate in Air

Aschmann et al. (2001) studied the atmospheric oxidation (photooxidation) of HHCB and reported a rate constant for hydroxyl radical oxidation of $k = 2.6 \pm 0.6 \times 10^{-11}$ cm^3 $molecule^{-1}$ $second^{-1}$. This leads to a calculated atmospheric half-life of 3.4 hours for HHCB (based on a 12-hour daylight period), and suggests that long-range atmospheric transport is unlikely to be an important process for HHCB. Recent work of Villa et al. (2014) agrees, but also suggests that "moderate-range" (this could be called regional) atmospheric transport may occur. Any HHCB in the atmosphere is likely to be in the gas (not particulate) phase. This is supported by consistent results from three different models that predict extent of sorption to atmospheric particulates (aerosols) based on chemical properties. The fraction of HHCB sorbed to airborne particulates from AEROWIN v1.00 (EPI Suite v4.11) were 0.00149, 0.00288 and 0.00329 for the Junge-Pankow, octanol/air (K_{oa}) and Mackay models, respectively.

2.3.2 Bioaccumulation and Bioconcentration

Table 2-7 summarizes published bioaccumulation data for aquatic organisms, from Dietrich and Hitzfeld (2004) and Reiner and Kannan (2011).

Fish

Dietrich and Hitzfield (2004) summarized information on the bioconcentration and bioaccumulation potential of HHCB. BCFs and BAFs varied with species but were generally lower than the commonly used criterion of 1,000 (log = 3) for characterizing bioaccumulation potential as moderate (EPA, 1999a; 1999b; Boethling et al., 2009). Measured BCFs of 620, 862 and 1,584 were reported for zebra fish, eel and bluegills, respectively (Table 2-7), whereas BAF values of 290, 510, 580, and 620 were reported for eel, tench, crucian carp and zebra mussels, respectively. These and other (chiefly monitoring) studies suggest that HHCB does not biomagnify.

A bluegill study was performed according to OECD Test Guideline 305 and using Good Laboratory Practice (GLP) standards (EC, 2008; Rimkus, 1999). Bluegill sunfish (*Lepomis macrochirus*) were exposed in a flow-through system to two concentrations of radiolabeled HHCB. A solubilizer was used to prepare a solution (concentration 0.001 percent (w/v)). Identification of the parent compound in water and fish was performed using TLC/HPLC. Nominal exposure concentrations were 1 and 10 µg/L. The fish were exposed for 28 days; the elimination period was also 28 days. The elimination rate constant (k_2) was estimated from the elimination curve (first-order kinetics), and determination of an uptake rate constant (k_1) was attempted using the following formula: $k_1 = BCF \times k_2$. However, this value could not be directly calculated from the increase of concentrations in fish due to rapid attainment of the final plateau level. Therefore, the BCF was derived from measured concentrations of parent compound in exposure water and the plateau concentration in fish. Based on the concentration of parent material, the BCF for whole fish was calculated to be 1,584 L/kg wet wt.

Reiner and Kannan (2011) and Nakata et al. (2007) studied bioaccumulation of HHCB in organisms from the upper Hudson River, USA; and marine tidal and shallow-water organisms from a Japanese site, respectively. In the first study, field (monitoring)-based BAFs were calculated as shown in Table 2-7). These and other (chiefly monitoring) studies suggest that HHCB is not subject to biomagnification, which occurs when a substance achieves higher concentrations on a lipid-adjusted basis in organisms than in their food (prey). Aquatic food chain modeling was conducted by the EPA using the BCFBAF© program (which uses the Arnot-Gobas model) in EPI Suite© v4.11. Consistent with the monitoring data, estimated BAFs for middle and upper trophic fish were successively lower, not higher, than the estimated BAF for HHCB at the lowest trophic level.

Metabolism may account for the observation that measured BCFs and BAFs are lower than would be estimated based on the log K_{OW} of HHCB (5.9). In this study, HHCB was metabolized to one or more polar metabolites in fish in a relatively short time and then excreted to the water phase. Recent work of Fernandes et al. (2013) provides evidence of metabolism of HHCB to a hydroxylated metabolite in European sea bass.

Table 2-7. BCFs and BAFs (L/kg ww) of HHCB in Aquatic Vertebrates[a]

Organism	Common Name	BCF	BAF
Scardinius erythrophthalmus	Rudd		20
Tinca tinca	Tench		510
Carassius carassius	Crucian carp		580
Anguilla anguilla	Eel	862	290
Dreissena polymorpha	Zebra mussel		620
Lepomis macrochirus	Bluegill sunfish	1,584	
Danio rerio	Zebrafish	620	
	Smallmouth bass[b]		31-106
	Largemouth bass[b]		30-146
	White perch[b]		21-333
	Catfish[b]		18-371

[a]source: Dietrich and Hitzfeld (2004) unless otherwise noted
[b]source: Reiner and Kannan (2011)

Invertebrates

Bioaccumulation has been studied with two benthic organisms (Table 2-8). Non-biting midges (*Chironomus riparius*) exposed to 9.8 $\pm$ 1.4 µg/L, the concentrations in the organisms increased to a maximum level between days 1 and 3, and then the level rapidly decreased to a new steady state with BCF values ranging from 85 to 138 L/kg wet wt. (Artola-Garciano et al., 2003). With the addition of piperonyl butoxide (PBO), a cytochrome P450 inhibitor, the BCF was 525, and it was concluded that the lower BCF values were likely caused by biotransformation of HHCB in this organism. In a flow-through experiment with blackworms (*Lumbriculus variegatus*), exposed to 4.6 $\pm$ 0.6 µg/L, uptake of HHCB reached a plateau after 3 days, and the measured BCF was 2,692 L/kg wet wt.

Jager (1998) (as cited in EC, 2008) reported a calculated BCF for earthworms (*Lumbricus terrestris*) (Table 2-8). The steady-state concentration of a substance in earthworms is assumed to be proportional to the soil pore water concentration and a BCF can be calculated using an equation given by Jager (1998) (as cited in EC, 2008):

$$BCF_{worm} = (0.84 + 0.012 \times K_{ow})/RHO_{earthworm}$$

Where:

BCF_{worm} = the earthworm bioconcentration factor (L/kg wet wt);
K_{OW} = the n-octanol:water partition coefficient; and
$RHO_{earthworm}$ = the density of the earthworm set at 1 kg wet wt/L.

Table 2-8. BCFs (L/kg ww) of HHCB in Benthic and Terrestrial Invertebrates

Organisms	Common Name	BCF	Reference
Chironomus riparius	Midge	85-138	Artola-Garicano et al. (2003)
Lumbriculus variegatus	Blackworm	2,692	Artola-Garicano et al. (2003)
Lumbricus terrestris	Earthworm	2,395[a]	Jager (1998)
[a]Calculated			

Plants

Application of biosolids or reclaimed wastewater to agricultural land or crops may provide a pathway for uptake of HHCB by plants. Land application of reclaimed wastewater seems not to have been studied as a pathway for HHCB introduction into the environment, but as reported in the EU 2008 assessment, in an unpublished study (Müller et al., 2002), transfer coefficients were determined for lettuce and carrots growing on biosolids-amended soil samples. The authors concluded that in spite of an unrealistically high concentration of HHCB in biosolids, no relevant accumulation in leaves was observed and that uptake ratios (the ratio C_{plant}/C_{soil}) were still well below the critical level of 1. Even for carrot root, transfer ratios were 0.1 and 0.48 in two different soils. Further, the low observed concentrations in the above ground parts of the carrot plant showed that there was no transport within the plant. It was concluded that under normal conditions transfer of HHCB from the soil to plants is not likely to be an important accumulation pathway.

2.3.3 Conclusions of Environmental Fate

HHCB is hydrophobic and partitions strongly to solid phases. Therefore, HHCB is removed in WWTPs predominantly due to sorption to sludge and biodegradation, but the degree of removal is considered suboptimal. HHCB has low persistence in water and is moderately persistent in soil and sediment. Based on available data, HHCB is considered to be of low to moderate concern for bioaccumulation. BCF values of 1,584 for bluegills and 2,692 for *Lumbriculus* indicate moderate bioaccumulation potential. However, BAF values are available for several aquatic organisms are in the range of 20 to 620, indicating low bioaccumulation. These studies, together with results of aquatic food-chain modeling (Arnot-Gobas model) and monitoring data for biota, suggest that HHCB is not subject to biomagnification.

3 ENVIRONMENTAL ASSESSMENT

3.1 INTRODUCTION

This assessment evaluated the environmental risk to the aquatic and terrestrial environments from the use of HHCB as a fragrance ingredient in consumer and commercial products. The HHCB environmental risk assessment is composed of the following: (A) an exposure assessment; (B) an ecological hazard assessment; (C) an environmental risk characterization; and (D) a discussion of uncertainties and data limitations. Environmental risks were assessed by comparing levels of HHCB measured in the environment to the COCs determined from aquatic and benthic toxicity studies.

3.2 ENVIRONMENTAL EXPOSURE ASSESSMENT

For this assessment, environmental monitoring data consisting of measured levels of HHCB in wastewater, surface water, sediment, soil, and biota were used to characterize environmental exposure to HHCB. The data for concentrations of HHCB measured in the environment in the US are from multiple sources, locations, and dates.

3.2.1 Estimated Environmental Releases

Releases to the environment potentially occur throughout the life cycle of HHCB. In the US, the life cycle of HHCB consists of processing of imported HHCB as an ingredient in the compounding of fragrance oils, blending of these fragrance oils into end-use products for commercial and consumer use, consumer or commercial use of products, and disposal of HHCB-containing products to the environment. Compounding of fragrance oils and formulation of end-use products occurs at industrial sites. A small amount of HHCB is released from these sites to the environment, including to water, mostly as a result of cleaning of compounding and blending equipment and cleaning of containers used to transport material. Specifically, the amount released from industrial sites was estimated at a maximum of 10 percent of the use volume (see Appendix E). EPA/OPPT assumed that the entire HHCB content of all of the various types of end-use products was released down-the-drain after use. Therefore, 90% of HHCB is assumed to be released to municipal wastewater after commercial and consumer use of products containing HHCB.

3.2.2 Measured Levels in the Environment

Studies published between 2004 and May 2012 that reported measurements of HHCB in the US environment were reviewed for this assessment. Data quality criteria included currency, geographic scope, accuracy/reliability, representativeness, lack of bias, comparability and applicability. Details regarding the QA/QC of each individual study, laboratory reporting limits, and brief summaries of the studies from the published literature are provided in Appendix G.

Additionally, monitoring data in US waterways were collected from the USGS NWIS database up to May 2012. All data collected from 2001-2012 contained in three USGS NWIS parameter codes (62075, 62823, 63209) for HHCB were included in this assessment. This database includes monitoring data as well as data collected for studies of organic anthropogenic waste indicators, organic wastewater contaminants, and biologically-active chemical mixtures in urban streams. USGS data from the NWIS database was accepted with the assumption that their internal methodologies were consistent and robust. These data were presumed to be collected under the guidance of the USGS National Field Manual for the Collection of Water-Quality Data, a publication which documents the methods, protocols, procedures and recommended practices for the collection of water-quality data (USGS, variously dated). Data reporting procedures were presumed to follow USGS guidance (Oblinger Childress et al., 1999).

Although ten times more data was available from the USGS as compared to data from published literature studies for effluent, surface water and sediment, the measured HHCB concentrations were within the same order of magnitude. EPA/OPPT assumed that data from scientific publications were comparable to the USGS data and were used along with the USGS data as the basis of the exposure and risk characterizations. Although sampling techniques varied depending upon the environmental media, gas chromatography/mass spectrometry (GC/MS) was the primary analytical methodology employed in the detection and quantification of HHCB across all media. HHCB found in wastewater, surface water, sediment, biosolids, soil, and aquatic biota are summarized below and in Table 3-1. More detailed information regarding extraction, recovery, and reporting levels for the monitoring data is provided in Appendix G.

Table 3-1. Measured Concentrations of HHCB in Biota

Environmental Compartment	Number of samples (all US studies)	Minimum	Maximum	Range of Reported Means	Reference
Wastewater (µg/L)					
Influent	>135	0.043	12.7	0.42 – 9.03	Hope et al. (2012), Horii et al.(2007), Chase et al.(2012), Reiner et al. (2007a), Osemwengie and Gerstenberger (2004)
Effluent	>135	0.010	13.0	0.02 – 5.31	
Effluent discharged to ocean	n/a	ND[e]	2.5	---[d]	Anderson et al. (2012)
Effluent (at stream or outfall)	47	0.090[a]	3.4[b]	0.98 – 1.18	USGS (code 62075 and 62823)
Surface Water (µg/L)					
Freshwater	94	ND[f]	1.6	0.00036 – 1.6	Osemwengie and Gerstenberger (2004), Anderson et al.(2012), Barber et al. (2011), Chase et al. (2012)Peck and Hornbuckle(2004), Reiner and Kannan (2011), USGS (2011), Klecka et al.(2010),
Outfall, Stream, Lake, Reservoir or Impoundment	4720	0.02[a]	2.3[b]	0.12 – 1.08	USGS NWIS (code 62075 and 62823)
Groundwater (well)	1548	0.04[a]	0.35[b]	0.32	USGS NWIS (code 62075)
Sediment (µg/kg dw)					
Freshwater	>59	ND[e]	388	1.1 - 144[c]	USGS (2008), Klecka (2010), Sapozhnikova (2010), Chase (2012), Reiner et al. (2011), Peck et al. (2006)
Bottom material (Lake, Resevoir, Impoundment, Stream)	606	17.68[a]	212.87[b]	67.99-87.46	USGS NWIS (code 63209)
Biosolids/Sludge (µg/kg dw)	>58	13	427,000	13 - 177,000[c]	Kinney et al.(2006, 2008), Buyuksonmez et al. (2005), DiFrancesco et al. (2004)
Soil (µg/kg dw)					
Biosolids amended	6	1050	2770	---[d]	Kinney et al. (2008),
Wastewater treated	n/a	<0.33	5.69	---[d]	Chase et al. (2012)
Biota (Fish) µg/kg					
Fish (wet weight)	93	<1	5.4	3.0-4.8	
Fish (lipid weight)	23	<1	51.1	---[d]	
Fish (tissue conc.)	30	n/a	2100	100 - 1800	Kannan et al.(2005), Reiner and Kannan (2011),
Eel (lipid weight)	1	125	125	---[d]	Ramirez (2009), Kinney (2008)
Biota (other) (µg/kg ww)					
Earthworm (tissue conc.)	9	ND[g]	3340	---[d]	Kinney et al.(2008)
Zebra Mussel (lipid weight)	4	10.3	19.3	---[d]	Reiner and Kannan (2011)
Shrimp, farmed (lipid weight)	3	n/a	330		
Shrimp, wild (lipid weight)	6	n/a	424	---[d]	Sapozhnikova et al.(2010)
Marine mammal (wet weight)	25	<1	25	2.8 - 14	Kannan (2005)
River Otter (wet weight)	3	2.4	3	2.8	
Mink (wet weight)	4	2.2	5.3	3.7	Kannan (2005)
Bird (wet weight)	5	1.9	4.2	2.3 - 4.0	

ND not detected
n/a not available
[a] 5th percentile value
[b] 95th percentile value
[c] range of averages
[d] Range of means not available
[e] method detection limit not specified
[f] detection range =0.038-2.16 µg/L
[g] method detection limit =12.5 µg/kg dw

3.2.2.1 Wastewater

In published studies, measured concentrations of HHCB in US WWTP influent and effluent varied widely. Influent values ranged from 0.043 to 12.7 µg/L and effluent values ranged from 0.010 to 13.0 µg/L (Table 3-1; for paired effluent/influent values see also: Appendix G, Table G-1). Effluent concentration was dependent on the type of waste treatment process employed and varied depending upon the season or month when the sample was collected; however, aerobically digested sludge reduced nitro and polycyclic musk concentrations more rapidly than anaerobically digested sludge (Smyth et al., 2008). Reported values from a Canadian study confirmed that effluent values were process dependent and concentrations were seasonally dependent and within an order of magnitude of those measured in the US (Smyth et al., 2008). Reported values summarized in the 2008 EU RAR were comparable to those found in the US, with one study from the UK that included a variety of treatment processes indicating a slightly wider range of influent concentrations (7.8-19.2 µg/L). The effluent values reported in the 2008 EU RAR were also comparable to those in the US, with the highest value (13.3 µg/L) reported for a study of 5 sewage treatment plants in Germany.

The mean HHCB concentrations in wastewater effluent from stream and outfall discharge sites calculated from the USGS NWIS data (more than 40 data points) were 0.98-1.18 µg/L. The highest values were measured at outfall sites (95^{th} percentile: 3.4 µg/L). The mean effluent concentrations were comparable at stream and outfall sites, but the range of concentrations was greater at outfall sites.

Very few studies have reported measuring metabolites of HHCB other than HHCB-lactone, the apparent principal degradation product of HHCB. However, measured values of HHCB-lactone in the influent (maximum 1.15 µg/L) and effluent (maximum 4 µg/L) of wastewater have been reported by Horii et al. (2007), and Reiner et al. (2007). In these studies, the range of concentrations of HHCB-lactone in the effluent were more than twice the concentration in the influent and were reported to be comparable to those measured in Switzerland.

3.2.2.2 Surface Water

In published studies, surface water concentrations of HHCB were found to range from non-detect (ND) to 1.6 µg/L and were dependent on their proximity to WWTP outfalls (Table 3-1; see also: Appendix G, Table G-2). The lowest reported concentrations were ND levels (reported detection range = 0.038-2.16 µg/L) at locations upstream from WWTPs; higher concentrations of HHCB were found downstream from the WWTPs. A maximum value of 1.6 µg/L was reported by Barber (2011) for a location at the North Shore Channel of the Chicago River at an area impacted by water reclamation plant effluent. These data support the assertion that the efficiency of HHCB removal in WWTPs plays a significant role in the observed surface water concentrations.

From more than 6000 data points for HHCB in surface water (collected by the USGS from locations in 46 states), the mean calculated value for HHCB concentration in surface water was < 1.1 µg/L for all sites; the highest concentrations were measured at outfall sites. The 95th percentile groundwater concentrations at well sites and surface water concentrations at streams and lakes, reservoirs, and impoundment sites were ≤0.35 µg/L (Table 3-1; see also: Appendix G, Table G-3 and Figures G-2 and G-3). Lower concentrations were consistently recorded in surface water samples collected from streams and lakes, reservoirs, and impoundment sites when compared to values measured at outfall sites.

Values reported for surface water concentrations in the EU (EC, 2008) were similar to those found in the US, with the exception of areas of the water system in Berlin, Germany where very high proportions of effluents are present. The median concentration in sections with a high contribution of effluents was 1.48 µg/L.

3.2.2.3 Sediment

Sediment concentrations varied from ND to 388 µg/kg dw in published studies (Table 3-1; see also: Appendix G, Table G-3). Aside from ND values reported in three tidal tributaries of the Chesapeake Bay, the lowest reported concentrations of HHCB (1.43 to 2.13 µg/kg dw) were found in a lake in Texas, a non-effluent impacted site. One of the highest concentrations (388 µg/kg dw) was reported along the upper Hudson River in New York, a river that receives treated wastewater discharge. Likewise, sediment along the Cuyahoga River in Ohio was found to have higher concentrations of HHCB in samples collected downstream of seven WWTPs (average= 144 µg/kg dw) when compared to those collected upstream of the same WWTPs (average = 37 µg/kg dw). These studies suggest that effluents from WWTPs are a major source of synthetic musks that enter the environment and are present in the sediments of various water bodies. Reported sediment concentrations in the EU (EC, 2008) and Asia (Lee et al., 2014) were similar to those in the US, with higher values found in Berlin, Germany and in the sediment of contaminated brooks in Hessen, Germany.

Over 600 bottom material measurements (collected from USGS locations in 25 states) were available from the USGS NWIS (Table 3-1; see also: Appendix G, Table G-7 and Figure G-3). The 95th percentile concentration at lake/reservoir/impoundment sites was 213 µg/kg, and the mean concentration was 87 µg/kg. The 95th percentile concentration at stream sites was 200 µg/kg, and the mean concentration was 68 µg/kg. The HHCB concentrations (5%ile, mean, and 95%ile) were similar at both types of sites.

3.2.2.4 Biosolids and Soil

Published concentrations of HHCB in biosolids ranged from <100 to >100,000 µg/kg dw (Table 3-1; see also: Appendix G, Table G-4). These values were dependent on a number of factors, including: season, date of collection, location, population served, WWTP operations (*e.g.*, municipal or industrial receiving waste stream, water volume, treatment type) and preparation methodologies prior to land application. Because these factors cannot be readily separated

from all values within the reported data set, it was not possible to determine which single treatment variable had the largest impact on the reported environmental concentrations. However, EPA observed that the US values for measurements of biosolids were similar to those obtained in Ontario, Canada and likewise showed differences depending upon location, treatment process, and season (Smyth et al., 2007). Reported concentrations of HHCB in sludge in the EU (EU RAR, 2008) were generally lower than those reported in the US.

Soil concentrations of HHCB have been reported in a limited number of studies, with values ranging from <0.33 to 2,770 µg/kg dw in biosolid amended soils (Table 3-2; see also: Appendix G, Table G-5). The available data indicate that land application of treated waste water effluent or biosolids results in detectable quantities of HHCB in those soils.

3.2.2.5 Biota

The measured concentrations of HHCB in wildlife varied by location, species, and method of reporting (*e.g.*, lipid weight, tissue weight, or wet weight) (Table 3-1; see also: Appendix G, Table G-6). Monitoring studies of aquatic biota were available only from the scientific literature, and the greatest number of sampling measurements was collected for fish (146 sampling measurements). On a lipid weight basis, measured values across various fish species and locations ranged from <1 to 51.1 µg/kg, whereas levels in wild caught and farm raised shrimp were 330 and 424 µg/kg, respectively (Appendix G, Table G-6). Mean tissue weight concentrations of HHCB in different fish species ranged from 100 to 1800 µg/kg (Appendix G, Table G-6). On a wet weight basis, HHCB has been reported in a number of different species and trophic levels. The reported values were relatively consistent across these species and ranged from <1 to 25 µg/kg (Appendix G, Table G-6).

3.2.2.6 USGS Data Analysis

In addition to reported quantitative values, the USGS dataset includes values that are reported as less than the USGS laboratory reporting level[10] (LRL); data that are between the LRL and the long-term method detection level (LT-MDL); and data that are below the LT-MDL (Oblinger Childress et al., 1999). The value of the LRL is reported with a "less than" remark code for samples in which the analyte was not detected. "Estimated" remark codes are noted for all values falling outside the calibration range because of increased measurement uncertainty or values below the LT-MDL determined using information-rich methods[11].

For monitoring data sets where the geometric standard deviation was less than 3.0, values recorded as "less than LRL" or "estimated" were replaced by the LRL divided by the square root of two, as per the EPA/OPPT's guidance (EPA, 1994). Where the geometric standard deviation

[10] The LRL for water sampling was 0.5 µg/L for sampling dated 7/16/2001 – 9/30/2009 and was updated to 0.05 µg/L for samples dating from 10/1/2009 to the present.

[11] The USGS defines *information rich methods* as "Classified as organic methods that use either mass spectrometric or photodiode array ultraviolet/visible spectroscopic detection. These methods have qualifying information that allows enhanced analyte identification."

was greater than 3.0, EPA/OPPT replaced values recorded as “less than LRL” or “estimated” with the LRL divided by two (EPA, 1994). This practice presents a conservative low end value protecting against false negative values; therefore, it should be noted that these values do not necessarily represent quantitative measured concentrations and are biased towards the LRL.

High quality monitoring data (greater than 6800 data points) were available from the USGS NWIS database for surface water and sediment. Data was available from the USGS NWIS for effluent to a lesser degree (47 data points) as shown in Table 3-1. Documented protocols and guidelines for sample collection and analysis were employed by the USGS such that these data sets are deemed to be acceptable for use in the exposure assessment.

3.2.3 Conclusions of Environmental Exposure

HHCB has been detected and measured in wastewater, surface water, sediment, sewage sludge, soil, and aquatic biota in numerous studies. These data strongly suggest that HHCB is ubiquitous and potentially widespread in the environment. However, these data reflect discrete locations and times, and the extent to which they are representative of the overall distribution of HHCB is not known.

3.3 ECOLOGICAL HAZARD ASSESSMENT

The environmental hazard assessment is based on previous hazard assessments including Balk and Ford (1999a, b); EU RAR (EC, 2008); HERA Project Report (HERA, 2004a); and Robust Summaries submitted under the US EPA HPV program (IFRA, 2003). In addition, a literature search was performed to identify peer-reviewed articles on ecotoxicity published between 2007 and May 2012. The search terms included freshwater and saltwater fish, aquatic invertebrates, and aquatic plants; pelagic and benthic organisms; acute and chronic sediment toxicity in freshwater and saltwater and terrestrial toxicity to soil organisms, birds, and mammals. The test species, test conditions, toxicity endpoints, statistical significance, and strengths/limitations of the study were summarized and evaluated for data quality. Data quality inclusion criteria included: use of appropriate analytical and test controls, identification of test substance and test organism, stated exposure duration time and administration route, a clear description of the effect endpoints, and transparent reporting of effect concentrations. Guideline studies as well as studies using other protocols were included if they met data quality criteria. Specific criteria for exclusion are: studies that included HHCB as part of a mixture in wastewater effluent or surface water and studies described only in abstract form or in a language other than English.

Application of uncertainty factors based on established EPA/OPPT methods (EPA, 2012f; 2013) were used to calculate lower bound effect levels (referred to as the concentration of concern; COC) that would likely encompass more sensitive species not specifically represented by the available experimental data. Uncertainty factors are included in the COC calculation to account for differences in inter- and intraspecies variability, as well as laboratory-to-field variability.

These uncertainty factors are dependent upon the availability of datasets that can be used to characterize relative sensitivities across multiple species within a given taxa or species group, but are often standardized in risk assessments conducted under TSCA, since the data available for most industrial chemicals is limited (Ahlers et al., 2008).

A summary of the available ecotoxicity data for HHCB that were deemed adequate for consideration in this assessment are provided in tables and the studies selected for use in calculating risk quotients are described in more detail in the appropriate section below.

3.3.1 Acute Toxicity to Aquatic Organisms

Acute aquatic toxicity studies considered for this assessment are summarized in Table 3-2.

Additional acute toxicity values for were reported in Deitrich and Hitzfield (2004). For 96-hour toxicity tests using fathead minnow and zebrafish embryos, an EC_{50}s of 0.39 mg/L and an LC_{50} of >0.67, were reported, respectively. These data were not included in EPA's consideration because they were reported in a secondary source and sufficient study details were not provided in the report or found during the search for information performed by EPA/OPPT. The zebrafish study appears to be described in the EU RAR (EC, 2008), but not used due to lack of experimental details.

The acute toxicity study in *Daphnia magna* (Wüthrich, 1996 as cited in Balk and Ford, 1999b and provided to EPA in IFRA, 2003) was selected from the available acute toxicity studies to calculate an RQ because *Daphnia* were the most sensitive species for acute toxic effects to HHCB. Although the lower end of the effects concentration range for freshwater mussel is lower than the Daphnid effects concentration, the authors reported highly variable concentrations during the test as reflected by the effects concentrations being reported as a range, the upper end of which is approximately three times higher than the Daphnid effects concentration. EPA notes that the differences in sensitivity between freshwater and marine organisms appears, based on available data, to be less than an order of magnitude. Daphnid is a more representative species for this assessment because the available monitoring data used for estimating exposure is largely freshwater. Furthermore, the study is reliable and demonstrates the acute effects (i.e. survival/immobilization) using appropriate, reproducible protocols.

The *Daphnia magna* 48-hour EC_{50} of 0.282 mg/L was divided by an assessment factor (uncertainty factor [UF]) of 5 for invertebrates, as per established EPA/OPPT methods (EPA, 2012f; 2013), to give an acute concentration of concern (COC) of 0.0564 mg/L or 56.4 µg/L.

Table 3-2. Aquatic Toxicity Data for HHCB - Acute Toxicity

Test Organism	Fresh/ Salt Water	Test Guideline/ Study Type	Duration (hr-hour)	End-point	Concentration (mg/L)	Chemical Analysis	Effect	References
Fish - Freshwater								
Bluegill sunfish *Lepomis macrochirus*	Fresh	OECD TG 204/Flow-through	96-hr[a]	LC_{50}	0.452	Measured	Mortality	Wüthrich (1996)[c]
Aquatic Invertebrates - Freshwater								
Water flea (*Daphnia magna*)	Fresh	OECD TG 202/Semi-static	48-hr[a]	EC_{50}	0.282	Measured	Immobilization	Wüthrich (1996)[c]
Freshwater mussel (*Lampsilis cardium*)	Fresh	Static[b]	48-hr glochidia	LC_{50}	0.999 - >1.75	Measured	Mortality	Gooding et al. (2006)
			96-hr juvenile	EC_{50}	0.153 - 0.831		Growth	
Aquatic Invertebrates - Saltwater								
Estuarine Copepods (*Nitocra spinipes*)	Brack-ish	SIS 1991/ Static	96-hr	LC_{50}	1.9	Measured	Mortality	Breitholtz et al. (2003)
Marine copepod (*Acartia tonsa*)	Salt	ISO 1997/ Static	48-hr	LC_{50}	0.47	Nominal	Mortality	Wollenberger et al. (2003)
Aquatic Plants - Freshwater								
Green algae (*Pseudokirchneriella subcapitata*)	Fresh	OECD TG 201/Static	72-hr	EC_{50}	>0.85	Measured	Growth	Van Dijk (1997)[c]
					0.72		Biomass	

Note: The shaded row indicates the principal study used for assessing acute risks to aquatic organisms.
[a] Value was calculated from the 21-day study
[b] Guideline not reported
[c] as reported in IFRA (2003), Balk and Ford (1999b) and EC (2008)

3.3.2 Chronic Toxicity to Aquatic Organisms

Chronic aquatic toxicity studies considered of acceptable quality are summarized in Table 3-3.

Chronic toxicity values for rainbow trout (*Oncorhynchus mykiss*) and zebrafish (*Danio rerio*) with reported 21-day $EC_{50\text{-repro}}$ and 21-day LC_{50} of 0.282 mg/L and 0.452, respectively are reported. However, these data were not included in EPA's consideration because they were reported in a secondary source (Deitrich and Hitzfield, 2004) and sufficient study details were not provided in the report or found during the search for information performed by EPA/OPPT.

Chronic aquatic studies with HHCB are difficult to conduct in static or semi-static systems because the tendency of HHCB to associate with organic matter and surfaces may affect exposure concentrations. For example, the measured concentrations at the end of two chronic

copepod tests (Breitholtz et al., 2003; Wollenberger et al., 2003) were only 2 to 19 percent of the nominal concentration. Volatilization or sorption to organic material likely accounts for the disappearance of HHCB from the water phase. HHCB has been reported to have a half-life of hours to days in river water tests using radiolabeled HHCB; HHCB may also be lost to the atmosphere via volatilization (Langworthy et al., 2000; Federle et al., 2002; as cited in EC, 2008). Additionally, HHCB will bind strongly to organic material and sediment (average half-life of 128 days) (Envirogen, 1998; as cited in EC, 2008). Therefore, chronic toxicity values based on nominal (not measured) concentrations from otherwise well-documented studies were not considered for this endpoint and the Concentration of Concern because the actual concentration of HHCB in the test system is unknown.

Following adequacy review of the chronic tests summarized in Table 3-3, the fish prolonged early life stage toxicity test (OECD TG 210) using fathead minnow (*P. promelas*) reported by Croudace et al. (1997) was selected as the study from which to calculated a chronic RQ. The marine copepod (*Acartia tonsa*) study by Breitholtz et al. (2003) was selected in EPA's draft assessment for HHCB; however, upon consideration of multiple comments regarding the use of this study for chronic RQ calculation, EPA/OPPT reconsidered this selection. The marine copepod (*Acartia tonsa*) study by Wollenberger et al. (2003) was considered less reliable than both the study of Breitholtz et al. (2003) and the fathead minnow study due to the fact that concentrations of HHCB were not measured in the study and due to other test design limitations described in the EU RAR (EC, 2008). Although EPA notes that the differences in sensitivity between freshwater and marine organisms appears, based on available data, to be less than an order of magnitude, the fathead minnow is a more representative species for this assessment because the available monitoring data used for estimating exposures is largely freshwater. Furthermore, the study is reliable and demonstrates the chronic effects (i.e., survival and growth) using appropriate, reproducible protocols.

The study was conducted using a flow-through system, wherein fathead minnow eggs (<24 hours old) were exposed to nominal concentrations ranging from 0.0125 to 0.2 mg/L for 36 days. Corresponding mean measured concentrations (measured via HPLC or GC 13 times during the test) were: 0.0091, 0.019, 0.037, 0.068 and 0.140 mg/L. Egg hatchability was not affected at any concentration. HHCB did have an effect on larval survival and growth at 0.140mg/L. Compared to controls, larvae exposed to 0.140 mg/L experienced a 20 and 54% reduction in mean length and weight, respectively. Survival was reported to be 78% and of those that survived, larvae were generally smaller, underdeveloped and displaying erratic swimming behaviors. The authors identified 0.068 mg/L as the NOEC for the study (based on survival and growth); the LOEC would be 0.140 mg/L.

EPA/OPPT calculated at a Maximum Acceptable Toxicant Concentration (MATC) effect concentration of 0 0.097 mg/L for survival. To derive a chronic COC, the MATC was divided by an assessment factor (UF) of 10, according to established EPA/OPPT methods (EPA, 2012f; 2013), to yield a chronic COC of 0.0097 or 9.7 mg/L for survival for aquatic organisms.

Table 3-3. Aquatic Toxicity Data for HHCB - Chronic Toxicity

Test Organism	Fresh/ Salt Water	Test Guideline/ Study Type	Duration	Endpoint	Concentration (mg/L)	Chemical Analysis	Effect	References
Fish - Freshwater								
Bluegill sunfish (*Lepomis macrochirus*)	Fresh	OECD TG 204/Flow Through	21-day	NOEC	0.093	Measured	Clinical Signs	Wüthrich (1996)[d]
				LOEC	0.182			
Fathead minnow (*Pimphales promelas*)	Fresh	OECD TG 210/Flow Through	36-day	NOEC	0.068	Measured	Survival Growth Development	Croudace et al. (1997b)[c]
				LOEC	0.140			
				MATC	0.097			
Aquatic Invertebrates - Freshwater								
Water flea (*Daphnia magna*)	Fresh	OECD TG 202/ Semi-static	21-day	NOEC	0.111	Measured	Reproduction	Wüthrich (1996)[d]
				LOEC	0.205			
				EC_{50}	0.293		Immobilization	
Aquatic Invertebrates - Saltwater								
Marine copepods (*Acartia tonsa*)	Salt	OECD TG Draft Invert Life Cycle/ Static	6-day	EC_{10}	0.044	Measured	Development	Bjornestad (2007)[b]
				EC_{50}	0.131			
				NOEC	0.038			
				LOEC	0.075			
			5-day	EC_{10}	0.037	Nominal	Development	Wollenberger et al. (2003)
				EC_{50}	0.059			
Estuarine copepods (*Nitocra spinipes*)	Brack-ish	___[a]/Static Renewal	22-day	NOEC	0.007	Measured	Development	Breitholtz et al. (2003)
				LOEC	0.02			
Aquatic Plants - Freshwater								
Green algae (*Pseudokirch-neriella subcapitata*)	Fresh	OECD TG 201/Static	72-hr	NOEC	0.201	Measured	Growth Biomass	Van Dijk (1997)[c]
				LOEC	0.466			

Note: The shaded row indicates the principal study used for assessing chronic risks to aquatic organisms.
LOEC = Lowest Observed Effect Concentration
NOEC = No Observed Effect Concentration
MATC = Maximum Acceptable Toxicant Concentration
[a] Test guideline and/or test type not reported
[b] As reported in EC (2008)
[c] As reported in Balk and Ford (1999b) and EC (2008)
[d] As reported in IFRA (2003); Balk and Ford (1999b); and EC (2008)

3.3.3 Toxicity to Sediment-Dwelling Organisms

Toxicity studies in sediment-dwelling organisms including amphipods, midges, oligochaete worms, polychaete worms, and mud snails are summarized in Table 3-4.

The Amphipod, *Hyalella azteca,* was selected for calculating an RQ because it is a well-established test species for evaluating the effects of chemicals in the sediment environment and is widely distributed across North America (Pennak, 1978). The study was conducted using established EPA and OECD test guidelines for measuring lethal and sublethal effects of chemical exposure (i.e., growth, survival, and reproduction). Although the sediment-dwelling species identified as most sensitive in Table 3-4 is the New Zealand mud snail, it is an invasive species in the US and currently is not ubiquitously distributed throughout the US. Toxicity data are also available for the polycheate (*Capitella sp.*) for relevant endpoints of growth and reproduction and the effects concentrations are similar to those for *Hyallela*. Although EPA notes that the differences in sensitivity between freshwater and marine organisms appears, based on available data, to be less than an order of magnitude, the freshwater *Hyalella* is a more representative species compared to *Capitella* for freshwater. Furthermore, the study is reliable and demonstrates the chronic effects (i.e., survival and growth) using appropriate, reproducible protocols.

Toxicity of HHCB to *H. azteca* was evaluated using five nominal concentrations of 0, 6, 14.5, 35, 83, 200 mg/kg and the measured concentrations were on average 49% of the nominal (i.e., 0, 3, 7.1, 16.3, 41 and 98 mg/kg, respectively) (Egeler, 2004 as cited in EC, 2008). Ten animals 7 to 14 days old (four replicates/concentration) were exposed for 28 days. A solvent control was used in the test. There were no mortalities at 35 mg/kg (measured 16.3 mg/kg) during the study. At day 28, mortality was 88% at 83 mg/kg (measured 40.67 mg/kg) and 100% at 200 mg/kg (measured 98 mg/kg). Growth (decreased length) was 9% below the control and total biomass decreased by 15% per replicate at 35 mg/kg (measured 16.3 mg/kg). The reported LOEC was 35 mg/kg (measured, 16.3 mg/kg) and NOEC was 14.5 mg/kg (measured, 7.1 mg/kg).

Table 3-4. Sediment Toxicity Data for HHCB

Test Organism	Fresh/ Salt Water	Test Guideline/ Study Type	Duration	End-Point	Concentration (mg/kg dw)	Test Analysis	Effect	Reference
Freshwater								
Amphipod (*Hyalella azteca*)	Fresh	OECD 218/ Renewal	28-day	NOEC	7.1[b]	Measured	Growth	Egeler (2004)[c]
				LOEC	16.3[b]			
				MATC	10.8			
Midge (*Chironomus riparius*)	Fresh	OECD 218/ Renewal	28-day	NOEC	200[b]	Measured	Emergence	Egeler & Gilberg (2004a)[c]
				LOEC	400[b]			
				EC_{50}	335[b]		Development	
Oligochaete (*Lumbriculus variegatus*)	Fresh	OECD 218/ Renewal	28-day	NOEC	16.2	Measured	Reproduction	Egeler & Gilberg (2004b)[c]
				LOEC	36.5			
				EC_{50}	44.5		Reproduction	
Saltwater								
New Zealand mud snails (*Potamopyrgus antipodarum*)	Fresh/ Brackish	Renewal[a]	120-day	NOEC	0.81	Measured	Reproduction	Pedersen et al. (2009)
				LOEC	7.0			
				NOEC	7.0		Juvenile Growth	
				LOEC	19.3			
Polychaete (*Capitella sp.*)	Salt	Renewal[a]	120-day	NOEC	1.5	Measured	Reproduction	Ramskov et al. (2009)
				LOEC	26			
				NOEC	26		Juvenile Survival	
				LOEC	123			

Note: The shaded row indicates the principal study used for assessing chronic risks to sediment-dwelling organisms.
[a] Test guideline not reported
[b] Concentration adjusted based on measured concentrations (recovery)
[c] as reported in EC, 2008

EPA/OPPT calculated at a Maximum Acceptable Toxicant Concentration (MATC) effect concentration of 10.8 mg HHCB/kg dw from this study. To derive a chronic COC for sediment-dwelling organisms, the MATC was divided by an assessment factor (UF) of 10, according to established EPA/OPPT methods (EPA, 2012f; 2013), to yield a chronic COC of 1.08 mg/kg dw.

3.3.4 Toxicity to Terrestrial Organisms

The toxicity data for terrestrial organisms considered for this assessment is summarized in Table 3-5.

Invertebrates

The earthworm (*Eisenia fetida*) was the most sensitive terrestrial (soil) invertebrate species tested (Chen et al., 2011a). In a two part test, Chen et al. (2011a) observed the lethality of earthworms exposed to HHCB in a 14-day study and the chronic effects in a 28-day study. In the acute study, adult earthworms were exposed for 14 days to concentrations of 0, 100, 140, 196, 274.4, 384.2, 537.8, 752.9, 1,054.1, and 1,475.8 mg HHCB (99 percent purity)/kg (air-dried soil) in acetone (Chen et al., 2011a). Observations were taken on days 7 and 14. No mortalities

occurred at the lowest concentrations of 100 and 140 mg/kg. However, there was 100% mortality at ≥ 1,054.1 mg HHCB /kg. The 7-day LC_{50} was reported as 489 mg HHCB/kg and the 14-day LC_{50} as 392.4 mg HHCB/kg.

In the 28-day chronic test, earthworms were exposed to 3, 10, 30, 50, and 100 mg HHCB (99 percent purity)/kg (air-dried soil) in acetone. After 28-days of exposure to HHCB there was a significant decrease in growth rate of earthworms which occurred at 100 mg HHCB /kg. Additionally, a significant decrease in reproduction rate and cocoon production in earthworms exposed to 50 and 100 mg/kg were noted. A LOEC of 50 mg/kg was reported; the NOEC is 30 mg/kg. A MATC of 38.7 mg/kg was calculated for the earthworm. Because exposure data are insufficient to assess HHCB risk to terrestrial invertebrates, EPA/OPPT did not calculate a COC for the earthworm.

Plants

For plants, two studies (An et al., 2009 and Chen et al., 2010; summarized in Table 3-5) were located that evaluated the toxicity of HHCB to wheat growth and development. However, these studies were not conducted using EPA, OECD or other established or widely recognized protocols, thereby making it difficult for EPA/OPPT to evaluate their adequacy or the relevance of the endpoints measured. This lack of adequate toxicity data for terrestrial plants coupled with only limited soil concentration data and external expert advice, does not support assessing risks to terrestrial plants and hence, COCs were not derived from these studies.

Table 3-5. Soil Toxicity Data for HHCB

Test Organism	Duration hr=hour	Test Guideline /Study Type	Endpoint	Value	Unit	Test Analysis	Effect	Reference
Terrestrial Organisms								
Springtail (*Folsomia candida*)	ISO/CD 11267	4-week	NOEC	45	mg/kg dw	Nominal	Mortality and reproduction	Klepka (1997)[a]
			LOEC	105				
Earthworm (*Eisenia fetida*)	ISO 11268-1/OECD 207/Not Refreshed	8-week	NOEC	45	mg/kg dw	Nominal	Reproduction	Gossmann (1997)[a]
			LOEC	105				
	OECD 207/Not reported	24-hr	LC_{50}	32.60	µg/cm^2	not reported	Mortality	Chen et al. (2011b)
		48-hr	LC_{50}	11.87				
		72-hr	LC_{50}	6.14				
	OECD 207/222*/ Not reported	7-day	LC_{50}	489	mg/kg	not reported	Mortality	Chen et al. (2011a)
		14-day	LC_{50}	392.4				
		28-day	NOEC	30			Reproduction	
			LOEC	50				
			MATC	38.7				
Nematode (*Caenorhab-ditis elegans*)	Not reported	24-hr	LC_{50}	>194.6	mg/L	Nominal	Mortality	Mori et al. (2006)
		60-hr	LOEC	9.8			Reduced growth and maturation	
		72-hr	LOEC	19.5			Decreased fecundity	
Wheat (*Triticum aestivum*)	Not reported/ Renewed	Contingent on control	EC_{50}	143.4	mg/L	Not reported	Shoot elongation	An et al. (2009)
				422.3			Root elongation	
		Contingent on control	LOEC	50	mg/L	Not reported	Seedling development – shoot height	
		21-Day	LOEC	0.2	mg/L	Not reported	Reduced Chlorophyll synthesis	
	Not reported	Contingent on control	NOEC	77	mg/kg dw	Not reported**	Reduced seed germination	Chen et al. (2010)
			LOEC	194				
			LC_{50}	846.6				
			EC_{50}	2,123				
			EC_{50}	928.5				

[a] As cited in Balk and Ford (199b) and EC(2008)
* Slightly modified guideline reported in the study
**HHCB reported at 77.4% pure (Chen et al. 2010)
LOEC=Lowest Observed Effect Concentration
NOEC=No Observed Effect Concentration
MATC=Maximum Acceptable Toxicant Concentration

3.3.5 Conclusions of Environmental Hazard Assessment

Ecotoxicity studies for HHCB have been conducted in fish, aquatic invertebrates, aquatic plants, sediment invertebrates, soil invertebrates, and terrestrial plants. HHCB was found to have high acute toxicity and moderate to high chronic toxicity to aquatic organisms. The COCs derived for aquatic, soil, and sediment-dwelling organisms are summarized in Table 3-6.

Table 3-6. Concentrations of Concern (COCs) for Environmental Toxicity

Environmental Toxicity	Concentration of Concern (COC)
Acute Toxicity, aquatic organisms	56.4 µg/L
Chronic Toxicity, aquatic organisms	9.7 µg/L
Chronic Toxicity, sediment-dwelling organisms	1.08 mg/kg dw (1080 µg/kg dw)

3.4 ENVIRONMENTAL RISK CHARACTERIZATION

3.4.1 Calculation of Risk Quotient (RQ) Values

The goal of this environmental risk characterization was to determine whether there are risks to the aquatic and benthic environments from the measured levels of HHCB found in wastewater, surface water, or sediment. The data for environmental monitoring and toxicity were used in this risk assessment to address the following key questions:

1. Does acute exposure to levels of HHCB measured in wastewater effluent in the US pose risks for adverse effects in aquatic invertebrates, fish, or plants?
2. Does chronic exposure to levels of HHCB measured in surface water in the US pose risks for adverse effects in aquatic invertebrates, fish, or plants?
3. Does chronic exposure to levels of HHCB measured in sediment in the US pose risks for adverse effects in sediment-dwelling invertebrates?

To answer these key questions, environmental risk was characterized by calculating risk quotients or RQs (EPA, 1998; Barnthouse *et al.*, 1982); the RQ is defined as:

RQ = Environmental Concentration / Effect Level

An RQ equal to 1 indicates that the exposures are the same as the concentration that causes effects. If the RQ is above 1, the exposure is greater than the effect concentration. If the RQ is

below 1, the exposure is less than the effect concentration. The Concentrations of Concern (COCs) for aquatic and benthic organisms shown in Table 3-6 and the environmental concentrations shown in Table 3-7 were used to calculate RQs. The environmental concentration for each compartment (*i.e.*, wastewater, surface water, sediment,) was determined based on measured concentrations of HHCB (Table 3-1). RQs were not calculated for terrestrial invertebrates or plants due to insufficient data for environmental concentrations.

Frequency, timing, and duration of exposure affects the potential for adverse effects in aquatic or benthic organisms. Aquatic exposure is assumed to be continuous based on constant wastewater production and a 2 to 4 day half-life of HHCB in water. This assumption is supported by the fact that HHCB is frequently measured in surface water downstream from WWTPs even though some fate studies indicate that a significant percentage of HHCB may be lost to volatilization and metabolism. Exposure of sediment dwelling-organisms to HHCB is also assumed to be continuous.

In order to characterize the degree of potential risk due to HHCB exposure, RQs were calculated based on measured environmental concentrations for the maximum published levels and range of means from published studies of surface water and sediment, and also for the mean and 95th percentile for surface water at outfall sites and bottom material from the USGS NWIS data (see Table 3-1 and Appendix G, Table G-8). Surface water concentrations of HHCB at outfalls are assumed to represent the maximum environmental aquatic concentrations for HHCB that would be experienced acutely in streams that receive wastewater.

Table 3-7. Environmental Concentrations Used to Calculate RQs

	Maximum Published Concentration	**Range of Mean Published Concentrations**	**95th Percentile USGS NWIS**	**Mean USGS NWIS**
Surface Water (µg/L)	1.6	0.36 – 1.6	2.3	1.08
Sediment (µg/kg dw)	388	1.1 - 144	213	87

The calculated RQs are presented in Table 3-8 and reflect the range of concentrations of HHCB measured in the environment. In addition to the maximum published surface water values, the acute and chronic RQs for aquatic organisms in surface water were also calculated from the 95th percentile USGS NWIS data using the concentrations measured in surface water at outfall sites. Though the concentration of HHCB in effluent discharge may be diluted in surface water downstream from the WWTP outfall, the calculated RQs for chronic risk to aquatic organisms are considered conservative estimates based on actual surface water concentrations.

Table 3-8. Calculated Risk Quotients (RQs) for HHCB

Calculated RQs	Maximum Published Concentration	Range of Mean Published Concentrations	95th Percentile USGS NWIS	Mean USGS NWIS	Range of RQs
Acute RQ, aquatic organisms Surface Water	0.03	0.006 to 0.03	0.04	0.02	0.006 to 0.04
Chronic RQ, aquatic organisms, Surface Water	0.16	0.04 to 0.16	0.24	0.11	0.04 to 0.23
Chronic RQ, sediment-dwelling organisms	0.36	0.001 to 0.13	0.20	0.08	0.001 to 0.36

The range of RQs for acute toxicity to aquatic organisms was 0.006 to 0.04 (Table 3-8). The highest RQ of 0.04 was calculated using the 95th percentile value from the USGS NWIS dataset for HHCB in surface water as measured at an outfall site (see Table 3-1 and Appendix G, Table G-8). All RQs for acute toxicity to aquatic organisms in Table 3-8 range from 25 to 167 times or 1 to 2 orders of magnitude below 1.

The range of RQs for chronic toxicity to aquatic organisms was 0.04 to 0.23 (Table 3-8). The highest RQ of 0.23 was calculated using the 95th percentile value from the USGS NWIS dataset for HHCB in surface water as measured at an outfall site (see Table 3-1 and Appendix G, Table G-8). All RQs for chronic toxicity to aquatic organisms in Table 3-8 range from 4 to 25 times or an order of magnitude below 1.

The maximum concentration for surface water as measured at an outfall site from the USGS dataset is 4.4 µg/L, a value well below both the chronic and acute concentration of concern for aquatic organisms.

The range of RQs for sediment-dwelling organisms was 0.001 to 0.36 (Table 3-8). The highest RQ of 0.36 was calculated using the maximum published level of HHCB in sediment of 388 µg/kg dw (Table 3-1). This HHCB sediment concentration is from a single sample taken from the upper Hudson River near Troy, NY (Reiner, 2011) and a second sample (from the same study) taken near Albany, NY was 351 µg/kg. Both of these values are above the 95th percentile value from the USGS monitoring data for bottom material. It should be noted that of the more than 600 bottom material samples available in the USGS dataset, a single value was identified as greater than the COC of 1080 µg/kg dw. This value is presumed to represent a "hotspot" as >99.9% of the remaining values were below the COC for sediment dwelling organisms. Aside from this single value, all RQs for acute toxicity to aquatic organisms in Table 3-8 range from 3 to 1000 times or up to 3 orders of magnitude below 1.

Insufficient exposure data were available to calculate risk for terrestrial invertebrates and plants *via* contact with contaminated water, sediment, or soil.

3.4.2 Key Sources of Uncertainty and Data Limitations

The strength of this assessment is the calculation of risk based on a considerable quantity of surface water and sediment monitoring data and an adequate ecological toxicity data set. However, a number of limitations in the approach create uncertainties associated with this assessment.

3.4.2.1 Representativeness of Exposure Concentrations

Available monitoring data for HHCB in water, sediment, and soil were used in the assessment. A robust dataset of over 6800 sampling measurements were available from the USGS NWIS for wastewater effluent, surface water, and bottom material along with over 500 sampling measurements from the scientific literature. The data from both published studies and the USGS NWIS database were used because the mean values for surface water and sediment were within the same order of magnitude. The concentrations reported in the literature and in the USGS NWIS data are assumed to represent reasonable estimates, however the data may not reflect the actual distribution of HHCB in the environment. Calculation of the RQ based on the maximum value, the 95th percentile, and the highest mean value was performed in an effort to capture the full range of concentrations in the environment.

3.4.2.2 Variability in Environmental Concentrations

Although calculation of the RQ based on the maximum reported value from the literature, the 95th percentile USGS NWIS value for surface water at effluent sites, and the highest mean value was performed in an effort to capture the full range of concentrations observed in this data set, higher values may exist in the environment. Variation of use practices across the population and differences in sewage treatment plant inputs (industrial, commercial or residential), removal efficiencies and receiving streams, may affect the final concentrations present in any single location or point in time. Unless actual environmental concentrations of HHCB in surface water or sediment are greater than one or two orders of magnitude higher than expected, additional measurements or estimates will not affect the risk calculation.

Use of all available data obscures trends such as effects of seasonality, differences in WWTP removal efficiencies, and differences in use trends; therefore the ability in this assessment to predict or attribute concentrations to any particular variable was not possible. WWTP removal efficiencies for HHCB are reported to vary from 58 to >99%, therefore environmental concentrations may vary to a similar extent depending upon other factors such as input and receiving environment. Well-designed studies that follow these factors over a period of time are needed to provide more definitive understanding of how these variables may be interrelated or interact to effect final concentrations in any given media or location.

3.4.2.3 Anaerobic Degradation

Waste (settled) activated sludge in WWTPs is generally sent to anaerobic digesters to reduce sludge volume and organic load before disposal of the concentrated material. Removal of HHCB under anaerobic treatment conditions has been addressed in several studies. Xue et al. (2010) reported poor overall removal of HHCB in a full-scale anaerobic/anoxic/aerobic system combined with membrane bioreactor for municipal wastewater reclamation, and little removal was attributed to biodegradation. In contrast Carballa et al. (2007) reported good removal of HHCB (60-80%) in mesophilic and thermophilic anaerobic sludge digestion. Kupper et al. (2006) observed apparent anaerobic degradation of HHCB in the sludge line of a full-scale treatment system, but the mechanism was not confirmed. Therefore, no direct evidence of anaerobic (methanogenic) biodegradation of HHCB exists, and this is therefore an uncertainty in characterizing biodegradation.

3.4.2.4 Volatilization

As noted in the document, volatilization seems not to be a major process in activated sludge treatment, although available data are limited. EPI Suite's WWTP model (STPWIN) predicts negligible removal due to volatilization. Two studies are cited in the section on fate in water, and they may appear at first glance to be in conflict, however the amount of volatilization in lab studies versus that in the environment is highly dependent on the test conditions. For lab studies, specifically, values will be dependent on design of test vessels, degree of mechanical aeration if any; and incubation temperature.

3.4.2.5 Isomers and Metabolites

Information (hazard and exposure) of HHCB isomers and metabolites is very limited. The measurement of specific isomers of HHCB were not described in the collected studies or USGS data set reviewed for this assessment and only a very limited number of studies described the occurrence of HHCB-lactone. Hazard as it relates to specific isomers of HHCB is also unknown. More information on isomers and metabolites would be needed to refine this assessment and provide a fuller understanding of if or how specific HHCB isomers or metabolites contribute to the observed HHCB behavior in environmental media and their impact on organisms present in water, sediment and soil.

3.4.2.6 Deriving Concentrations of Concern from Single Species Tests

Ecological hazard of industrial chemicals is routinely evaluated using a single species and within a controlled laboratory setting in the form of a toxicity test. There is uncertainty associated with extrapolating these single-species laboratory test results to concentrations intended to be protective of all species and environments. Application of uncertainty factors (or assessment factors) based on established EPA/OPPT methods (EPA, 2012; 2013) were used to calculate lower bound effect levels (referred to as the concentration of concern; COC) that would likely encompass more sensitive species not specifically represented by the available experimental data. Uncertainty factors are included in the COC calculation to account for differences in inter- and intraspecies variability, as well as laboratory-to-field variability. These uncertainty factors are dependent upon the availability of datasets that can be used to characterize relative sensitivities across multiple species within a given taxa or species group, but are often

standardized in risk assessments conducted under TSCA, since the data available for most industrial chemicals is limited (Ahlers et al., 2008).

3.4.2.7 Assessment of Risk to Terrestrial Invertebrates or Plants

Two studies with six total samples reported HHCB concentrations in soil following land application of either biosolids or wastewater effluent. The locations are limited to two sites and may not representative of overall US soil concentrations given differences in sludge application practices, WWTP contaminant removal, or seasonal factors. Measured concentrations of HHCB in non-amended soil were not available for comparison. The limited data available on measured levels of HHCB in biosolids or wastewater-amended soil, prevented quantitative assessment of risk to terrestrial invertebrates and terrestrial plants.

3.4.3 Conclusions of Risk Characterization

RQs were calculated based on measured values for the maximum published levels and range of means from published studies of surface water and sediment, and also for the mean and 95th percentile for surface water at outfall sites and bottom material from the USGS NWIS data in an effort to capture the spectrum of environmental concentrations. Uncertainty factors were used to account for inter-species and lab-to-field extrapolation of hazards for organisms exposed HHCB in the ambient environment. Risk estimates for terrestrial environments were not performed due to very limited monitoring and toxicity data.

3.5 CONCLUSIONS OF ENVIRONMENTAL ASSESSMENT

This assessment evaluated the environmental risk to aquatic organisms from the use of HHCB as a fragrance ingredient in consumer and commercial products. Scenarios of interest included release of HHCB in wastewater to surface water or application of biosolids to land. The assessment endpoints were acute and chronic toxicity to aquatic organisms and chronic toxicity to sediment organisms. The risk assessment considered only direct exposure to aquatic organisms and sediment-dwelling organisms.

Acute and chronic RQs indicated that maximum measured surface water concentrations did not exceed COCs for aquatic organisms. The chronic COC was not exceeded for sediment-dwelling organisms at the upper range of measured environmental concentrations for the maximum published values or the more than 600 sediment measurements from the USGS. Low risk concerns were identified for both acute and chronic toxicity to aquatic organisms and for sediment-dwelling organisms. Therefore, unless environmental concentrations increase by factor of 1 to 2 orders of magnitude, the assessment does not indicate risk concerns.

Insufficient data were available to calculate chronic risk for terrestrial invertebrates and plants *via* contact with contaminated water, sediment, or soil.

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APPENDICES

Appendix A HUMAN HEALTH TOXICITY STUDIES, BIOMONITORING DATA, AND RISK ASSESSMENTS

The human health hazard reviewed and summarized in this section (see Table A-1) relies heavily on the hazard information extracted from the 2008 EU RAR on HHCB (EC, 2008). Other reviews are also available (OECD, 2009; EPA, 2003; HERA, 2004a; 2004b). This review is not intended to be an exhaustive discussion of the toxicity of HHCB, but rather a summary of the available data. The reader is referred to the original documents for more detailed information.

A-1 Human Hazard Characterization

A-1-1 Toxicokinetics (Absorption, Distribution, Metabolism, Excretion)

Available toxicokinetics data indicate that HHCB has poor dermal absorption and is extensively metabolized and excreted, with no evidence of significant bioaccumulation. An *in vivo* human study reported approximately 0.1 percent dermal absorption following exposure to HHCB (Hawkins et al., 1996a; Ford et al., 1999; as cited in EC, 2008), while an *in vitro* dermal absorption study with human epidermal membranes showed 5.2 percent absorption (Green and Brian, 2001; as cited in EC, 2008). Human biomonitoring data show that HHCB has been detected in blood, milk, and adipose tissue following dermal exposures (see Table A-2). Data in humans after oral and inhalation exposures, including half-life data, are not available. In animals, dermal exposures in rats showed 16 percent absorption, with distribution to the stomach, liver, fat, plasma, adrenal glands, kidneys, and thyroid, with excretion primarily in the feces (Ford et al., 1999; Hawkins et al., 1995; as cited in EC, 2008). Intravenous studies in rats and pigs indicated that HHCB is rapidly distributed and excreted primarily in the feces in the rat and in the urine in pigs (Hawkins et al., 1997; Hawkins, 1997; as cited in EC, 2008). No evidence of accumulation in the feces or urine was seen in either the rat or pig. Apparent half-lives of elimination in the blood were longer in the rat than in the pig, with clearance from fat and skin considerably slower. HHCB and metabolites were found in the milk of pregnant and lactating rats exposed *via* the oral route (Hawkins *et al.*, 1996a; as cited in EC, 2008). Toxicokinetics data in animals after oral and inhalation exposures are not available.

A-1-2 Acute Toxicity

Several acute toxicity studies in rats and rabbits by the oral and dermal routes with HHCB are available (Minner and Foster, 1977; Moreno, 1975; as cited in EC, 2008). Acute toxicity studies by the inhalation route are not available. The acute toxicity of HHCB is low *via* the oral route in rats and low *via* the dermal route in rabbits, with LD_{50} values >3,000 mg/kg-bw.

A-1-3 Subchronic/Repeated-Dose Toxicity

An oral range-finding study in rats with mean achieved intakes up to 829 mg/kg-bw/day reported signs of liver toxicity at 347 mg/kg-bw/day (lowest-observed-adverse effect concentration [LOAEL] (Api and Ford, 1999; Hopkins et al., 1996; as cited in EC, 2008); however, the follow-up 13-week repeated-dose oral (dietary) toxicity study showed no adverse, treatment-related effects on any parameters measured. The no-observed-adverse-effect level (NOAEL) for systemic toxicity was 150 mg/kg-bw/day, the highest dose tested (Api and Ford, 1999; Hopkins et al., 1996; as cited in EC, 2008). In that same study, no effects on organ weight or histopathology were reported in the reproductive organs at any dose level. Repeated-dose subchronic toxicity studies by the inhalation and dermal routes were available, but were considered to be of limited value because they were not conducted according to guidelines or GLPs or they used fragrance mixtures in which HHCB was only present at low levels.

A-1-4 Reproductive Toxicity and Fertility

No combined repeated-dose/reproductive/developmental or multigenerational reproductive toxicity studies on HHCB are available. Information on reproductive organs is available from the repeated-dose dietary toxicity study in rats; this study did not show any signs of toxicity to the reproductive organs examined at doses as high as ~150 mg/kg-bw/day.

A modification to the ICH Guideline on Detection of Toxicity to Reproduction for Medicinal Products (http://www.ich.org/products/guidelines/safety/article/safety-guidelines.html) was conducted in rats in order to assess the potential for adverse effects to the neonate following exposure to HHCB through nursing. In this comprehensive study, the basis for the dose selection for this study was a pharmacokinetic analysis by Hawkins et al. (1996b) (as cited in EC, 2008) that determined the oral doses required to produce levels in the milk of lactating rats similar to those reported in human milk and several orders of magnitude higher. At oral doses of 2 and 20 mg/kg-bw/day, HHCB was found in the milk of lactating dams at 2.28 and 32.8 mg/L, respectively (see Human Biomonitoring below for comparison to levels in human breast milk). Five groups of pregnant Sprague-Dawley rats (28/dose) were administered 0, 2, 6, or 20 mg/kg-bw/day HHCB (>95 percent purity) by gavage from gestation day (GD) 14 through weaning on lactation day 21 (Ford and Bottomley, 1997; Jones et al., 1996; as cited in EC, 2008). Dams were allowed to litter and rear their offspring to weaning. F_1 offspring were exposed to HHCB *in utero* from GD 14 through lactation *via* the dam's milk, and exposures ceased after weaning in the F_1 offspring and in the F_2 generation. Since milk consumption was not measured in the lactation study, actual intake of HHCB could not be determined.

After parturition, the offspring were counted, sexed, weighed, and examined for external abnormalities. On postnatal day 4, the pups were weighed and the litters were culled to four males and four females. During the pre-weaning period, pups were examined for several developmental milestones, including surface righting reflex, startle reflex, air righting reflex, and pupil reflex. Selected offspring from these litters (24/sex/dose) were allowed to reach sexual maturity and were then assessed for behavioral changes in motor coordination and

balance, activity, and avoidance. When the F_1 generation offspring reached 84 days of age, they were mated (avoiding sibling pairings) and the reproductive capacity was assessed by examining the females before and after mating to determine time of pregnancy, estrous cyclicity, pre-coital time, pregnancy rates, and duration of gestation. F_2 generation offspring were examined for abnormalities at parturition and then periodically until study termination on postnatal day 21. No adverse, treatment-related effects were reported in the dams or in either generation of offspring for any parameters assessed up to and including 20 mg/kg-bw/day. The NOAEL for maternal, reproductive, and developmental toxicity was 20 mg/kg-bw/day, based on no effects observed at the highest dose tested.

A-1-5 Developmental Toxicity

In a pilot range-finding study of prenatal developmental toxicity (Christian et al., 1997; Christian et al., 1999; as cited in EC, 2008), four groups of 25 pregnant Sprague-Dawley rats were exposed to 0, 100, 250, 500, and 1,000 mg/kg-bw/day HHCB (purity not specified) by gavage on GDs 7 to 17. Three dams were found dead at 1,000 mg/kg-bw/day. Maternal body weights were reduced (approximately 10 percent) in the three highest dosage levels. No other effects were reported in the dams. No signs of gross fetal malformations were reported; however, fetal body weights were reduced at the two highest doses.

Based on the results of the dose range-finding study described above, pregnant Sprague-Dawley rats (25/dose) were administered 0, 50, 150, and 500 mg/kg-bw/day HHCB (>95 percent purity) by gavage on GDs 7 to 17 (Christian et al., 1997; Christian et al., 1999; as cited in EC, 2008). The dams were observed for clinical signs of toxicity. Food and water consumption and body weights were recorded at various intervals. On GD 20, the dams were sacrificed and a gross necropsy was performed. The numbers of corpora lutea, implantation sites, resorption sites, live and dead fetuses, and live pup body weights were recorded. Fetuses were examined for sex and underwent a gross examination. One half of the fetuses was examined for visceral anomalies and the other half was examined for skeletal abnormalities. Signs of maternal toxicity in the dams consisted of significant increases in salivation, urine-stained fur, red or brown substance on the forepaws, and alopecia beginning at 150 mg/kg-bw/day, with statistical significance at 500 mg/kg-bw/day. At ≥150 mg/kg-bw/day, statistically significant reductions in food consumption and maternal body gain were observed, with the most severe reductions in body weight occurring during GDs 7 to 10. After completion of the dosing period (GDs 18 to 20), a significant rebound in food consumption and body weight gains occurred, with values reported as being comparable to controls. Signs of developmental toxicity were confined to the highest dose of 500 mg/kg-bw/day. Significant reductions in offspring body weight were reported at the high dose; however, according to the study authors, when severity, dose relationships, and historical ranges were taken into consideration, these body weight reductions were not definitively considered to be treatment-related. The number of litters or fetuses with morphological alterations (malformations and variations) reported at the high dose was not statistically significantly increased when compared to the control group. Malformations that did occur were few in number and were considered spontaneous in origin, except for the vertebral/rib malformations reported in only three fetuses that were not litter

mates. No other parameters were reported to be affected by treatment at any dose level. The LOAEL for maternal toxicity was 150 mg/kg-bw/day, based on increases in clinical signs of toxicity and decreases in body weight, and the NOAEL was 50 mg/kg-bw/day. Although there is uncertainty concerning the effects reported in the offspring, a conservative LOAEL for developmental toxicity of 500 mg/kg-bw/day was concluded and the NOAEL was 150 mg/kg-bw/day.

A-1-6 Genetic Toxicity

Following a wide array of *in vitro* and *in vivo* mutagenicity studies, HHCB did not induce gene mutations in bacterial cells or chromosome aberrations in mammalian cells (San et al., 1994; Api and San, 1999; Mersch-Sundermann et al., 1998a; 1998b; Kevekordes et al., 1997; Curry and Putman, 1995; Gudi and Ritter, 1997; San and Sly, 1994; Kevekordes et al., 1998; as cited in EC, 2008).

A-1-7 Carcinogenicity

A 2-year cancer study is not available for HHCB. As an alternative, EPA/OPPT evaluated the weight of the evidence from mutagenicity, subchronic, and endocrine studies, to support the conclusion that cancer data are not a critical data need.

A-1-8 Additional Information

Irritation and sensitization studies in humans and animals showed that HHCB was not corrosive, irritating, or sensitizing in rabbits and humans. No data on respiratory tract irritation were available. HHCB was considered to be a minimal eye irritant in rabbits and a possible photo-irritant in rabbits and guinea pigs, but not in humans (Guillaume et al., 1973a; 1973b; Rubenkoenig and Ede, 1964; Lindstrum et al., 1978a; 1978b; 1978c; Harrison and Stolman, 1986; Gabriel and Mark, 1987; Folk and Dammers, 1987; Shanahan and Alworth, 1987; Haynes, 1984; 1985; 1986; Sauer, 1980; Levenstein, 1973a; 1973b; 1975a; 1975b; Wolven and Levenstein, 1963; Basketter, 1996; Parish, 1988; as cited in EC, 2008).

In a series of *in vitro* assays with HHCB using different protocols designed to test for potential endocrine disruption (*i.e.*, estrogenic and/or anti-estrogenic activity), HHCB showed weak estrogenic and anti-estrogenic activity *in vitro*, dependent on the estrogen receptor type (Seinen et al., 1999; Bitsch et al., 2002; Schreurs et al., 2002; Schreurs et al., 2004; Schreurs et al., 2005a; Schreurs et al., 2005b; Gomez et al., 2005; as cited in EC, 2008). Marginal repressing effects were also found *in vitro* on the androgen and progesterone receptor. However, no estrogenic effects were seen in the *in vivo* uterotrophic assay.

Possible anti-estrogenic effects in zebrafish were assessed (Schreurs et al., 2004; as cited in EC, 2008). Transgenic zebrafish were exposed to 0.01, 0.1, 1, and 10 µM HHCB with and without estradiol (E_2). The highest concentration of 10 µM HHCB was toxic. Concentrations of 0.01, 0.1, and 1 µM HHCB were not estrogenic. Dose-dependent antagonistic effects were reported at 0.1 and 1 µM levels.

Table_Apx A-1. Summary of Human Health Hazard Information

Endpoints	HHCB CASRN (1222-05-5)	References (as cited in EC, 2008)
Acute oral toxicity LD_{50} (mg/kg-bw)	 >3,000	Minner and Foster (1977); Moreno (1975)
Acute dermal toxicity LD_{50} (mg/kg-bw)	 >3,250	Minner and Foster (1977); Moreno (1975)
Acute inhalation toxicity LC_{50} (mg/L)	No data	
Repeated-dose toxicity Oral (mg/kg-bw/day) Dermal (mg/kg-bw/day) Inhalation (mg/L/day)	 NOAEL = 150 (highest dose tested) No adequate data No adequate data	Api and Ford (1999); Hopkins *et al.* (1996)
Reproductive toxicity Oral (mg/kg-bw/day) Maternal NOAEL/LOAEL Reproductive NOAEL/LOAEL Developmental NOAEL/LOAEL	 NOAEL = 20 (highest dose tested) NOAEL = 20 (highest dose tested) NOAEL = 20 (highest dose tested)	Ford and Bottomley (1997); Jones et al. (1996)
Developmental toxicity Oral (mg/kg-bw/day), rats Maternal NOAEL/LOAEL Developmental NOAEL/LOAEL	 NOAEL = 50/LOAEL = 150 NOAEL = 150/LOAEL = 500	Christian et al. (1997); Christian et al. (1999)
Genetic toxicity, gene mutation *In vitro* Genetic toxicity, gene mutation *In vivo*	 Negative Negative	San et al. (1994); Api and San (1999); Mersch-Sundermann et al. (1998a); Mersch-Sundermann et al. (1998b)
Genetic toxicity, chromosomal aberrations *In vitro* Genetic toxicity, chromosomal aberrations *In vivo*	 Negative Negative	Api and San (1999); Kevekordes et al. (1997); Curry and Putman (1995); Gudi and Ritter (1997); San and Sly (1994); Kevekordes et al. (1998)

Table_Apx A-1. Summary of Human Health Hazard Information

Endpoints	HHCB CASRN (1222-05-5)	References (as cited in EC, 2008)
Additional information		
Corrosivity/skin-eye Irritation/sensitization	Negative	See summary above
Respiratory tract irritation	No data	
Carcinogenicity	No data	
Endocrine disruption		
In vitro	Weak	
In vivo	Negative	

A-2 Human Biomonitoring

Biomonitoring data describing human exposure levels of HHCB are summarized in Table A-2. Data are preliminary and limited based on small numbers of subjects, limited geographic representation, high variability, and incomplete details on methodology.

Several small studies have reported concentrations of HHCB in limited numbers of the general population in Asia and Europe. Only two small studies have reported exposure levels in the US. To date, HHCB has been measured in adipose tissue, blood, breast milk, and umbilical cord blood. Most of these studies reported data for <100 samples and several of them did not provide data tables or details on the results of the data. However, HHCB was detected in a high majority of the samples collected in the studies reported here. Therefore, it can be assumed that exposure is widespread.

Both US studies took place in 2004 in the northeastern part of the US. A study on adipose tissue in 49 residents of New York City undergoing liposuction reported a mean concentration of 178 ng/g lipid weight (lw) (range: 12 to 798 ng/g lw) (Kannan et al., 2005), which falls between the reported concentrations of HHCB in adipose tissue from two other available studies (Moon et al., 2012; Schiavone et al., 2010). A study of 43 Korean women reported a mean of 81 ng/g lw in adipose tissue (range: 28 to 211 ng/g lw) (Moon et al., 2012), while a small study in Italy reported a mean of 361 ng/g lw (range: 28 to 211 ng/g lw) (Schiavone et al., 2010).

Several small studies on concentrations of HHCB in breast milk have been conducted. In the US, a mean of 227 ng/g lw was reported for 39 samples collected in Massachusetts (Reiner *et al.*, 2007b). The levels reported in this small study are much higher than those reported in other countries to date (see Table A-2). However, in order to compare the results, the timing of the collection of the samples must be considered (length of time after birth), and it is not clear from the report when the US samples were collected.

No studies in the literature reported blood concentrations of HHCB in the US. A collection of matched maternal and cord blood samples in the Netherlands indicated high detection and correlation between maternal and cord blood serum. Concentrations reported in studies in the Netherlands, Austria, and China are so variable that they cannot and should not be compared (see Table A-2). Little data were provided in these studies, which were primarily done in 2005. Given that HHCB is sequestered in the fat, blood may not be an appropriate matrix for biomonitoring.

High variance in measurements may be a result of the analytical methods for measuring HHCB in human matrices as they are still being developed and modified because the sample sizes are very small, the results should not be extrapolated to larger populations. Also, data from other countries may not represent those of the US since exposure patterns may vary greatly between countries, especially for personal care products such as those that contain HHCB.

Table_Apx A-2. Human Biomonitoring Data for HHCB

Population	Sampling Year(s)	Levels	Reference
New York City residents undergoing liposuction; n = 49 (12 males, 37 females)	2003-2004 HHCB detected in adipose tissue in all samples	Mean: 97 ± 88 ng/g ww Range: 6.1-435 ng/g ww Mean: 178 ± 166 ng/g lw Range: 12-798 ng/g lw	Kannan et al. (2005)
n = 43 women undergoing laparoscopy surgery for myoma	2007-2008 100 percent detection in adipose tissue	Mean: 81 ± 44 ng/g lw Range: 28-211 ng/g lw	Moon et al. (2012)
n = 12 surgical samples, Siena, Italy, 3 females, 9 males	2005-2006 HHCB detected in 92 percent of adipose tissue samples	Mean: 361 ± 467 ng/g lw	Schiavone et al. (2010)
n = 39 milk samples from Massachusetts women	2004 HHCB detected in 97 percent of breast milk samples; sample collection time not reported	Mean ± SD: 227 ± 228 ng/g lw Range: <5-917 ng/g lw	Reiner et al. (2007b)
10 primiparous mothers (25-29 years old)	1999 2003/2004 HHCB detected in all breast milk samples; collected 14-26 weeks after birth	Median: 147 µg/kg fat Range: 38-422 µg/kg fat Mean: 179 ± 111 µg/kg Large variability in individual samples	Duedahl-Olesen et al. (2005)
n = 101 random samples of 266 breast milk samples collected from 44 primiparous women, Uppsala County	1996-2003, 14-21 days postpartum HHCB detected in all breast milk samples	Median: 63.9 ng/g lw Range: 2.8-268 ng/g lw	Lignell et al. (2008)
40 mothers (24-38 years old), Munich	1997-1998; HHCB detected in 35/40 breast milk samples	Median: 64 ng/g lw Mean: 115 ng/g lw Range: 21-1,316 ng/g lw	Liebl et al. (2000)

Table_Apx A-2. Human Biomonitoring Data for HHCB

Population	Sampling Year(s)	Levels	Reference
Seoul, Korea n = 20 volunteers, >25 years old, 17 breast milk samples, 14 umbilical cord samples; 18 maternal serum samples	2007; breast milk samples collected 3-10 days after delivery; HHCB detected in 100 percent breast milk samples; 70 percent cord blood samples; 90 percent maternal serum samples	Breast milk mean: 0.055-0.515 ng/g lw Umbilical cord blood: 0.67-2.7 ng/g lw Maternal serum: 0.17-1.4 ng/g lw	Kang et al. (2010)
n = 110 mothers in southwest China taking children for vaccines; 110 breast milk samples	2009	Median: 11.5 ng/g lw Range: <1.1-456.7 ng/g lw	Yin et al. (2012)
n = 10 breast milk samples from Chengdu, China; validating analytical method	2009 HHCB detected in breast milk, all samples	Range: 11.7-67.6 ng/g lw	Wang et al. (2011)
100 volunteers in 3 cities in Yangtze River Delta, Shanghai, Shaoxing, and Wuxi	2006-2007 Breast milk samples collected 1-2 weeks after delivery; 99 percent detection	Median: 63 ng/g Ranges: Shanghai: <5-278 Shaoxing: 5-782 Wuxi: 24-281 ng/g lw	Zhang et al. (2011)
Serum samples from volunteers in the Netherlands n = 42 maternal serum samples, 27 cord blood samples	2005 report HHCB detected in 38/42 maternal serum samples and 26/27 cord blood samples	Maternal: 0.15-3.2 ng/g serum Cord blood: 0.11-1.6 ng/g serum	Peters (2005)
Students at Medical University of Vienna n = 100 plasma samples (55 female, 45 male); 19-43 years old	2005 report Detection in 91 percent of plasma samples; women had higher levels than men but lower values in 26-43 year olds	Plasma concentrations: Males, median: 260 ng/L Range: 98-540 ng/L Females, median: 580 ng/L Range: 290-885 ng/L	Hutter et al. (2005)

Table_Apx A-2. Human Biomonitoring Data for HHCB

Population	Sampling Year(s)	Levels	Reference
Dept of Angiology at Hanusch-Krankenhaus, Vienna; n = 53 women >50 years old (51-71 years)	Detection in 89 percent of plasma samples; older women had higher concentrations of HHCB	Maximum plasma concentration: 6,900 ng/L No other data provided	Hutter et al. (2010)
11 cities in China n = 204 (94 female, 110 male) 17-75 years old (median 25 years)	98 percent detection in blood samples (almost all above LOQ)	Whole blood Median: 0.85 µg/L Maximum: 1.63 µg/L No data tables provided	Hu et al. (2010)

A-3 Summary of 2008 EU Human Health Risk Assessment

The human health hazards and risks of HHCB have been extensively assessed by several other reliable entities (EC, 2008; OECD, 2009; EPA, 2003; HERA, 2004a; 2004b). Each of these well-documented, independent reviews concluded that there was no significant risk to human health from exposure to HHCB as used in household cleaning products. Moreover, the overall conclusions of the EU RAR (EC, 2008) were that there was no need for further information and/or testing and no need for risk reduction measures beyond those already being applied. The EPA/OPPT has thoroughly reviewed these other assessments and presents a summary of the most recent EU RAR.

A-3-1 Assumptions and Points of Departures Used in the EU RAR

The EU RAR (EC, 2008) assessed risk in three populations for relevant endpoints: *workers* (exposures from handling during production and dilution, compounding, formulation, and professional cleaning); *consumers* (exposures from a wide variety of consumer products such as perfumes, creams, toiletries, soaps, shampoos, and household and laundry cleaning products); and *humans exposed via the environment* (exposures *via* food and water including fish, root crops, and mother's milk).

Dermal absorption of 16 and 5.2 percent in rats and humans, respectively, was used. Since toxicokinetic data of HHCB following oral and inhalation absorption are not available in humans or animals, intermediate defaults of 50 percent for oral absorption and 100 percent absorption from inhalation exposures were used.

For general systemic effects following repeated exposures, the oral NOAEL of 150 mg/kg-bw/day from the 13-week subchronic toxicity study in rats was used as a point of departure. Since NOAELs are not available following dermal or inhalation exposures, the administered dose from the oral 13-week toxicity study was converted into an internal dose (body burden) by taking into account different absorption factors. Based on an oral absorption value of 50 percent in humans and animals, an internal NOAEL of ≥75 mg/kg-bw/day was used, with 100 percent absorption assumed for inhalation exposures and 5.2 percent absorption for dermal exposures.

For developmental/reproductive toxicity, the NOAEL of 20 mg/kg-bw/day (the highest dose tested) from the peri/post-natal oral (gavage) toxicity study was used as a point of departure (Ford and Bottomley, 1997; Jones et al., 1996; as cited in EC, 2008). Based on an oral absorption rate of 50 percent, an internal NOAEL of ≥10 mg/kg-bw/day was used, with 100 percent absorption assumed for inhalation exposures and 5.2 percent absorption assumed for dermal exposures.

Worst-case estimates were based upon combined (simultaneous) dermal and inhalation exposures for compounding workers only (the only scenario relevant for combined exposures).

A-3-2 Risk to Workers

Oral exposures in workers were assumed to be mitigated by personal hygiene measures, and therefore, only risks following dermal and inhalation exposures in the workplace were assessed.

For acute toxicity following dermal exposures, high LD_{50} values (>3,000 mg/kg-bw) indicated no concern for workers. Although acute toxicity studies by the inhalation route are not available, low-level, short-term exposures in workers, combined with the low acute toxicity by the oral route, suggested no concern for workers for acute toxicity by the inhalation route.

Given that HHCB showed no potential for skin/eye irritation, corrosivity, or sensitization/ photosensitization, it was concluded that there was no concern for local effects in workers following repeated exposures. Likewise, since there is a lack of skin and eye irritation potential, no significant respiratory tract irritation potential was expected.

For both the dermal and inhalation exposure scenarios for the general systemic toxicity endpoint, a minimal margin of safety (MOS) of 100 was used for comparison. The MOS was based on an interspecies factor of 10 (4 for metabolic size differences and 2.5 for remaining differences), an intraspecies factor of 5, and a factor of 2 for extrapolation from semi-chronic to chronic exposure. For each inhalation/dermal exposure scenario, the calculated MOS was well above the minimal MOS of 100 (≥2,600). Combined exposure routes for total body burdens for skin contact and inhalation for the compounding scenario resulted in MOS values well above 100 (≥2,000). As a result, it was concluded that for workers under these exposure scenarios for general systemic toxicity, there is, at present, no need for further information and/or testing and no need for risk reduction measures beyond those already being applied.

Given that HHCB was not found to be mutagenic in a wide array of studies, it was concluded that for mutagenicity for workers under these exposure scenarios, there is, at present, no need for further information and/or testing and no need for risk reduction measures beyond those already being applied. Although there are no carcinogenicity data for HHCB, it was concluded that there was no concern for workers under these exposure scenarios for carcinogenicity based on negative mutagenicity data and the lack of any apparent structural alerts that would raise a concern.

For both the dermal and inhalation exposure scenarios for the developmental/reproductive toxicity endpoint, a minimal MOS of 50 was used for comparison. The MOS was based on an interspecies factor of 10 (4 for metabolic size differences and 2.5 for remaining differences) and an intraspecies factor of 5. For dermal, inhalation, and combined exposure scenarios, the calculated MOS was above 50 (≥261). As a result, it was concluded that for workers under these exposure scenarios for developmental/reproductive toxicity, there is, at present, no need for further information and/or testing and no need for risk reduction measures beyond those already being applied.

A-3-3 Risk to Consumers

The main route of exposure for consumers was assumed to be dermal, with some inhalation exposures and no oral exposures.

Given that HHCB showed no potential for skin/eye irritation, corrosivity, or sensitization/ photosensitization, it was concluded that there was no concern for local effects in consumers following repeated exposures. Since there is a lack of skin and eye irritation potential, no significant respiratory tract irritation potential was expected.

For general systemic effects following repeated exposures, a MOS of 200 (based on a factor of 10 for intraspecies differences, 4*2.5 for interspecies differences, 2 for duration extrapolation, and 1 for dose-response) was used. The calculated MOS for dermal exposure was well above 200 (≥1,400). Therefore, it was concluded that there was no concern for general systemic toxicity in consumers following repeated dermal exposures.

Given that HHCB was not found to be mutagenic in a wide array of studies, it was concluded that for mutagenicity for consumers, there is, at present, no need for further information and/or testing and no need for risk reduction measures beyond those already being applied. Likewise, although there are no carcinogenicity data for HHCB, it was concluded that there was no concern for consumers under these exposure scenarios for carcinogenicity based on negative mutagenicity data and the lack of any apparent structural alerts that would raise a concern.

For the dermal exposure scenario for the developmental/reproductive toxicity endpoint, a minimal MOS of 100 was used for comparison. The MOS was based on an intraspecies factor of

10, an interspecies species factor of 10 (4 for metabolic size differences and 2.5 for remaining differences), and factor of 1 for dose-response. The calculated MOS was above 100 (MOS ≥189). It was concluded that for consumers with a dermal exposure scenario for developmental/ reproductive toxicity, there is, at present, no need for further information and/or testing and no need for risk reduction measures beyond those already being applied.

A-3-4 Risk to Humans Exposed Indirectly via the Environment

Exposure to HHCB by the inhalation route (*via* air) was considered to be negligible. The main route of exposure to humans in the environment was oral (*via* fish and root crops). Exposures were based on local and regional daily intake estimates following production. A separate risk characterization for breast-fed babies was conducted for exposure *via* mother's milk (see next section for more information).

For general systemic effects following repeated exposures, an internal NOAEL of ≥75 mg/kg-bw/day and a MOS of 200 (based on a factor of 10 for intraspecies differences, 4*2.5 for interspecies differences, 2 for duration extrapolation, and 1 for dose-response) were used. The calculated MOS for exposure *via* food was >3E+4 for the local production scenario and >8E+5 for the regional production scenario. Therefore, it was concluded that for the local and regional exposure scenarios, there was no concern for general systemic toxicity in humans exposed indirectly *via* the environment.

As with the other populations, since HHCB was not found to be mutagenic in a wide array of studies, it was concluded that for mutagenicity, there is, at present, no need for further information and/or testing and no need for risk reduction measures beyond those already being applied. Likewise, although there are no carcinogenicity data for HHCB, it was concluded that there was no concern for carcinogenicity based on negative mutagenicity data and the lack of any apparent structural alerts that would raise a concern.

For the oral exposure scenario for the developmental/reproductive toxicity endpoint, an internal NOAEL of ≥10 mg/kg-bw/day and a MOS of 100 were used. The MOS was based on an intraspecies factor of 10, an interspecies species factor of 10 (4 for metabolic size differences and 2.5 for remaining differences), and factor of 1 for dose-response (based on the lack of effect at the highest dose tested). The calculated MOS values for local and regional oral exposure scenarios were 3,846 and 10,000, respectively, indicating no concern for developmental/ reproductive toxicity in humans exposed indirectly *via* the environment.

A-3-5 Assessment of Risk for Breast-Fed Babies Exposed via Mother's Milk

The presence of HHCB in human milk was considered to be the result of indirect environmental exposures from a variety of sources, including maternal exposure to consumer products; intake *via* food, water, or air; and occupational exposures.

The concentrations of HHCB in milk samples for humans and rats were compared. By using the dose level in dams (20 mg/kg-bw/day, the highest dose tested from the oral peri/postnatal developmental toxicity study), the measurement of levels of HHCB in the milk of dams (17.6 and 5.0 µg/mL at four or eight hours post dosing, respectively), with the maximum level of HHCB found in human milk samples (1,316 µg/kg fat), the EU RAR (EC, 2008) concluded that pups in the high-dose group were estimated to be exposed to levels approximately 100 to 360 times the maximum level found in human milk samples.

The amount of milk consumed by infants and rat pups was compared. Assuming 50 percent absorption of the ingested HHCB, the average daily uptakes (ADUs) for the breast-feeding infant for 0 to 3 and 3 to 12 months, as well as the average daily milk consumption for the rat pup, were estimated. A comparison of these two estimates of uptake showed a difference of approximately three orders of magnitude between the levels of HHCB exposure in the rat (in which no adverse effects were found) and the human infant exposure.

Total internal (worst-case) combined exposures were estimated and compared to the internal NOAELs for general systemic toxicity following repeated exposures and for developmental/ reproductive toxicity (≥75 and ≥10 mg/kg-bw/day, respectively) in order to calculate MOS values. The worst-case combined exposure was estimated from the sum of the worst-case estimates from three populations: (1) dermal and inhalation exposures for scenario 2 for compounding workers; (2) dermal and inhalation exposures for consumers; and (3) oral and inhalation, locally *via* the environment. For general systemic effects following repeated exposures, a MOS of 100 was used (based on 4*2.5 for metabolic size and other differences, a factor of 5 for intraspecies differences, and a factor of 2 for semichronic to chronic exposure extrapolation). The calculated MOS was well above 100 (798). For the developmental/ reproductive toxicity endpoint, a MOS of 50 (based on 4*2.5 for interspecies species differences and 5 for intraspecies differences) was used. The calculated MOS was above 50 (≥106).

As a result, the EU concluded that, overall, there was no concern for breast-fed babies exposed indirectly *via* the environment and no need for further information and/or testing or risk reduction measures beyond those already being applied.

A-4 Key Sources of Uncertainty and Data Limitations on Human Health

Overall, adequate screening-level animal toxicity data are available to characterize the human health hazard for HHCB. Toxicokinetics (by the dermal route), acute, repeated-dose, and developmental toxicity data are available to characterize the human health hazard of HHCB. Although no multigenerational reproductive toxicity studies on HHCB are available, information on developmental and reproductive toxicity was obtained from both the repeated-dose dietary toxicity study (reproductive organ data) and the peri/post-natal reproductive toxicity study with modified exposures. Several assays testing for endocrine disruption, genotoxicity, and irritation/sensitization (including several studies in humans) are also available. The database is

incomplete for toxicokinetic data in animals by the oral and inhalation routes; acute toxicity data by the inhalation route; repeated-dose toxicity data by the inhalation and dermal routes; and chronic toxicity/carcinogenicity. Data in humans on most toxicity endpoints are not available.

One area of uncertainty concerns the effects reported in offspring in the prenatal developmental toxicity study in rats (Christian et al., 1997; Christian et al., 1999; as cited in EC, 2008). When severity, dose relationships, and historical ranges were taken into consideration, the reductions in pup body weight were not definitively considered by the study authors to be treatment-related. Additionally, fetal malformations observed were reported in only three fetuses from separate litters. Even though there is uncertainty surrounding these endpoints, the study authors, as well as other reliable entities, have concluded a conservative LOAEL for developmental toxicity of 500 mg/kg-bw/day from this study.

Another area of uncertainty concerns the period of dosing in the peri/post-natal reproductive toxicity study (Ford and Bottomley, 1997; Jones et al., 1996; as cited in EC, 2008). This study was designed to determine effects of HHCB on the neonate when exposed through nursing. Exposures in the F_1 offspring occurred *in utero* from GD 14 through lactation; there were no exposures to HHCB beginning from weaning in the F_1 offspring throughout the F_2 generation. Dosing of pregnant animals should typically include the entire period of organogenesis, which in the case of the rat is GDs 6 to 15. Although dosing in this study began towards the end of organogenesis, it was considered to be adequate for screening-level purposes in order to characterize developmental toxicity (including endpoints such as pup weight, pup survival, and postnatal death) and reproductive toxicity (reproductive performance) as well as a battery of developmental neurotoxicity tests. Other reliable entities such as the 2008 EU RAR on HHCB (EC, 2008) and the 2009 SIDS Initial Assessment Profile (OECD, 2009) also concluded that this study was adequate for assessing hazard for these endpoints.

Human biomonitoring data indicate that HHCB is present in milk, fat, and blood, but the studies in the US are preliminary and of limited value for characterizing exposures. Specifically for the US, there are few biomonitoring studies in limited locations with small numbers of samples. HHCB has not been measured as part of the National Health and Nutrition Environmental Survey (NHANES), so the data are not representative. Exposures in the US are assumed to be similar to those in Europe, but it is unknown how manufacture and use of products containing HHCB may differ between regions or cultures since use of fragrances, personal care, and cleaning conventions may vary.

A-5 Conclusions of Human Health Assessment

The available toxicokinetic data indicate that HHCB is poorly absorbed through the skin; toxicokinetic data for the oral and inhalation routes are not available. Limited pharmacokinetic data have reported HHCB metabolites in the milk of pregnant and lactating rats. Human biomonitoring data have reported HHCB in milk, fat, and blood, but the studies are preliminary

and of limited value for characterizing exposures. The acute toxicity by the oral and dermal routes is low. Signs of systemic toxicity following repeated exposures have not been reported. None to weak estrogenic and anti-estrogenic activity has been demonstrated. HHCB is not mutagenic, corrosive, irritating, or sensitizing; however, minimal eye irritation and possible signs of photoirritation have been reported. Carcinogenicity data are not available.

HHCB was initially selected for review based on a moderate hazard concern for developmental toxicity and a high potential for exposure. However, following further review of the developmental and reproductive toxicity data, and taking into account several lines of evidence, the conclusion is an overall low concern for developmental toxicity. The uncertainty surrounding the pup body weights seen at 500 mg/kg-bw/day from the prenatal developmental toxicity study, the occurrence of only three fetuses from separate litters exhibiting fetal malformations at 500 mg/kg-bw/day from this same study, and the lack of fetal morphological changes observed in the pilot studies, even at maternally lethal dosages (up to 1,000 mg/kg-bw/day) lead to the conclusion that 500 mg/kg-bw/day is a conservative LOAEL. No effects were reported in fetuses in the perinatal toxicity study with lactational exposures at doses similar to those reported in human milk and several orders of magnitude higher (20 mg/kg-bw/day). Overall, a low concern for developmental toxicity is indicated by the data. Additionally, the human health hazards and risks of HHCB have been extensively reviewed and assessed by several other reliable entities, most recently the EU, and it has been concluded that the overall concern for human health hazards, including that for developmental toxicity, is low.

The review of human health hazard studies, biomonitoring studies in the US and elsewhere, and the EU RAR showed an overall low risk concern for human health, including the risk for developmental toxicity, from current use of HHCB.

Appendix B CHEMICAL SYNTHESIS OF HHCB

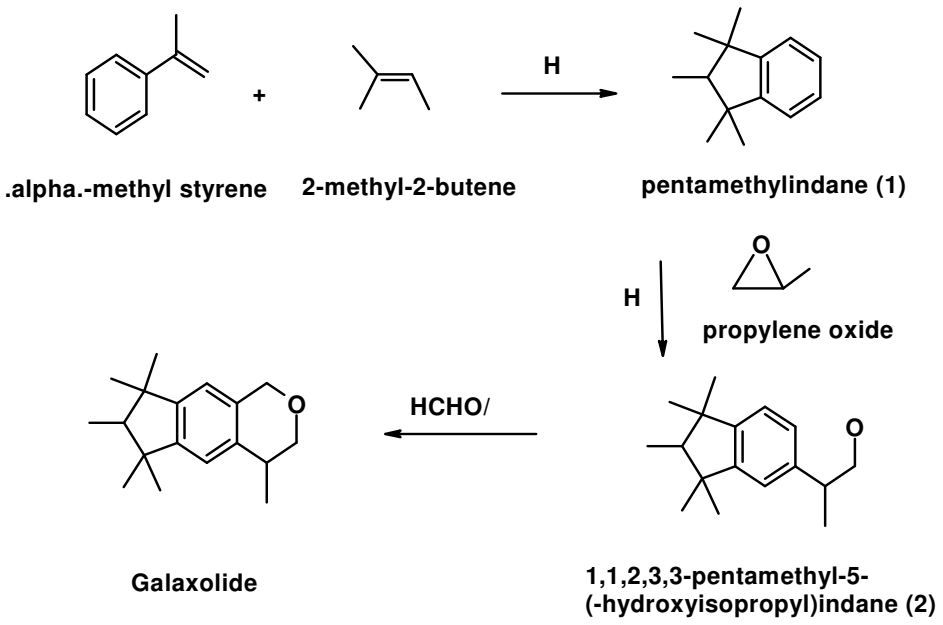


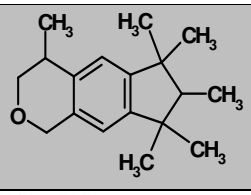
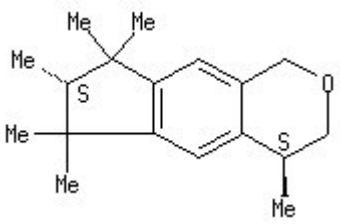
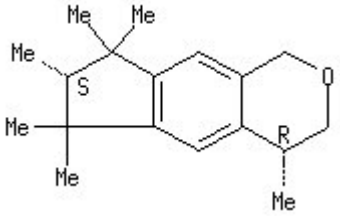
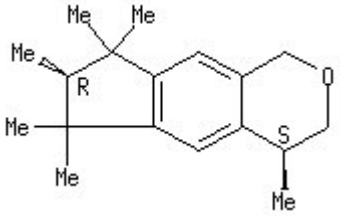
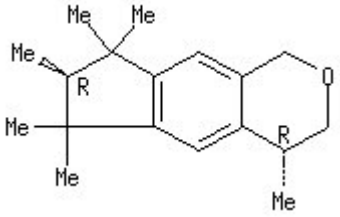
Figure Apx B-1. Chemical Synthesis of HHCB

HHCB is manufactured by a three-step reaction (Ullmann, 2003; Zviely, 2002). First, a cycloaddition reaction of .alpha.-methyl styrene and 2-methyl-2-butene (*i.e.,* amylene) is performed under acidic conditions to obtain 1,1,2,3,3-pentamethylindane (1). Second, the pentamethylindane (1) is hydroxyalkylated with propylene oxide in a Friedel-Crafts reaction using aluminum chloride as a catalyst. Third, the ring closure of the resulting 1,1,2,3,3-pentamethyl-5-(-hydroxyisopropyl)indane (2) to 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-γ-benzopyran (HHCB; Galaxolide) is accomplished with paraformaldehyde and a lower aliphatic alcohol *via* the acetal or with paraformaldehyde and a carboxylic acid anhydride *via* the acylate.

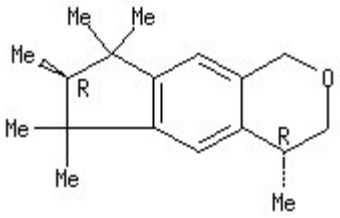
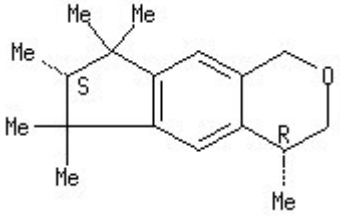
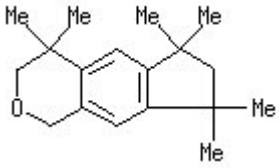
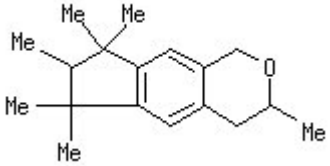
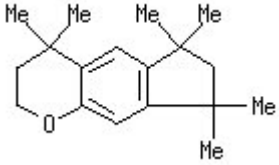
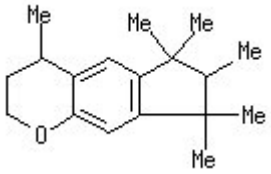
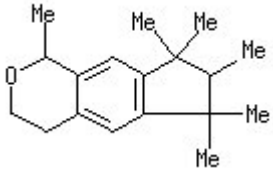
1) Ullmann's Encyclopedia of Industrial Chemistry (2003). 6th ed. Vol 1: Federal Republic of Germany: Wiley-VCH Verlag GmbH & Co. 2003 to Present, p. V14 145.
2) Zviely M; Kirk-Othmer Encyclopedia of Chemical Technology. (2005). NY, NY: John Wiley & Sons; Aroma Chemicals. Online Posting Date: Jan 25, 2002.

Appendix C HHCB (*), HHCB DIASTEREOISOMERS (#1 TO #6), AND RELATED STRUCTURAL ANALOGS (#7 TO #15)

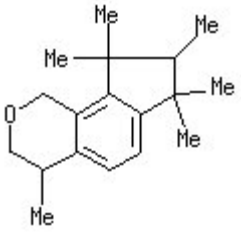
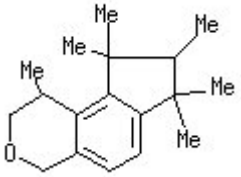
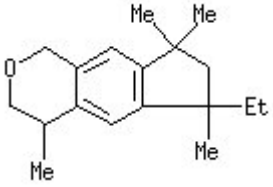
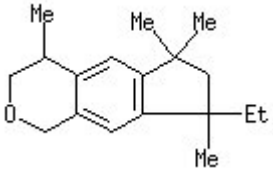
Table_Apx C-1. HHCB, HHCB Diastereoisomers, and Related Structural Analogs

	CASRN	Chemicals Structure	Chemical Index Name	On TSCA Inventory
*	1222-05-5		1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-γ-2-benzopyran	Y
1	172339-62-7		Cyclopenta[g]-2-benzopyran,1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-, (4S,7S)-	N
2	172339-63-8		Cyclopenta[g]-2-benzopyran,1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-, (4R,7S)-	N
3	252332-95-9		Cyclopenta[g]-2-benzopyran,1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-, (4S,7R)-	N
4	252332-96-0		Cyclopenta[g]-2-benzopyran,1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-, (4R,7R)-	N

Table_Apx C-1. HHCB, HHCB Diastereoisomers, and Related Structural Analogs

	CASRN	Chemicals Structure	Chemical Index Name	On TSCA Inventory
5	252933-48-5		Cyclopenta[g]-2-benzopyran,1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-, (4R,7R)-rel-	N
6	252933-49-6		Cyclopenta[g]-2-benzopyran,1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-, (4R,7S) rel-	N
7	1222-06-6		Cyclopenta[g]-2-benzopyran, 1,3,4,6,7,8-hexahydro-4,4,6,6,8,8-hexamethyl-	N
8	857091-61-3		Cyclopenta[g]-2-benzopyran, 1,3,4,6,7,8-hexahydro-3,6,6,7,8,8-hexamethyl-	N
9	102296-64-0		Cyclopenta[g]-1-benzopyran, 2,3,4,6,7,8-hexahydro-4,4,6,6,8,8-hexamethyl-	N
10	135546-43-9		Cyclopenta[g]-1-benzopyran,2,3,4,6,7,8hexahydro-4,6,6,7,8,8-hexamethyl-	N
11	135546-42-8		Cyclopenta[g]-2-benzopyran, 1,3,4,6,7,8-hexahydro-1,6,6,7,8,8-hexamethyl-	N

Table_Apx C-1. HHCB, HHCB Diastereoisomers, and Related Structural Analogs

	CASRN	Chemicals Structure	Chemical Index Name	On TSCA Inventory
12	114109-63-6		Cyclopenta[h]-2-benzopyran, 1,3,4,7,8,9-hexahydro-4,7,7,8,9,9-hexamethyl-	N
13	114109-62-5		Cyclopenta[f][2]benzopyran, 1,2,4,7,8,9-hexahydro-1,7,7,8,9,9-hexamethyl-	N
14	78448-48-3		Cyclopenta[g]-2-benzopyran, 6-ethyl-1,3,4,6,7,8-hexahydro-4,6,8,8-tetramethyl-	N
15	78448-49-4		Cyclopenta[g]-2-benzopyran, 8-ethyl-1,3,4,6,7,8-hexahydro-4,6,6,8-tetramethyl-	N
*This compound was evaluated in this assessment.				

Appendix D CDR DATA FOR HHCB

The information in Tables D-1 through D-4 is from EPA's non-CBI CDR database (EPA, 2014b) for the 2012 reporting cycle, for HHCB, CASRN 1222-05-5. The chemical name used in the CDR database is cyclopenta[g]-2-benzopyran, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-.

Table_Apx D-1. CDR National HHCB Information[a]

Production volume (aggregated)	3,126,728 lbs
Maximum concentration (at manufacture or import site)	> 90 percent
Physical form(s):	Liquid; Dry Powder, Liquid, Other
Number of manufacturing, processing, and use sites	Not completely reported.[b]
Number of reasonably likely to be exposed industrial manufacturing, processing and use workers	Not completely reported.[c]
Was industrial processing or use information reported?	Yes
Was commercial or consumer use information reported?	Yes

[a] More detailed information was not publically available on the CDR website because it was considered to be CBI.
[b] The total number of sites reported by two submitters is in the range of less than 65 to 123 but one submitter reported that the number of sites is "not known or reasonably ascertainable" and the other submitters did not report any information.
[c] The number of workers reported by one submitter is in the range of 25 – 49, but the other submitters either reported that the number of sites is "not known or reasonably ascertainable" or else did not report any information.

Table_Apx D-2. CDR HHCB Industrial Use Information

Type of Processing	Industrial Sector (Based on NAICS)	Industrial Function
Processing—incorporation into formulation, mixture, or reaction product	Soap, Cleaning Compound, and Toilet Preparation Manufacturing	Odor agents
Processing—incorporation into formulation, mixture, or reaction product	All Other Chemical Product and Preparation Manufacturing	Odor agents
Processing—incorporation into formulation, mixture, or reaction product	Other (requires additional information)	Odor agents
Processing—incorporation into article	Plastics Material and Resin Manufacturing	Odor agents

Table_Apx D-3. HHCB CDR Consumer Information

Commercial/Consumer Product Category	Maximum Concentration in Related Consumer/Commercial Product Category	Intended for Use in Children's Products in Related Product Category
Cleaning and Furnishing Care Products	Reported as "Not Known or reasonably ascertainable" and "<1% - <30%"	Reported as "Not known or reasonably ascertainable" and "No" by different submitters
Personal Care Products	Reported as "Not Known or reasonably ascertainable" and "<1% - <30%"	"Not known or reasonably ascertainable"
Air Care Products	Reported as "Not Known or reasonably ascertainable" and "<1% - <30%"	Reported as "Not known or reasonably ascertainable" and "No" by different submitters
Laundry and Dishwashing Products	Reported as "Not Known or reasonably ascertainable" and "<1% - <30%"	Reported as "Not known or reasonably ascertainable" and "No" by different submitters
Plastic and Rubber Products not elsewhere Covered	<1%	Not known or reasonably ascertainable
Non-TSCA Use	1% - <30%	Not known or reasonably ascertainable
Source: EPA (2014b)		

Table_Apx D-4. CDR Company Site Information (2012)

Company	Site	City	State	Zip Code	Manufacture	Import	Site Limited
Berje, Inc.	Berje, Inc.	Carteret	NJ	07003	No	Yes	N/A
International Flavors & Fragrances, Inc.	Ashland, Inc.	Hazlet	PA	19067-3701	No	CBI	N/A
Firmenich, Inc.	Firmenich, Inc.	Plainsboro	NJ	08543	CBI	CBI	--
Symrise, Inc.	Symrise, Inc.	Branchburg	NJ	08773	No	Yes	N/A
S C Johnson & Son, Inc.	S C Johnson & Son, Inc.	Sturtevant	WI	53177	CBI	CBI	--
Source: EPA (2014b)							

Appendix E MODELED RELEASE ESTIMATES ACCORDING TO STAGE OF PRODUCTION AND USE

This Appendix contains modeling estimates of releases of HHCB to the environment from industrial sites of all stages of the HHCB life cycle and estimates of releases from consumer and commercial use of end-use products.

In general, the life cycle of a fragrance ingredient such as HHCB includes:

- Manufacturing of the chemical substance,
- Compounding of fragrance oils containing the chemical substance,
- Blending of fragrance oils containing the chemical substance into commercial and consumer products (*e.g.*, perfumes, cosmetics, soaps, detergents),
- Use of these commercial and consumer products.

The release estimates are summarized in Table E-1 and indicate that the majority of the use volume of HHCB (>90%) is released to WWTP influent as a result of consumer and commercial use of HHCB-containing products. These release estimates are based on the estimated 2011 use volume of 1,700 metric tons (3.74 million lbs) for HHCB (IFRA, 2012b). This volume represents the vast majority of the quantity of HHCB used to prepare fragrance oils or fragrance compounds within the United States, based on data collected from IFRA's Volume of Use Survey (IFRA, 2012d).

Because total releases as a fraction of production volume is independent of the production volume or the number of sites, the estimation of release amounts was not revised to account for the CDR data subsequently reported in 2012 (3.1 million lbs). A discussion of the release estimates according to stages of the life cycle follows.

E-1 Estimated Release from Manufacture and Import

As discussed in Chapter 2, HHCB is not currently manufactured in the US (IFRA, 2012a). For this release assessment, four companies are assumed to import HHCB to the US at five sites (see Appendix D, Table D-4). Releases are not expected to result from import activities, but may occur at import sites if HHCB is also diluted and compounded onsite after import, as further discussed below.

Table_Apx E-1. Summary of Estimated Environmental Releases

Release Activity	Release Media	Release Factor (Amount Released per Amount Used, Percent)	Number of Sites	Number of Release Days at Each Site (Days/Year)	Daily Release (kg/Site-Day)	Combined Annual Releases of All Sites (kg/Year)	EPA Models and Assumptions
Import and Compounding of Fragrance Oils							
Unloading 100 percent of HHCB at 75 °C from bulk containers into dilution tank	Air	NA	5-49	2-18[a]	9.02×10^{-3}	0.812-0.884	EPA AP-42 Model (EPA, 1991; Fehrenbacher and Hummel, 1996)
Transferring 65 percent of (diluted) HHCB at 75 °C from dilution tank into totes for distribution to customers or indoor vessels for compounding	Air	NA	5-49	26-251[a]	3.22×10^{-4}-6.45×10^{-4}	0.405-0.821	EPA AP-42 Model (EPA, 1991; Fehrenbacher and Hummel, 1996)
Cleaning of bulk containers	Water	0.07-0.2[b]	5-49	2-18[a]	13.2-37.8[c]	1,190-3,400[d]	EPA Bulk Transport Residual Model (EPA, 1988; 1992)
Cleaning compounding equipment	Water	0.07-1[b]	5-49	250	0.097-13.6	1,190-17,000	EPA Single Process Vessel Residual Model (EPA, 1988; 1992)

Table_Apx E-1. Summary of Estimated Environmental Releases

Release Activity	Release Media	Release Factor (Amount Released per Amount Used, Percent)	Number of Sites	Number of Release Days at Each Site (Days/Year)	Daily Release (kg/Site-Day)	Combined Annual Releases of All Sites (kg/Year)	EPA Models and Assumptions
Blending of Fragrance Oils into Products							
Cleaning of transport containers	Water, incineration, land	0.3-3[e]	950	250	0.021-0.215	5,100-51,000	EPA/Office of Pollution Prevention and Toxics (OPPT) Drum Residual Model (EPA, 1988; 1992)
Cleaning of process vessels	Water, incineration, land	2	950	250	0.143	34,000	EPA/OPPT Multiple Vessel Residual Model (EPA, 1988; 1992)
Conveying, mixing, and packaging powder products	Air, water, incineration, land	3.83	950	250	0.274	65,110	4.5 percent dust losses from spray-drying unit with 85 percent air pollution control device efficiency (4.5 percent × 0.85 = 3.825 percent) (OECD, 2010)
Use of End Products							
Disposal of products	Water	90.0-93.7	Not known	250	Not known	1,530,000-1,592,900	Engineering judgment assuming a 100 percent release scenario
Total Releases	Water	90.1-94.9	NA	2-250	NA	1,532,380-1,613,300	
	Air	NA	NA	2-251	NA	1.22-1.71	
	Water, incineration, land (uncertain)	2.3-5	NA	250	NA	39,100-85,000	
	Air, water, incineration, land (uncertain)	3.83	NA	250	NA	65,110	

Table_Apx E-1. Summary of Estimated Environmental Releases

Release Activity	Release Media	Release Factor (Amount Released per Amount Used, Percent)	Number of Sites	Number of Release Days at Each Site (Days/Year)	Daily Release (kg/Site-Day)	Combined Annual Releases of All Sites (kg/Year)	EPA Models and Assumptions

NOTES:

NA – not applicable. All release calculations assume an annual HHCB volume of 1,700,000 kg/year (IFRA, 2012b).

Note: containers may be cleaned by a third-party (*i.e.*, contractors); therefore, container cleaning releases may or may not occur on-site.

[a]The number of unloading days, which is equal to the number of release days, is calculated by dividing the average HHCB throughput per site by the container volume, assuming that one container is unloaded per site per day. Bulk containers are assumed to be 5,000 gallons and totes are assumed to be 550 gallons. The calculation for the cleaning of bulk containers is presented as a sample calculation of this variable:

Low-end of range of number of unloading days (or release days) per site:

HHCB throughput per site = (1,700,000 kg/year ÷ 49 sites) ÷ (1 g/cm^3 × kg/1,000 g × cm^3/0.0002642 gallons) = 9,166 gallons per site.

Number of release days per site = 9,166 gallons/site-year ÷ 5,000 gallons/site-day = 1.83 days ~2 days.

Upper High-end of range of number of unloading days (or release days) per site:

HHCB throughput per site = (1,700,000 kg/year ÷ 5 sites) ÷ (1 g/cm^3 × kg/1,000 g × cm^3/0.0002642 gallons) = 89,828 gallons per site.

Number of release days per site = 89,828 gallons/site-year ÷ 5,000 gallons/site-day = 18 days.

[b]The release factor, or the amount released per unit amount used, is equal for this release activity to the residual amount in a transport container or process equipment. This residual amount is expressed as a fraction of container or equipment volume and has the following range of values:

(1) container unloading and equipment cleaning by gravity drain: 0.07 percent (central tendency) and 0.2 percent (high-end).

(2) equipment cleaning by pumping: 1 percent (conservative).

[c]The calculation of this range of releases per site is presented as a sample calculation of this variable:

Low-end of range of amount released per site-day:

5,000 gallons/container-site-day × 0.0007 residual gallons/container gallon × (1 g/cm^3 × kg/1,000 g × cm^3/0.0002642 gallons) = 13.2 kg/site-day

UpperHigh-end of range of amount released per site-day =

5,000 gallons/container-site-day × 0.002 residual gallons/container gallon × (1 g/cm^3 × kg/1,000 g × cm^3/0.0002642 gallons) = 37.8 kg/site-day

[d]The calculation of this range of combined releases of all sites per year is presented as a sample calculation of this variable:

Lower-end of range of amount released per year = 13.2 kg released /site-day × 5 sites × 18 days of release = 1,190 kg/year

Upper-end of range of amount released per year = 37.8 kg released/site-day × 5 sites × 18 days of release = 3,400 kg/year

[e]Residual amount as a fraction of drum volume: (1) containers unloaded by pumping: 3 percent (high-end) and 2.5 percent (central tendency); (2) containers unloaded by pouring: 0.6 percent (high-end) and 0.3 percent (central tendency).

E-2 Estimated Release from Compounding

The number of compounding sites in which HHCB is processed is not known. Therefore, the number of sites was estimated using two alternative approaches, resulting in an estimated range of per-site release from compounding sites. EPA/OPPT expects that imported HHCB may be delivered to compounding sites. Compounding sites are classified under North American Industry Classification System (NAICS) code 32562, Toilet Preparation Manufacturing, and specifically under the Perfume Oil Mixtures and Blends Manufacturing subcategory. This NAICS code is not mentioned in the 2006 non-CBI IUR data on HHCB; therefore, EPA/OPPT assumed that HHCB compounding may occur only at the five import sites. This approach resulted in a high estimate of per-site release. Alternatively, the number of sites was estimated to be equal to the number of IFRA member companies. The estimated HHCB volume used to compound fragrance oils was collected through surveys of IFRA member companies, which represent the vast majority of fragrance volume produced in the US. IFRA North America currently has 49 member companies (IFRA, 2012d). If each member company owns at least one site in the US, then each site may compound HHCB for fragrance oils. Alternately, the 2002 Economic Census estimates 33 companies under this NAICS subcategory (USCB, 2004). The 2007 Economic Census does not provide a detailed breakdown of NAICS 32562 by subcategory, but shows that the total number of companies within NAICS 32562 increased by four percent from 2002 to 2007. EPA/OPPT infers that the number of companies under the Perfume Oil Mixtures and Blends Manufacturing subcategory also increased by four percent from 2002 to 2007, and estimates a total of 34 companies under this subcategory in 2007.[12] Therefore, the number of compounding sites is estimated to include 5 to 49 sites. The geographic distributions of all sites under NAICS 32562 are shown in Table E-2.

In summary, compounding could occur at these sites under two scenarios: (1) after import, HHCB is diluted and compounded at the five import sites; or (2) HHCB is delivered to and compounded at the 49 compounding sites identified in the Census. During compounding, EPA/OPPT assumes that imported HHCB is unloaded into large outdoor storage tanks for dilution to a 65 percent solution and is subsequently transferred to an indoor tank in the compounding facility for further processing. Typically, HHCB is present in the compounded fragrance oils at two to four percent (HERA, 2004a).

At room temperature (25 °C), HHCB is a non-volatile liquid with a vapor pressure of 5.47×10^{-4} mmHg (see Table 2-1). Because HHCB is a highly viscous liquid at concentrations ≥65 percent, concentrated HHCB may be heated to become less viscous during transfer activities, during which HHCB may have sufficient vapor pressure to volatilize (EC, 2008). The unloading and transfer are expected to occur at temperatures between 25 and 75 °C (EC, 2008). Therefore, there is potential for releases to air due to volatilization from unloading and transfer

[12] Because the number of establishments under this subcategory is not known, and most companies under NAICS 32562 are small companies with no more than one establishment, the 34 companies are assumed to represent 34 establishments (sites).

Table_Apx E-2. Geographic Distribution for Facilities under NAICS 32562 Toilet Preparation Manufacturing

State	Number of Establishments	Establishments with 20 Employees or More	Number of Employees
California	188	59	7,572
New Jersey	97	56	10,227
New York	62	24	6,243
Texas	60	20	3,134
Florida	59	16	1,531
Illinois	41	22	3,860
Pennsylvania	30	10	1,575
Georgia	24	6	652
Connecticut	22	11	2,582
Minnesota	18	10	1,469
Missouri	17	6	1,091
North Carolina	17	9	3,984
Ohio	17	11	2,370
Colorado	14	3	283
Tennessee	14	9	2,033
Washington	14	1	106
Wisconsin	12	3	190
Arizona	10	6	—[a]
Massachusetts	9	4	249
Arkansas	8	4	1,545
Maryland	8	3	500
Idaho	7	3	318
Indiana	7	3	378
Michigan	7	5	2,795
Utah	7	3	—[b]
Virginia	7	4	1,208
Kentucky	5	2	—[a]
Iowa	4	2	—[b]
Delaware	2	1	—[b]
Vermont	2	1	—[c]

[a]250 to 499 employees.
[b]500 to 999 employees.
[c]100 to 249 employees.
Source: USCB (2007a).

of HHCB at elevated temperatures. Releases to other environmental media may also occur from cleaning of transport containers and compounding vessels. The environmental release media may include water or incineration, depending on the method of cleaning (IFRA, 2012c).

No quantitative release information was found for the compounding scenarios. Compounding releases were assessed using US EPA release models related to cleaning and transfer activities (Fehrenbacher and Hummel, 1996; EPA, 1988; 1991; 1992). In the absence of information to indicate otherwise, EPA/OPPT assumed that container and equipment residues containing HHCB were released to water. EPA/OPPT estimated the number of release days per year for container unloading and transfer activities at compounding sites, assuming that one container is unloaded per site per day and using a default unloading rate of approximately 200 gallons per minute. EPA/OPPT estimated the number of release days per year for equipment cleaning by assuming that cleaning occurs after each batch, there is one batch per site per day, and there are 250 days per year operation at each site.

E-3 Estimated Release from Blending of Fragrance Oils

After compounding, fragrance oils containing HHCB are blended with other materials to formulate commercial and consumer products. EPA/OPPT assumed that HHCB may be present in the formulated products at a maximum of 0.9 percent (HERA, 2004b). The 2006 non-CBI IUR reported a range of 100 to 999 industrial processing and use sites specifically associated with HHCB. The estimate of 100 to 999 sites includes both compounding and blending sites. Because there are approximately 49 compounding sites, EPA/OPPT estimated that there may be up to 950 blending sites (999 sites – 49 sites = 950 sites). Blending sites are classified under NAICS codes 32562, 325611, and 325612. A geographic distribution of these sites, many of which are located in California, New York, and Texas, is provided in Tables E-3 and E-4. Note that the data include sites within the NAICS industry sectors, and may include sites that do not specifically handle HHCB.

Table_Apx E-3. Geographic Distribution for Facilities under NAICS 325611 Soap and Other Detergent Manufacturing

State	Number of Establishments	Establishments with ≥20 Employees	Number of Employees
Texas	72	11	1,111
California	69	11	851
Illinois	43	14	2,123
Ohio	39	15	3,075
New Jersey	34	16	1,787
Pennsylvania	32	8	900
Florida	31	5	—[a]
Missouri	31	8	1,553
Georgia	28	10	862
New York	28	5	356
Michigan	27	2	255
North Carolina	23	6	1,341
Wisconsin	21	3	182
Indiana	20	6	903
Tennessee	15	3	392
Louisiana	14	4	—[b]
Minnesota	13	1	150
Oregon	11	1	—[c]
Arizona	10	2	—[b]
Massachusetts	10	3	196
Colorado	9	1	—[c]
Kansas	8	4	—[a]
Utah	8	1	—[d]
Connecticut	7	3	270
Kentucky	6	2	—[b]
Maryland	6	2	—[a]
Rhode Island	6	3	—[a]
Mississippi	4	1	—[c]
Vermont	2	1	—[c]
Maine	1	1	—[c]
West Virginia	1	1	—[c]
Wyoming	1	1	—[c]

[a]250 to 499 employees.
[b]500 to 999 employees.
[c]100 to 249 employees.
[d]1,000 to 2,499 employees.
Source: USCB (2007b).

Table_Apx E-4. Geographic Distribution for Facilities under NAICS 325612 Polish and Other Sanitization Goods Manufacturing

State	Number of Establishments	Establishments with ≥20 Employees	Number of Employees
California	68	21	1,492
New York	36	11	1,077
Texas	36	11	876
Ohio	34	15	1,243
Florida	31	8	459
Illinois	28	9	629
Pennsylvania	25	10	564
Wisconsin	25	11	4,459
Georgia	21	7	1,082
Missouri	18	7	—[a]
Indiana	17	6	497
Minnesota	17	1	—[b]
New Jersey	16	5	454
North Carolina	16	7	264
Michigan	14	4	255
Tennessee	14	4	281
Colorado	13	2	151
Washington	12	1	133
Massachusetts	11	4	209
Mississippi	9	3	—[b]
Oregon	9	2	195
Maryland	6	3	439
Virginia	5	2	128
Iowa	2	2	—[b]
West Virginia	2	1	—[b]
Delaware	1	1	—[c]

[a]1,000 to 2,499 employees.
[b]100 to 249 employees.
[c]500 to 999 employees.
Source: USCB (2007c).

During the blending of fragrance oils into commercial and consumer products, releases may result from the cleaning of transport containers and process vessels. Additionally, releases may

occur as a result of conveying, mixing, and packaging of powder products containing HHCB. No quantitative release information was found for the scenario of blending of fragrance oils; therefore, these releases were estimated using the OECD Emission Scenario Document (ESD) on the *Blending of Fragrance Oils into Commercial and Consumer Products* (OECD, 2010). The ESD assumes that fragrance oils may be transported to blending sites in drums. Further, the ESD indicates a potential for dust losses if the operation involves the blending of solid commercial and consumer products (*e.g.*, powder detergent) (OECD, 2010). Because the volume of HHCB formulated into solid and powdered products is not known, EPA/OPPT conservatively assumes that the entire volume is formulated into powder products, which results in an overestimate of the release amount. EPA/OPPT estimated 250 release days per year (OECD, 2010), assuming that container cleaning occurs on a daily basis. In some cases, containers may be sent to contractors for cleaning, and actual releases may not occur onsite. EPA/OPPT also assumes that one batch of fragrance product is blended per site per day and that equipment is cleaned after each batch.

E-4 Estimated Release from Use of Commercial and Consumer Products

No quantitative release information was found for the use of commercial and consumer products containing HHCB. EPA/OPPT assumed that the use of all end-use products containing HHCB results in down-the-drain release of HHCB to water. The basis for this assumption is the information on the type of end-use products that fragrance oils are used in, as summarized in Table 2-7. All of the types of end-use products listed with the possible exception of "other" and "fine fragrances" are products whose use is likely to result in down-the-drain release of these products, and these products together represent 89% of the use volume of HHCB. It is noted that the data in Table 2-7 is neither specific to HHCB nor to the use of HHCB in the US. However, a 100% release to water from the use of end-use products has also been assumed in other HHCB risk assessments (EC, 2008 and HERA, 2004b). This assumption is deemed to be adequate for the purpose of this release assessment as well. These releases, combined with upstream losses from compounding and blending, account for 100 percent of the HHCB use volume. Wastewater containing detergent and cleaning products is discharged to the sewer and routed to industrial and municipal wastewater treatment facilities. The release estimates represent the quantity released prior to treatment (*e.g.*, wastewater influent). Because HHCB has uses in many different types of products and an accurate estimate of the number of commercial sites that may use these products is not available, release calculations for use of commercial and consumer products are presented as combined releases from all sites on an annual basis.

Appendix F ADDITIONAL STUDIES

F-1 Endocrine Mechanisms and Molecular Pathways

Studies were available on the effects of HHCB on endocrine mechanisms and other molecular pathways and are briefly summarized here.

The ability of HHCB to bind and interfere with steroid hormone receptors was investigated in liver cells of several aquatic organisms and also in cell lines. Weak estrogenic activity of HHCB (purity unknown) was observed in competitive estrogen receptor binding assays with rainbow trout, carp, and the amphibian, *Xenopus laevis*, and only at relatively high concentrations (Dietrich and Chou, 2001; as cited in EC, 2008; Schreurs et al., 2002; Schreurs et al., 2004). HHCB showed no *in vivo* estrogenic activation of vitellogenin production in carp (*Cyprinus carpio*) (Seinen *et al.*, 1999). HHCB did induce vitellogenin gene expression and protein synthesis in the livers of male medaka, *Oryzias latipes*, at 0.5 mg/L (nominal) (Yamauchi et al., 2008). Antiestrogenic activity has been observed in various cell lines at lower concentrations (Schreurs et al., 2002). HHCB produced a dose-dependent antagonistic effect on estrogen binding in juvenile zebrafish at concentrations at or below the no-observed-effect levels from early-life stage or growth studies in fish (Schreurs *et al.*, 2004). No *in vivo* estrogenic activity was detected in a transgenic zebrafish assay, but anti-estrogenic effects were observed *in vivo* (Schreurs et al., 2004). HHCB (53.5 percent in DEP) was shown to inhibit estrogen-induced vitellogenin production in rainbow trout within the range of concentrations that have been detected in tissues of fish from contaminated locations (Simmons et al., 2010). A study of the interactions of HHCB with fish metabolic systems in carp showed that HHCB significantly inhibited Phase I and Phase II enzymes involved in the synthesis and metabolism of steroids (Schnell et al., 2009).

The ecdysteroid agonist and antagonist activity of HHCB was assessed in an assay with the *Drosophila melanogaster* BII-cell line. HHCB did not show specific agonistic or antagonistic activity in this bioassay (Breitholtz et al., 2003).

HHCB inhibited multixenobiotic resistance (mxr) transporters in gills of the marine mussel, *Mytilus californianus*, an effect that may increase the accumulation of other toxicants in the tissues (Luckenbach et al., 2004). The inhibitory effects of HHCB were only partially reversed after a recovery period in clean seawater (Luckenbach and Epel, 2005).

Appendix G ENVIRONMENTAL MONITORING DATA ANALYSIS

G-1 Measured Concentrations in Wastewater

The majority of synthetic musk fragrances are used in consumer products that enter WWTPs through down-the-drain disposal. Therefore, the concentration of HHCB in influent is dependent on the source of waste received (municipal, commercial, industrial) and the population served by the WWTP. The concentrations of fragrance materials in effluent, however, are dependent upon the ability of these compounds to be eliminated during treatment as well as WWTP size, type, and process.

In the draft report of the Science Advisory Panel for Chemicals of Emerging Concern in California's Aquatic Ecosystems, the maximum concentration of HHCB in wastewater effluent reported from within the state of California was 2.78 µg/L (Anderson et al., 2012). The maximum aqueous concentration of HHCB in treated municipal wastewater effluent discharged to the ocean was 2.5 µg/L. Details regarding the extraction methods and individual QA/QC procedures for the specific studies were not provided in this summary document.

Quarterly samples of wastewater influent and effluent were taken over a one year period at two WWTPs in Texas, and synthetic musk fragrance concentration was measured (Chase et al., 2012). HHCB was consistently one of the more abundant synthetic musk fragrances present throughout quarterly sampling, though seven other musk fragrances were also detected. The authors concluded that the low concentration of the other musk fragrances, as compared to HHCB, could be due in part to a low influx of those compounds into the WWTP or sampling time. From samples prepared using solid phase extraction methods, the influent concentration of HHCB was as high as 5.7 µg/L and the effluent concentration was as high as 6.1 µg/L as shown in Table G-1. Recoveries were reported to be consistently over 50%. The method detection limit was 0.004 µg/L and the method quantitation limit was 0.040 µg/L. Where synthetic musk fragrances were detected in blank samples, the amount present was below the calculated method detection limit.

A state-wide survey in Oregon of trace metals and organic chemicals in municipal effluent was published in 2012 (Hope et al., 2012). Oregon's Senate Bill 737, enacted in 2007, required the state's 52 largest municipal WWTPs and water pollution control facilities to collect effluent samples in 2010 and analyze them for persistent organic pollutants. These facilities are located state-wide and represent a variety of treatment types, service population sizes, geographic areas, and flow conditions. HHCB was detected in 2/102 (the reported limit of quantitation was 10 µg/L) samples with a median value of 12.5 µg/L and a maximum value of 13.5 µg/L. The samples were filtered and analyzed using mass spectrometry by directly injecting the sample without extraction. Each sampling batch included a laboratory method blank and a laboratory control sample, with the data reported as estimated if the sample result did not exceed ten times the level in the blank.

Wastewater influent and effluent concentrations were determined at two WWTPs, one located in a rural area in Kentucky and the other from an urban site in Georgia (Horii et al., 2007). These two WWTPs treat primarily domestic and commercial wastewater. Concentrations of HHCB in influent from both WWTPs were 3 to 60 times higher than those in effluent. Mean concentrations of HHCB in influent of the Kentucky plant (2.499 µg/L) were up to six times higher than the mean concentrations for the Georgia plant (0.42 µg/L). However, mean concentrations of HHCB in the effluents did not differ significantly between the two sites (0.044 and 0.055 µg/L, respectively). Liquid-liquid extraction was performed on the wastewater samples and the concentration of HHCB was determined by GC/MS. Procedural blanks analyzed with the samples as a check for contamination during analysis did not reveal the presence of the target polycyclic musks. The limit of quantification was reported as 10 ng/L and the average recovery for HHCB was 87 ± 4%.

Wastewater and sludge samples were collected over a five-day period in October 2005 from two WWTPs in the state of New York that employ identical treatment processes and serve cities of moderate population size (approximately 100,000 people) (Reiner et al., 2007a). Both WWTPs receive domestic and commercial discharge, and although one also receives industrial discharge, the measured concentration of HHCB did not differ. HHCB was found in wastewater samples with influent and effluent concentrations in the ranges of 4.76 to 12.7 and 0.010 to 0.098 µg/L, respectively. Liquid-liquid extraction was performed on the wastewater samples and the concentration of HHCB was determined by GC/MS. Field blanks and procedural blanks were run with samples as a check for contamination and the limit of quantitation, 10 ng/L, was set to be 10 times the standard deviation found in the blanks. The average recoveries for HHCB were 85 ± 4.3%.

WWTP effluent was sampled at two sites over a two-year period: Taylors Falls WWTP at Taylors Falls, Minnesota and St. Croix Falls WWTP at St. Croix Falls, Wisconsin (USGS, 2011). The sites were chosen because the WWTPs discharge into the St. Croix River upstream from endangered mussel populations. Mean concentrations of HHCB over the sampling period were 0.048 µg/L at Taylors Falls and 1.33 µg/L at St. Croix Falls. Water samples then were stored on ice and extracted within 48 hours of sampling using disposable, polypropylene solid-phase extraction (SPE) cartridges. Field and laboratory blank samples were analyzed to ensure that environmental samples were not contaminated during collection and processing and to assess potential contamination. The average concentration in blank samples was reported as 0.012 µg/L. Recovery with spiked samples was 84.4 with a standard deviation of 22.1%.

Osemwengie and Gerstenberger (2004) analyzed surface water from the confluence of three municipal sewage treatment effluent streams in the state of Nevada, for a period of 7 to 12 months. HHCB was consistently detected in higher monthly average concentrations (0.032 to 0.098 µg/L) relative to the other polycyclic musks. The sewage effluents were sampled from a dedicated effluent receiving stream (one that receives only sewage effluent and rarely runoff). Twice filtered water was drawn by and passed through a peristaltic or diaphragm pump and finally through a cartridge containing a 1:1 polymethyl methacrylate:polystyrene crossed linked with 50% divinylbenzene sorbent. Fragrance free soaps were used during extraction and

analysis. Prepared field blanks and laboratory blanks were used to check for contamination. The method reporting limit for HHCB was 0.02 ng/L based on a signal-to-noise ratio of 3 to 1. The average recovery was 97% for STP effluent and 99% for nanopure water.

Smyth et al. (2008) measured the HHCB concentration in the influent and effluent over a one-year period at six different WWTPs, located in Ontario Canada, employing four different treatment processes. Influent concentrations were as high as 40.3 µg/L and effluent concentrations were as high as 3.73 µg/L. Samples were subjected to liquid-liquid extraction with petroleum ether and cleaned up on deactivated silica gel. Concentrations of HHCB in field blanks were 2-3 orders of magnitude below concentrations measured in wastewater influent and 1-2 orders of magnitude below concentrations measured in effluent, therefore background contamination was considered negligible. Mean recovery of a deuterated surrogate (anthracene or phenanthrene) was 93% with a standard deviation of 23%. The MDL was reported as 0.011 µg/L. Lagoon treatment produced the lowest effluent concentration, although the authors noted that process temperature may have influenced the removal efficiency. Three WWTPs that utilize conventional activated sludge processes were studied, with only small differences in effluent concentrations observed. Overall, effluent HHCB concentrations were an order of magnitude less than the influent HHCB concentrations, as summarized in Table G-1.

A limited number of studies have evaluated the differences between different types of wastewater treatment and their ability to remove HHCB. A study by Simonich et al. (2002) of six wastewater treatment processes (activated sludge, carousel, oxidation ditch, trickling filter, rotating biological contactor, and lagoon) in the US and Europe concluded that overall removal (primary and secondary treatment) of fragrance materials ranged from 58.6-99.9%, with the lowest removal occurring at carousel plants. The highest removal, 96.7-99.9%, was for lagoon plants. In Canada, Smyth's 2008 study (Smyth et al., 2008) of fragrance material removal by four WWTP processes (activated sludge, extended aeration, oxidation ditch, lagoon) similarly found that lagoon treatment yielded the highest removal (>95%). Both authors suggested that the long retention times associated with lagoon treatment is a likely factor in the high removal values, but suggest that other factors such as the ability of given treatment plant to nitrify may have a greater influence on fragrance material removal than retention time alone.

Table_Apx G-1. Measured Concentrations of HHCB in Wastewater

Location	Year	n	Influent Concentration (µg/L)	Effluent Concentration (µg/L)	Comments	Reference
CA	2007	—[a]	—[b]	2.78 maximum	Effluent dominated inland waterway	Anderson et al. (2012)
				ND to 2.5 maximum	Treated municipal wastewater discharged to ocean	
TX	2009-2010	—[a]	4.772-5.735	2.928 - 6.136	Effluent from two WWTPs	Chase et al. (2012)
OR	2010	102, 2 detects	—[b]	12.5 median; 13.0 maximum	52 municipal WWTPs sampled	Hope et al. (2012)
KY	2005	—[a]	0.043-7.032 2.499 mean	0.017-0.024; 0.021 mean 0.010-0.037; 0.044 mean	Before chlorination	Horii et al. (2007)
GA			0.284-0.522, 0.420 mean	0.051-0.418; 0.189 mean 0.028-0.098; 0.055 mean	Primary	
NY(a)	2005	—[a]	4.76-12.7 9.030 mean	4.120-8.150; 3.730 mean 2.810-3.730; 3.350 mean	Primary	Reiner et al. (2007a)
NY(b)	2005		1.8-11.5 5.940 mean	2.950-6.820; 5.310 mean 2.360-3.310; 2.700 mean	Primary	
NV	2000-2001	24	—[b]	0.0326-0.0979; 0.0569 mean		Osemwengie and Gerstenberger (2004)
MN WI	2007	7	—[b]	0.048 mean 1.330 mean	Taylors Falls WWTP St. Croix Falls WWTP	USGS (2011)
Ontario, Canada	2003-2004	3	7.75-14.6 8.38-14.0 1.81-7.21 5.03-40.30 6.64-10.8 3.78-19.0	0.0546-0.673 2.477-3.730 0.497-1.130 2.290-3.240 2.230-3.340 0.914-1.680	Lagoon Extended aeration Oxidation ditch Activated sludge Activated sludge Activated sludge	Smyth et al. (2008)

[a] Not reported.
[b] Not measured.
ND: not detected, detection limits not specified.

G-2 Measured Concentrations in Surface Water

Because synthetic musk fragrances, such as HHCB, are used in many consumer products, these compounds enter WWTPs through down-the-drain practices. The ability of fragrance materials to be eliminated during treatment depends on a variety of factors inherent to the WWTP, such as WWTP size and type (municipal or industrial), processes utilized to treat waste, and populations served (rural versus urban). Consequently, effluents from WWTPs are a primary source of fragrance materials into the environment. These compounds are found in surface water and groundwater located near wastewater discharge areas, with peak environmental concentrations typically occurring near effluent discharge points (Heberer et al., 1999; Peck et al., 2006). Although the concentration of HHCB in wastewater and surface water may vary, HHCB is continuously present and constitutes a constant exposure source.

A science advisory panel convened by the Southern California Coastal Water Research Project was charged to identify potential sources of chemicals of emerging concern, evaluate their fate and effects, and ultimately provide guidance for developing monitoring programs that assess those chemicals with the highest potential to cause effects in the state's receiving waters (Anderson et al., 2012). All data were blank censored to ensure that the reported compounds were in the sample at the time of collection and not artifacts of sample processing and analysis. The established reporting level for HHCB was 0.5 µg/L. Three scenarios were evaluated: Scenario 1, a WWTP effluent-dominated inland (freshwater) waterway; Scenario 2, a coastal embayment that receives both WWTP effluent and stormwater discharge; and Scenario 3, offshore ocean discharge of WWTP effluent. The maximum values reported for each of these scenarios are recorded in Tables G-1 and G-2.

Water samples were taken from the North Side water reclamation plant effluent and the North Shore Channel of the North Branch of the Chicago River, both located in the Chicago, Illinois metropolitan area (Barber et al., 2011). This plant collects sewage from residential (96 percent) and commercial/industrial (3.4 percent) sources. Following treatment, the water reclamation plant effluent is discharged to the North Shore Channel. There were no statistical differences in concentrations of HHCB in these two locations, indicating minimal in-stream dilution or transformation. The mean concentration was reported as 1.6 µg/L. Glass fiber filtered samples were extracted using polystyrene divinylbenzene solid phase extraction and eluted with methylene chloride. Field blanks, laboratory blanks and distilled water matrix spikes were analyzed for qualtiy assurance, however, specific details regarding laboratory reporting limits for the water samples were not provided.

HHCB was detected in the surface waters of the Canyon Lakes System and North Fork Brazos River near Lubbock, Texas in 2010 (Chase et al., 2012). Reported concentrations in the lake ranged from ND to trace amounts, whereas concentrations in the river were appreciably higher, ranging from 0.077 to 0.794 µg/L. The low lake concentrations were expected to be similar to groundwater since the source was groundwater from a land applied site, whereas occurrence in the stream water was attributed to direct release of WWTP effluent. The semi permeable membrane device used for sampling was dialyzed into hexane and reduced in volume prior to

analysis by GC/MS. Recoveries were reported to be consistently over 50%. The method detection limit was 0.001 µg/L and the method quantitation limit was 0.005 µg/L. Where synthetic musk fragrances were detected in blank samples, the amount present was below the calculated method detection limit.

Peck and Hornbuckle (2004) characterized the concentration of several synthetic musk fragrances in western Lake Michigan with water samples that were collected from 1999 to 2001. A lake-wide annual mass balance analysis showed that WWTP discharge was the major source of the synthetic musks, whereas major loss mechanisms were outflow and volatilization. The average measured concentration of HHCB over this time period was 4.7 ng/L. The resin and glass fiber filters used in water sampling were extracted with acetone/hexane. Separations were performed with hexane, dichloromethane/hexane and methanol followed by analysis using GC/MS techniques. The average recovery of fluoranthene-d_{10} was 56±34%. Field and laboratory blanks were utilized with the average sample to blank ratio for the dissolved–phase listed as 7.9. The limit of detection was not reported.

Water concentrations were measured in 2006 for HHCB from three locations along the upper Hudson River, with mean concentrations for HHCB ranging from 0.00395 to 0.0251 µg/L (Reiner and Kannan, 2011). The highest concentration was found near Albany, New York, which corresponded to the location with the highest population. Liquid-liquid extraction with hexane and dichloromethane was performed prior to analysis by GC/MS. Average recoveries of HHCB ranged from 85-98%, with the standard deviation below 15% for all analytes. Field blanks and procedural blanks were analyzed with the samples as a check for contamination. The limit of quantitation for HHCB was 0.001 µg/L.

The USGS (USGS, 2011) and the National Park Service cooperated on a study to determine the occurrence of wastewater indicators in the St. Croix National Scenic Riverway in Minnesota and Wisconsin. Samples of treated wastewater effluent from two WWTPs, located in Taylors Falls, Minnesota and St. Croix Falls, Wisconsin were collected from 2007 to 2008, with concentrations up to 1.33 µg/L; however HHCB in surface water sampled from the St. Croix River at two locations in MN was not detected. Semi-permeable membrane devices (SPMD) were used in conjunction with polar organic chemical integrative samplers (POCIS). Analytes were extracted from SPMDs by means of a two stage dialysis with a solvent. Analytes were separated from the POCIS extracts by means of a liquid chromatography gradient elution. The concentration of HHCB in the field blank sample collected at the St. Croix River above Rock Island near Franconia, MN was 0.012 µg/L. The method reporting limit for HHCB was listed as “0.5e” where *e* denotes variable performance during initial method validation.

Klecka et al., 2010 in a report prepared to a multi-board work group, summarized studies on chemicals of emerging concern that could pose threats to water quality in the Great lakes watershed. The mean value of HHCB in surface water was 0.008 µg/L from 50 samples with a detection frequency of 70%. Specific information regarding detection limits and analytical methodologies was not provided as the report provided summary statistics only.

Concentrations of HHCB were detected in low concentrations in Lake Mead, with a mean value of 0.00036 ± 0.0003 µg/L in 2001, although this waterbody is downstream of the confluence of three WWTPs in Las Vegas, Nevada (Osemwengie and Gerstenberger, 2004). The low concentrations of HHCB, compared to the concentrations found in the municipal sewage effluent, may be due in part to the dilution of the discharge of the combined sewage treatment plant effluents, as the effluent constitutes only 1.37 percent of the total lake volume. Twice filtered water was drawn by and passed through a peristaltic or diaphragm pump and finally through a cartridge containing a 1:1 polymethyl methacrylate:polystyrene crossed linked with 50% divinylbenzene sorbent. The low concentrations of the analytes in the Lake Mead extracts were analyzed using GC/MS in the selected-ion mode. Fragrance free soaps were used during extraction and analysis. Prepared field blanks and laboratory blanks were used to check for contamination. The method reporting limit for HHCB was 0.02 ng/L based on a signal-to-noise ratio of 3 to 1. The average recovery was 97% for STP effluent and 99% for nanopure water.

Passive sampling devices were deployed within two nearshore study zones along the north shore of Lake Ontario, Canada near the municipalities of Pickering and Ajax, Ontario, and Port Hope, Ontario (Helm et al., 2012). Time-weighted average dissolved water concentrations were estimated based on chemical amounts sequestered in the device. Organic wastewater chemicals, like HHCB, were greatest in the vicinity of the Pickering-Ajax WWTP, and then dramatically declined. Calculated sampling device-based concentrations of HHCB ranged from approximately 0.0002 to 0.010 µg/L near Pickering-Ajax and from 0.1 to 0.3 ng/L near Port Hope. Recovery of HHCB was >79% as determined by extracting passive sampling devices spiked with the target compound. Measured concentrations in the laboratory and field blanks were 5 to >10 times lower than the samples.

HHCB concentration was measured in a limited set of samples from Hamilton Harbor and the open waters of Lake Ontario (Andresen et al., 2007). The results were obtained from two replicate extractions of each sample by means of liquid–liquid extraction. The measured HHCB concentration within the bay of Hamilton (highly influenced by WWTP effluents) was 0.007 µg/L. With increasing distance from Hamilton Harbor, the measured concentration decreased and stabilized at 0.002 µg/L. The authors indicated that the decrease of the measured concentrations might be traced back to dilution effects or that musk fragrances are stable under the conditions found in Lake Ontario; however, they concluded that further investigation is needed.

Table_Apx G-2. Measured Concentrations of HHCB in Surface Water

Location	Date	n	Concentration (µg/L)	Comments	Reference
CA	2008	—[a]	0.970 maximum	Resevoir	Anderson et al. (2012)
Chicago, IL	2006-2007	3	1.6 mean	North Shore Channel of the Chicago River (Impacted by water reclamation plant effluent)	Barber et al. (2011)
Lubbock, TX	Fall 2010 Spring 2010	—[a]	0.045 0.077-0.794	Canyon Lakes System North Fork Brazos River	Chase et al. (2012)
Michigan	1999-2001	13	0.0047 ±0.0025	Lake Michigan, background locations	Peck and Hornbuckle (2004)
Catskill, NY Troy, NY Albany, NY	7/2006 5/2006 5/2006	2 2 2	0.00395 mean 0.00736 mean 0.0251 mean	Upper Hudson River	Reiner and Kannan (2011)
Taylors Falls, MN St. Croix Falls, WI Franconia, MN Sunrise, MN	2007-2008	8	0.048 1.33 ND ND	Taylors Falls WWTP at Taylors Falls, MN St. Croix Falls WWTP at St. Croix Falls, WI St. Croix River above Rock Island St. Croix River below Sunrise River	USGS (2011)
Las Vegas, NV	2000-2001	14	0.00006-0.001, 0.00036 mean	Lake Mead	Osemwengie and Gerstenberger (2004)
Great Lakes		50	0.00008-0.180; 0.0008 median; 0.00844 mean; 0.027 95th%ile	Great lakes watershed, summary of studies	Klecka et al. (2010)
Lake Ontario, Canada	June 2008	—[a]	0.00012-0.00034 estimated 0.00021-0.010 estimated	Port Hope Pickering-Ajax	Helm et al. (2012)
Lake Ontario, Canada	2005	4	0.007	Hamilton Harbour	Andresen et al. (2007)

[a]Not reported.
ND: not detected (detection range =0.038-2.16 µg/L)

G-3 Measured Concentrations in Sediment

Studies of measured environmental concentrations of fragrance compounds in the sediment in North America are limited. Synthetic musk fragrances are known to be present in wastewater effluent and the aquatic environment and have been detected in downstream surface water sediment of streams and lakes. Aquatic biota may be subsequently exposed to HHCB from suspended sediment intake.

Chase et al. (2012) measured the concentration of HHCB in the sediment of a playa lake, a non-effluent impacted study site located in proximity to an area treated by a WWTP. The primary water sources are rain and urban runoff, as these playas are integral for storm water retention. In this study, sampling was performed in the summer and fall timeframes of 2010. The measured concentrations of HHCB were 2.13 and 1.43 µg/kg dw in the summer and fall of 2010, respectively, as shown in Table G-3. Samples were extracted with 1:1 acetone/hexane. Recoveries were reported to be consistently over 50%. The method detection limit was 0.3 µg/kg and the method quantitation limit was 0.33 µg/kg. Where synthetic musk fragrances were detected in blank samples, the amount present was below the calculated method detection limit.

The USGS investigated the occurrence and distribution of organic waste contaminants in the Tinkers Creek watershed and two other tributaries to the Cuyahoga River in northeastern Ohio (USGS, 2008; also summarized in Klecka *et al.,* 2010). The effluent from WWTPs constitutes a continuous and sometimes high proportion of the flow in Tinkers Creek and its tributaries (sometimes ≥80 percent). HHCB was detected in the streambed sediment in 29 percent of upstream sites and 86 percent of downstream sites. The range of values is 20 to 390 µg/kg dw with an estimated median value of 60 µg/kg dw. The reported upstream concentrations were <60 µg/kg dw, whereas the downstream concentrations ranged from 20 (estimated value) to 390 µg/kg dw with three of the seven downstream samples having measured values >200 µg/kg dw. Sediment samples were extracted using a pressurized water/isopropyl alcohol extraction. For HHCB, the long term method detection level was reported as 16.5 µg/kg and the laboratory reporting level was listed as 50 µg/kg. Method and replicate blanks were used to check for contamination.

Water and sediment samples were collected in May and July 2006 at three locations along the upper Hudson River: Troy, Albany, and Catskill, New York (Reiner and Kannan, 2011). WWTPs in each of these New York cities discharge effluents into the Hudson River. HHCB was detected in all sediments sampled. Concentrations of HHCB were 72.8 µg/kg dw from Catskill, 351 µg/kg dw from Albany, and 388 µg/kg dw from Troy. Samples were extracted with a 1:3 hexane and dichloromethane mixture. Average recoveries of HHCB ranged from 85-98%, with the standard deviation below 15% for all analytes. Field blanks and procedural blanks were analyzed with the samples as a check for contamination. The limit of quantitation for HHCB in sediment was 5 µg/kg. The authors noted that these sediment concentrations were similar to concentrations that have been measured in Germany (Fromme et al., 2001; Dsikowitzky et al., 2002).

Sediment samples from three tidal tributaries of the Chesapeake Bay, US (Magothy, Corsica, and Rhode Rivers) were collected in 2007 (Sapozhnikova et al., 2010). The Rhode River is the only one that receives effluent from a WWTP. Concentrations of HHCB detected in sediments were as high as 9.2 µg/kg dw for the Magothy River, 2.3 µg/kg dw for the Rhode River, and 1.6 µg/kg dw for the Corsica River. The reported average HHCB concentration was 1.1±2.2 µg/kg dw. The Magothy River is the most urbanized river among the three tributaries studied and was reflected in the concentration of HHCB found in the sampling. Thirty-nine total samples were collected, but details regarding the number of samples for each location were not

provided. Samples were extracted with a 1:1 mixture of methylene chloride and acetone prior to GC/MS analysis. Reagent blanks and replicate samples were analyzed with each batch of samples and blank subtracted as needed. HHCB recoveries were 71% and recoveries in blank samples spiked with musk standards were 96±12%. The limit of detection was not specified.

Sediment samples from Lake Erie and Lake Ontario were measured in August of 2003 (Peck *et al.*, 2006). The concentration in the Lake Erie core sample were blank corrected using the average mass of each compound found in four method blanks. Sample extraction was performed with dichloromethane and exchanged into hexane. The LOD for the core was defined as 3 times the standard deviation. The LOD in the Lake Ontario core was 5.1 µg/kg. The HHCB concentration measured in Lake Ontario (16 µg/kg dw) was an order of magnitude higher than that of Lake Erie (3.2 µg/kg dw). This difference may be attributed to multiple factors, including distance from principal sources (*e.g.*, wastewater outfalls) and differences in loss mechanisms (*e.g.*, photolysis, outflow, or volatilization). The concentration of HHCB over time was extrapolated from the collected sediment core samples and the HHCB concentration was correlated to US fragrance consumption from 1980 to 2006. The authors concluded that HHCB concentration found in the sediment samples reflected the trend of fragrance consumption over this time period.

Table_Apx G-3. Measured Sediment Concentrations at Locations in the US

Location	Year	Concentration µg/kg dw	Comments	Reference
Lubbock, TX	Summer 2010 Fall 2010	2.13 1.43	Canyon Lake System	Chase et al. (2012)
Tinkers Creek, OH Cuyahoga River, OH	2006	20[a]-<60, 37 average 20[a]-390, 144 mean	Upstream of 7 WWTPs Downstream of 7 WWTPs	USGS (2008); Klecka et al. (2010)
Troy, NY Albany, NY Catskill, NY	2006	388 351 72.8	Upper Hudson River, NY	Reiner and Kannan (2011)
Magothy River, MD Rhode River, MD Corsica River, MD	2007	ND - 9.2 ND - 2.3 ND - 1.6 1.1 avg	Tributary, Chesapeake Bay	Sapozhnikova et al. (2010)
Lake Erie Lake Ontario	2003	3.2 16	Great Lakes	Peck et al. (2006)

[a] Reported as an estimated value.
ND = not detected, limit of detection not specified

G-4 Measured Concentrations in Biosolids and Sludge

The removal of fragrance compounds from wastewater has been reported in the US, Canada, and EU in several studies, with overall removal efficiencies ranging from as low as 29.9 percent

to as high as 99.7 percent (typical values were 70 to 80 percent) depending upon the type of wastewater treatment (Simonich et al., 2002; Deblonde et al., 2011; Smyth et al., 2008). A fraction of HHCB is expected to preferentially partition to organic-rich biosolids during wastewater treatment based on the high measured log K_{OW} values of 5.3 to 5.9 (see Table 2-1) and the estimated K_{OC} values of 4.1 to 4.3 (EPI Suite v4.11, see Chapter 2, Section C, Environmental Fate). These solids, produced through the process of wastewater treatment, are subsequently disposed of by either landfill or incineration. They may also be utilized for land application to fertilize plants or to improve the quality of soil, and may then become available for plant and terrestrial biota uptake.

In a study by Kinney et al. (2006), the presence and concentration of organic waste contaminants was determined in solid materials produced during wastewater treatment. Nine different biosolid products from municipal WWTPs in seven different states were analyzed. Six of these products were intended for commercial, homeowner, and municipal use and are available to the public for purchase. Three of the products are used in agriculture. HHCB was detected in all nine biosolids. HHCB was extracted from the biosolid using accelerated solvent extraction and measured by GC/MS. Each sample was composited from bulk material and samples were analyzed in triplicate. All samples had similar treatment (secondary activated sludge and chlorine disinfection); however, the preparation and applied form of the solid varied. The concentration of HHCB varied, with one site sample measuring as high as 177,000 µg/kg dw. All other measured HHCB concentrations were below 4,000 µg/kg dw. As this high value was more than 40 times higher than the next highest measured concentration, and more than four orders of magnitude higher than the lowest reported concentration, the authors considered it as an "outlier." Excluding this value, the reported median value for HHCB was 1,461 µg/kg dw. Extractions were performed using accelerated solvent extraction wand a 70:30 acetonitrile: water solvent mixture. At least one laboratory spike and laboratory blank were evaluated for each set of extractions and quantifications; however HHCB was not detected in any of the blanks hence no contamination was indicated. The method detection limit was noted as 16.5 µg/kg and the recoveries ranged from 54-107%. Differences in biosolid preparation techniques and WWTP characteristics (*e.g.*, location, population served, volumes of wastewater, and retention times) likely account for the wide variation in HHCB concentrations measured, with no one factor being clearly delineated in this study.

A second study by Kinney et al. (2008) was performed to examine bioaccumulation in earthworms from soil obtained from three Midwest agricultural fields, which had been amended with biosolids or swine manure. The biosolid source had a concentration of 427,000 µg/kg dw, although details regarding this source were not provided. HHCB and tonalide were detected in one of the blank samples at a concentration 1 to 3 orders of magnitude lower than reported values. The method detection limit for HHCB was reported as 12.5 µg/kg dry weight.

Biosolid samples were collected from the North City Wastewater Reclamation Plant of the City of San Diego to study biosolid degradation on a weekly schedule for 19 weeks (Buyuksonmez and Sekeroglu, 2005). Samples were taken from the return activated sludge line after purging

the stagnant sludge from the sampling line. The presence of HHCB was determined using GC/MS following extraction (sequentially with methylene chloride, ethyl acetate and hexane) with a Soxhlet apparatus for 24 hours. The average concentration of HHCB in biosolids was reported to be 7,840 µg/kg dw, although the number of samples taken and information regarding blanks and the limit of detection were not provided.

In a study by DiFrancesco et al. (2004), digested sludge was collected from two sites over a period of two years. Anaerobically digested and dewatered sludge was obtained from two activated sludge plants in Delaware: Georgetown WWTP and Wilmington WWTP. Background concentrations of fragrance materials in anaerobically digested sludge from the municipal WWTPs were determined in July 2000 and again in March (Georgetown) and October (Wilmington) 2002. Sludge was dewatered using a gravity separator at Georgetown WWTP and a belt filter press at Wilmington WWTP. Both WWTPs added polymers to assist in sludge dewatering, and the Georgetown facility also used aluminum chloride for phosphorus removal. Samples were extracted using accelerated solvent extraction (dichloromethane) and were analyzed using GC/MS. The HHCB concentrations ranged from 21,800 to 86,000 µg/kg dw. The overall concentrations in this limited sampling were higher in samples collected in 2000 and at the Georgetown site. Table G-4 includes a summary of measured concentrations of HHCB in biosolids. Only data with all matching confirmation ions and a signal-to-noise ratio greater than 5 were accepted.

For comparison, studies of biosolids from Canada are also included in Table G-4. HHCB concentrations in digested biosolid were studied by Smyth et al. (2007a, 2007b) in Canada. One oxidation ditch with tertiary sand filtration, one extended aeration activated sludge plant, and three conventional activated sludge plants that discharge into the Grand River, Ontario, Canada were used as sampling sites with four sampling events from November 2003 to July 2004. Extraction was performed using supercritical fluid (5% acetone in hexane) or microwave assisted techniques (acetone and hexane), measurement was performed using GC/MS. Method blanks were employed to confirm the absence of contamination. 3 out of 15 blanks showed concentrations of HHCB that were 2-3 orders of magnitude below concentrations measured in sludge samples. The method detection limit was reported as 41 µg/kg. HHCB concentrations in digested biosolid ranged from 9,430 to 55,500 µg/kg dw. Smyth et al. (2007a) reported that these values were in range with previous reported values in Canada (Lee et al., 2003) and the United Kingdom (Stevens et al., 2003).

Yang and Metcalfe (2006) also studied biosolids from WWTPs in Ontario, Canada. Samples were collected from the Peterborough WWTP over a one-year period, and the HHCB concentrations were measured in the raw, return activated sludge, and digested biosolids. Concentrations of HHCB did not vary significantly for any one sampling type over the one-year study. However, there was a trend for the synthetic musks to accumulate in the return activated sludge and to finally concentrate in the digested biosolids. The annual average concentrations of HHCB were 3,309.9 µg/kg dw in the return activated sludge and 6,788.4 µg/kg dw in the digested biosolid. Extractions were performed by accelerated solvent extraction using a 1:1 mixture of *n*-hexane and ethyl acetate. Field blank and method blank samples were analyzed and recovery studies

were conducted in triplicate with blank subtraction. All recoveries of synthetic musks were >80%. The limit of quantitation was determined from spiking experiments into surrogate matrices and corresponded to a 5:1 signal-to-noise ratio. Method LOQs were reported to vary between 0.4 and 4.0 ng/L for analytes in sewage samples; the specific value for HHCB was not reported.

Table_Apx G-4. Measured Concentrations of HHCB in Biosolids and Sludge

Location	Collection Year	N	Concentration µg/kg dw	Preparation/Comments	Reference
WI	5/2003 11/2003	3 3	1,400 (average) 1,470 (average)	Rotary kiln dried	Kinney et al. (2006)
CO	5/2003 11/2003	3 3	3,700 (average) 2,670 (average)	Composted + plant material	
TX	8/2003 11/2003	3 3	1,100 (average) 977 (average)	Composted + yard material	
WA	11/2003	3	933 (average)	Composted with sawdust	
WA	5/2004	3	13 (average)	Composted with yard waste	
KS	9/2003 11/2003	3 3	2,370 (average) 1,970 (average)	Air dried and turned	
AZ	6/2003 1/2004	3 3	1,300 (average) 697 (average)	biosolid	
AZ	6/2003 1/2004	3 3	1,100 (average) 767 (average)	Dewatered biosolid + polymer	
IA	4/2005	3	177,000 (average)	Thermophilic and mesophillic digestion + polymer	
Unknown	2008	3	427,000[b]	No details given	Kinney et al. (2008)
San Diego, CA	2005	—[a]	7,840 (average)	Return activated sludge line	Buyuksonmez and Sekeroglu (2005)
Wilmington, DE	7/2000 10/2002	—[a] 7	43,000[b] 21,800 (± 4,300)	Anaerobically digested sludge	DiFrancesco et al. (2004)
Georgetown, DE	7/2000 3/2002	—[a] 3	86,000[b] 37,600 (± 4,500)	Anaerobically digested sludge	
Ontario, Canada (5 locations)	2003-2004	7 10 12 11 11	9,430 (mean) 40,300 (mean) 42,000 (mean) 55,500 (mean) 46,300 (mean)	Aerobically digested biosolids Aerobically digested biosolids Anaerobically digested biosolids Anaerobically digested biosolids Anaerobically digested biosolids	Smyth et al. (2007a)
Canada (site 1/site 2)	2003-2005	—[a]	36,500/20,100 25,600/17,500 73,700/45,300 23,800/20,500	Primary sludge, wet Primary sludge, air dried Waste activated sludge, wet Waste activated sludge, air dried	Smyth et al. (2007b)

Table_Apx G-4. Measured Concentrations of HHCB in Biosolids and Sludge

Location	Collection Year	N	Concentration µg/kg dw	Preparation/Comments	Reference
Canada Peterborough, ON	2/2003	1	4,514.4	Raw sludge	Yang and Metcalfe (2006)
		3	3,638.8 (± 787.7)	Return activated sludge	
		3	7,896.7 (± 2,130.8)	Digested sludge	
	4/2003	3	2,857.4 (± 18.6)	Raw sludge	
		3	5,103.9 (± 635.0)	Return activated sludge	
		3	7,006.8 (± 931.3)	Digested sludge	
	7/2003	3	3,356.1 (± 1,058.6)	Raw sludge	
		3	2,156.6 (± 504.7)	Return activated sludge	
		3	6,477.6 (± 1,065.3)	Digested sludge	
	10/2003	3	2,482.4 (± 340.6)	Raw sludge	
		3	2,340.4 (± 360.2)	Return activated sludge	
		3	5,772.76 (± 115.2)	Digested sludge	
	Annual average		3,302.5	Raw sludge	
			3,309.9	Return activated sludge	
			6,788.4	Digested sludge	
			469.6 (± 22.4)	Trucked biosolid	

[a]Not reported.
[b]Value was not reported as mean, average, or median.

Studies summarized in Table G-4 reported analytical reliability using accelerated solvent extraction or supercritical fluid extraction followed by GC/MS techniques. Studies of HHCB in biosolids are somewhat limited, however, because the studies that detected and measured HHCB reported concentrations in the range of <100 to >100,000 µg/kg dw. The measured concentration depended on a variety of factors, including WWTP operations such as receiving waste stream (municipal or industrial), date of collection, location, population served, water volume, treatment type, and preparation methodologies prior to land application. Because these factors cannot be independently separated from values within the reported data set, it is not possible to assign sensitivity to a single factor. Comparison with values reported for studies in Ontario, Canada are within the reported range for those from the US and also similarly show differences depending upon location, treatment process, and season.

G-5 Measured Concentrations in Soil

Limited data were available that described concentrations of HHCB in soil. Though many studies are designed to understanding the biodegradation or fate of HHCB in the soil compartment, there are data showing that land application of wastewater effluent or amendment of soils with biosolids resulted in measurable concentrations of HHCB. The high frequency of HHCB detection in biosolids and wastewater suggests that they may be an important organic waste contaminant source to terrestrial environments, particularly when applied to soil. Table G-5 summarizes the measured concentrations in HHCB in soil.

In 2008, Kinney et al. (2008) reported HHCB concentrations in agricultural soil amended with biosolids. Three sites were studied. Site 1 (minimally affected site) was a non-irrigated soybean field that had not been amended with either human or livestock waste for at least the previous seven years. Soil and earthworm samples were collected from this field in June and September of 2005. Site 2 (biosolid amended site) was a no-till, non-irrigated soybean field receiving biosolid as a fertilizer for the first time. Site 3 (manure-amended site) was a non-irrigated cornfield receiving liquid swine manure as an organic fertilizer. While HHCB was not detected at site 3, HHCB was detected at site 1 in June 2005 at measurable levels, up to 633 µg/kg dw. HHCB concentrations were highest at site 2 and ranged from 1,050 to 2,770 µg/kg dw. Extractions were performed with a 70:30 acetonitrile: water solvent mixture. HHCB and tonalide were detected in one of the blank samples at a concentration 1 to 3 orders of magnitude lower than reported values. The method detection limit for HHCB was reported as 12.5 µg/kg dry weight.

In a study by Chase et al. (2012), soil core samples were collected quarterly over a two-year period at sites outside the city limits of Lubbock, Texas to determine the amount of sorption after land application of treated wastewater and groundwater recharge. The wastewater at this site is treated through primary and secondary treatment processes for removal of suspended solids and organic carbon through different operating flow streams at the WWTP. Some of the treated effluent is released to a secondary effluent reservoir, which is then applied *via* center pivot irrigation at a land application site. Two sites were studied both inside and outside the pivot irrigation system; however, small differences in measured HHCB concentrations were observed between the two locations. Three sample depths were studied: 0 to 6, 12 to 18, and 24 to 30 inches below the surface. Over the time period of the study, at locations inside the irrigation system, concentrations ranged from <0.33 to 1.98 µg/kg dw at 0 to 6 inches, <0.33 to 1.25 µg/kg dw at 12 to 18 inches, and ND to 1.25 µg/kg dw at 24 to 30 inches. For locations outside the irrigation system, concentrations ranged from <0.33 to 5.69 µg/kg dw at 0 to 6 inches, <0.33 to 0.77 µg/kg dw at 12 to 18 inches, and <0.33 to 0.79 µg/kg dw at 24 to 30 inches. The highest reported value (5.69 µg/kg dw) was measured outside the irrigation system in the winter of 2009; however, excepting this value, concentrations were below 1.98 µg/kg dw over the time period of the study. Soil concentrations were found to be lowest during the summer months, possibly due to volatilization. Extractions were performed with 1:2 acetone/hexane. Recoveries were reported to be consistently over 50%. The method detection limit was 0.3 µg/kg and the method quantitation limit was 0.33 µg/kg. Where synthetic musk fragrances were detected in blank samples, the amount present was below the calculated method detection limit. Groundwater was also sampled and found to range from ND to <5 ng/L.

Table_Apx G-5. Measured Concentrations of HHCB in Soil

Location	Year	Site	Soil Concentration (µg/kg dw)			Reference
Midwest, US Agricultural fields	2005	Minimally affected	ND-633			Kinney *et al.* (2008)
		Biosolid amended	1,050-2,770			
		Manure amended	ND			
Lubbock, TX Land application site	2009-2010		*0-6 inches*	*12-18 inches*	*24-30 inches*	Chase *et al.* (2012)
		Inside pivot irrigation	<0.33-1.98	<0.33-1.25	ND-0.90	
		Outside pivot irrigation	<0.33-5.69	<0.33-0.77	<0.33-0.79	
Ontario, Canada	2003	Biosolid amended	2.0 ± 0.1 (after 1 day) 2.8 ± 0.4 (after 2 weeks)			Yang and Metcalfe (2006)
ND: not detected; method detection limit = 12.5 ug/kg dw (Kinney); 0.3 µg/kg dw (Chase)						

A Canadian study of biosolid-amended agricultural fields similarly showed that HHCB persisted in soils for the first two weeks post application, but concentrations declined thereafter (Yang and Metcalfe, 2006). Extractions were performed by accelerated solvent extraction using a 1:1 mixture of *n*-hexane and ethyl acetate. Field blank and method blank samples were analyzed and recovery studies were conducted in triplicate with blank subtraction. All recoveries of synthetic musks were >80%. The limit of quantitation was determined from spiking experiments into surrogate matrices and corresponded to a 5:1 signal-to-noise ratio. Method LOQs were reported to vary between 0.2 to 1.9 µg/kg wet weight for analytes in soil; the specific value for HHCB was not reported.

More studies are needed, however, to understand the influence and integration of time, degradation mechanisms (such as volatilization, transformation, and leaching), and organic matter content on long-term concentrations. Soil studies, although clearly limited, do indicate that land application of treated wastewater effluent or biosolids results in detectable quantities of HHCB in soil and hence represents a potential route through which HHCB may enter terrestrial ecosystems.

G-6 Measured Concentrations in Biota

The liver, fillet, and fat of many aquatic organisms, including fish, shrimp, zebra mussels, and aquatic mammals and birds, have been sampled from US waters in a variety of locations (Table G-6). These data confirm the widespread occurrence of HHCB in aquatic media and subsequent exposure to wildlife.

Six geographical locations in various parts of the US were selected as sampling sites for five effluent-dominated rivers receiving discharge from WWTPs located in Chicago, Illinois; Dallas, Texas; Orlando, Florida; Phoenix, Arizona; and West Chester, Pennsylvania; and one reference site on the Gila River, New Mexico (Ramirez et al., 2009).The reference site was expected to be removed from anthropogenic point sources; therefore, no accumulation of HHCB was expected

or detected in fish collected from this site. A total of 18 to 24 adult fish of the same resident species were collected from each sampling location during late summer and fall of 2006. Fish sampled at each site were divided into six composites, each containing three or four adult fish of similar size. The composites revealed the presence of HHCB at maximum concentrations ranging from 300 to 2,100 µg/kg tissue. Lipid determinations were made gravimetrically and extractions were performed with 1:1 dichloromethane-hexane. Each analytical batch contained one blank, at least one continuing calibration verification sample and two laboratory control samples. No target pharmaceuticals were detected in blank samples. The method detection limit for HHCB was reported as 12 µg/kg tissue concentration.

Concentrations of polycyclic musks, including HHCB, in fish were collected in 2006 from Troy, Albany, and Catskill along the upper Hudson River in the eastern region of the state of New York (Reiner and Kannan, 2011). There are 148 WWTPs that discharge treated wastewater into the Hudson River, which flows southward from its headwaters in the Adirondack Mountains and ultimately empties into the Atlantic Ocean at New York City. The measured levels of HHCB varied across fish species, with a range of <1 to 39 µg/kg lipid weight; the overall highest concentrations were measured in white perch liver. Biological samples were extracted with 3:1 dichloromethane-hexane. Average recoveries of HHCB ranged from 85-98%, with the standard deviation below 15% for all analytes. Field blanks and procedural blanks were analyzed with the samples as a check for contamination. The limit of quantitation for HHCB in fish samples was 1 µg/kg.

Shrimp samples were collected from a seafood market survey of wild and farmed shrimp from the US and other countries (Mexico, India, Ecuador, Thailand, China, and others) in 2006 (Sapozhnikova et al., 2010). The shrimp were analyzed for the presence of synthetic musks. HHCB was detected in all samples, with concentrations ranging from 48 to 683 µg/kg lipid weight (average 199 µg/kg lipid weight) in farmed shrimp and 66 to 762 µg/kg lipid (334 ng/g lipid weight) in wild shrimp (HHCB in US wild shrimp max 330 µg/kg lipid weight, n=6). Farm-raised shrimp from Indonesia (n = 1) showed the highest concentration of HHCB, followed by farm-raised shrimp from the US (max 424 µg/kg lipid weight, n = 3). No trends could be discerned as the sampling was not statistically significant; however, the presence of HHCB in shrimp tissues, both farmed and wild caught, indicates the widespread exposure of aquatic biota to HHCB. Lipid content was determined gravimetrically and measured as dichloromethane extractible non-volatiles. Reagent blanks and replicate samples were analyzed with each batch of samples and blank subtracted as needed. HHCB recoveries were 71% and recoveries in blank samples spiked with musk standards were 96±12%. The limit of detection was not specified.

Kannan et al. (2005) reported the presence of HHCB in the tissues of higher trophic level aquatic organisms and humans (see Appendix A for summary of human biomonitoring studies). Among the wildlife species analyzed, tissue concentrations ranged from <1 to 25 µg/kg ww. Blubber tissue obtained from spinner and bottlenose dolphins from coastal waters off the state of Florida contained the highest levels of HHCB. Sample tissues were ground with anhydrous sodium sulfate and extracted with mixed solvents of dichloromethane and hexane (3:1).

Average recoveries of HHCB ranged from 85-98%. Procedural blanks were analyzed with every set of six samples to check for laboratory contamination and to correct sample values, if necessary. Procedural blanks contained trace levels of HHCB. The limit of quantitation (LOQ) was set to be twice the concentration that was found in blanks. In wildlife samples, the LOQ for HHCB was 1 µg/kg wet weight.

Seven to eight carp were collected 200 meters from the drinking water intake area on Lake Mead, Nevada on a monthly basis over a period of one year (Osemwengie and Gerstenberger, 2004). The measured HHCB concentration in the fish tissue ranged from 1.4 to 4.5 µg/kg ww with an annual mean concentration of 3.0 µg/kg ww. Lipids were extracted from carp tissue using chloroform. Prepared field blanks and laboratory blanks were used to check for contamination. The method reporting limit for HHCB was not specified.

Kinney et al. (2008) measured HHCB concentrations in earthworm tissue obtained from amended soils where biosolids or liquid swine manure was applied. These values were compared to values reported for worms collected from a soybean field, which had not been amended with human or livestock waste for at least the previous seven years. HHCB concentrations in worms 30 days post application (3,340 µg/kg ww) were an order of magnitude higher than the levels measured after 156 days (131 µg/kg ww). Tissue concentrations in worms from the manure amended site were 49 µg/kg after 139 days. Extractions were performed with a 70:30 acetonitrile: water solvent mixture. HHCB and tonalide were detected in one of the blank samples at a concentration 1 to 3 orders of magnitude lower than reported values. The mean earthworm spike recovery was reported to be 40% and the MDL was 12.5 µg/kg dw.

Table_Apx G-6. Measured Concentrations of HHCB in Biota[*]

Species	Year	n	Location	HHCB Concentration (µg/kg)	Reference
µg/kg, lipid weight					
White perch (liver)	2006 Hudson River	3	Troy, NY	6.27-19.9	Reiner and Kannan (2011)
			Albany, NY	7.58-22.5	
			Catskill, NY	13.7-27.9	
Channel catfish (liver)		3	Troy, NY	11.1-39	
		1	Catskill, NY	<1	
Smallmouth bass (liver)		3	Troy, NY	<1-11.1	
		3	Albany, NY	1-31.9	
		3	Catskill, NY	<1	
Largemouth bass (liver)		1	Albany, NY	10.9	
		1	Catskill, NY	8.22	
White catfish (liver)		1	Albany, NY	6.56	
		1	Catskill, NY	5.79	
Brown bullhead (liver)		3	Catskill, NY	<1-51.1	
Zebra mussel		4	Catskill, NY	10.3-19.3	
American eel (whole body)		1	Catskill, NY	125	
Shrimp, wild caught	2006	6	US	330 max	Sapozhnikova et al. (2010)
Shrimp, farm raised		3	US	424 max	
µg/kg, tissue concentration					
Earthworm	2005		Midwestern US		Kinney et al. (2008)
		3	-minimally affected site	61[a]; ND[b]	
		3	-biosolid amended site	3,340[c]; 131[d]	
		3	-manure amended site	ND[e]; 49[f]	
Largemouth bass	2006	6	Chicago, IL North Shore Channel	1,300 mean; 1,800 max	Ramirez et al. (2009)
Smallmouth buffalo fish		6	Dallas, TX Trinity River	800 mean; 1,800 max	
Bowfin		6	Orlando, FL Little Econlockhatchee River	100 mean; 300 max	
Common carp		6	Phoenix, AZ Salt River	1,800 mean; 2,100 max	
White sucker		6	West Chester, PA Taylor Run	1,800 mean; 2,000 max	
µg/kg, wet weight					
Sea otter (liver)	1993-1999	8	Monterey Bay, CA	<1-3.2[g]	Kannan et al. (2005)
Harbor seal (liver)	1996-1997	3	Central CA coast	4.4-5.5; 4.8 mean	
California sea lion (liver)	1993-1996	3	Central CA coast	1.5-4.4; 2.8 mean	
River otter (liver)	1997	3	Grand River, MI	2.4-3; 2.8 mean	

Table_Apx G-6. Measured Concentrations of HHCB in Biota*

Species	Year	n	Location	HHCB Concentration (µg/kg)	Reference
Bottlenose dolphin (blubber)	1994-2000	4	FL coast	4.2-20.5; 12 mean	
Striped dolphin (blubber)	1995-1997	3	FL coast	8.1-25; 14 mean	
Pygmy sperm whale (blubber)	2000	1	FL coast	6.6	
Atlantic sharpnose shark (liver)	2004	3	Indian River Lagoon, FL coast	4.6-5.2; 4.8 mean	
Mink (liver)	1997	4	Aurora/Plainfield, IL	2.2-5.3; 3.7 mean	
Common merganser (liver)	1999	2	Buffalo Harbor, NY	3.7-4.2; 4 mean	
Greater and lesser scaup (liver)	1995-1999	2	Niagara River, NY	1.9-2.7; 2.3 mean	
Mallard (liver)	1995	1	North Tonawanda Creek, NY	2.7	
Atlantic salmon (skin on fillet)	2003	6	Farmed and wild, local market NY	<1-3.2[h]	
Smallmouth bass (liver)	2003	3	Effley Falls Reservoir and Rock Pond, NY	4.3-5.4; 4.8 mean	
Carp	2001	84	Lake Mead, NV	1.4-4.5; 3.0 mean	Osemwengie and Gerstenberger (2004)

* Note that human biomonitoring studies are summarized in Appendix A, Section A-2
ND Not detected, method detection level = 12.5 µg/kg dw
[a] Measured June 2005.
[b] Measured September 2005.
[c] 31 days after application of biosolids.
[d] 156 days after application of biosolids.
[e] 30 days after application of liquid swine manure.
[f] 139 days after application of liquid swine manure.
[g] 38 percent of samples contained detectable concentrations.
[h] 83 percent of samples contained detectable concentrations.

G-7 USGS National Water Quality Information System Data

Monitoring data collected by the USGS NWQL and available from the NWIS database up to May 2012 were obtained for environmental concentrations of HHCB within the US (USGS, 2012). The following categories of HHCB data were available from the USGS NWIS: water, filtered recoverable; water unfiltered, recoverable; and solids recoverable, dw. These data were grouped by medium type and site type, as shown in Table G-8. The Medium and Site Code definitions are provided in Table G-9.

The data compiled herein include values that are reported as less than the USGS LRL; values that are between the LRL and the LT-MDL; and values that are below the LT-MDL (Oblinger Childress *et al.*, 1999). Similar categories of data were available for both types of water samples. However, for unfiltered water from effluent/stream, surface water/outfall, and groundwater/well sites, significantly fewer than 10 data points were available, and were thus not incorporated into the summary plots.

For this assessment, data contained in three NWIS parameter codes (*i.e.,* 62075, 62823, and 63209) for HHCB were included. The LRL for water sampling was 0.5 µg/L for sampling dated 7/16/2001 to 9/30/2009. The LRL was updated to 0.05 µg/L for samples dating from 10/1/2009 to the present based on a re-evaluation of the LT-MDL by the USGS. Interim reporting levels were recorded for data collected from solid samples, based on USGS practices for data interpretation. For monitoring data sets where the geometric standard deviation was <3.0, values recorded as "less than LRL" or "estimated" were replaced by the LRL divided by the square root of two, as per the EPA OPPT guidance document (EPA, 1994). Likewise, where the geometric standard deviation was >3.0, values recorded as "less than LRL" or "estimated" were replaced by the LRL divided by two (EPA, 1994). These values are summarized in Table G-7 below. It should be noted, therefore, that resulting low-end values are biased toward the LRL. This practice presents a conservative low-end value, which protects against false negative values. As such, values do not necessarily represent quantitative measured concentrations.

Table_Apx G-7. Summary of Substituted Values (µg/L) for Water Samples

	7/2001 to 9/2009 (LRL=0.5 µg/L)	10/2009-2012 (LRL=0.05 µg/L)
Geometric Standard Deviation < 3	0.35	0.035
Geometric Standard Deviation < 3	0.25	0.025

USGS data from the NWIS database was accepted with the assumption that their internal methodologies were consistent and robust. These data were presumed to be collected under the guidance of the USGS National Field Manual for the Collection of Water-Quality Data, a publication which documents the methods, protocols, procedures and recommended practices

for the collection of water-quality data (USGS, variously dated). Data reporting procedures were presumed to follow USGS guidance (Oblinger Childress et al., 1999).

For each of the data groupings (Table G-8), box plots were generated with the calculated mean, median, 1st (Q1) and 3rd (Q3) quartile, and 5th and 95th percentile values as shown in Figures G-1 to G-3. A summary of these calculated values is presented in Table G-8.

Table_Apx G-8. Summary of Box Plots for USGS HHCB Data

USGS Parameter Code Medium/Site	HHCB Concentration							
	5th Percentile	Q1	Median	Mean	Q3	95th Percentile	n	% of n <LRL
62075 Water, filtered, recoverable; HHCB (µg/L)								
Effluent/stream	0.35	0.35	1.40	1.18	1.82	2.16	10	40
Effluent/outfall	0.09	0.35	0.76	0.98	1.2	3.40	27	48
Surface water/outfall	0.35	0.35	0.65	1.08	1.70	2.30	41	48
Surface water/stream	0.10	0.35	0.35	0.35	0.35	0.35	2803	98
Surface water/lake, reservoir, impoundment	0.04	0.35	0.35	0.33	0.35	0.35	263	100
Groundwater/well	0.04	0.35	0.35	0.32	0.35	0.35	1548	99
62823 Water, unfiltered, recoverable; HHCB (µg/L)								
Effluent/outfall	0.14	0.14	1.02	1.01	1.85	2.26	10	40
Surface water/stream	0.02	0.05	0.14	0.12	0.14	0.15	1568	100
Surface water/lake, reservoir, impoundment	0.03	0.14	0.14	0.12	0.14	0.14	45	100
63209 Solids, recoverable, dry weight; HHCB (µg/kg)								
Bottom material/lake, reservoir, impoundment	27.93	41.02	65.42	87.46	106.08	212.87	122	99
Bottom material/stream	17.68	24.75	40.00	67.99	67.19	200.00	484	92
n: number of sample measurements; LRL: laboratory reporting level								

Table_Apx G-9. USGS Medium and Site Codes

Medium Codes	
Surface water	Water on the surface of the earth stored or transported in rivers, streams, estuaries, lakes, ponds, swamps, glaciers, or other aquatic areas. It also may refer to water in urban drains and storm-sewer systems.
Effluent	Treated or untreated wastewater after use at a facility or wastewater treatment plant, or from combined sources, such as combined-sewer overflows or tile drainage systems.
Groundwater	Water below the surface of the earth contained in the saturated zone. It does not include soil moisture or interstitial water.
Bottom material	A mixture of mineral and organic matter that compose the top bed deposits (usually the first few inches) underlying a body of water.
Site Codes	
Outfall	A site where water or wastewater is returned to a surface-water body (*e.g.*, the point where wastewater is returned to a stream). Typically, the discharge end of an effluent pipe.
Lake, reservoir, impoundment	An inland body of standing fresh or saline water that is generally too deep to permit submerged aquatic vegetation to take root across the entire body (*Cf*. wetland). This site type includes an expanded part of a river, a reservoir behind a dam, and a natural or excavated depression containing a water body without surface-water inlet and/or outlet.
Well	A hole or shaft constructed in the earth intended to be used to locate, sample, or develop groundwater, oil, gas, or some other subsurface material. The diameter of a well is typically much smaller than the depth. Wells are also used to artificially recharge groundwater or to pressurize oil and gas production zones. Additional information about specific kinds of wells should be recorded under the secondary site types or the Use of Site field. Underground waste-disposal wells should be classified as waste-injection wells.
Stream	A body of running water moving under gravity flow in a defined channel. The channel may be entirely natural, or altered by engineering practices through straightening, dredging, and (or) lining. An entirely artificial channel should be qualified with the "canal" or "ditch" secondary site type.

Figure G-1 shows box plots for filtered water from three different mediums and associated sites. HHCB concentrations are highest from effluent at outfall or stream sites and from surface water at outfall sites with higher values at outfall sites, although the range of values varied from ND to >3 µg/L. The mean effluent concentrations were comparable, but the range of concentrations was greater at outfall sites. This may be due to the limited data that were available for effluent/stream sites, such that data as summarized here encompasses only 10 data points collected from four individual sites. Mean values for filtered water were <1.5 µg/L for all medium and site types.

Groundwater concentrations at well sites and surface water concentrations at streams and lakes, reservoirs, and impoundment sites ranged from ND to <0.5 µg/L. For effluent/outfall

filtered/non-filtered samples, the HHCB concentrations were higher in unfiltered samples (Figure G-2). This may be attributed to the presence of HHCB in suspended solids. Again, significantly lower concentrations were consistently recorded in filtered surface water samples collected from streams and lakes, reservoirs, and impoundment sites—areas that are unaffected or nominally affected by wastewater treatment outfall.

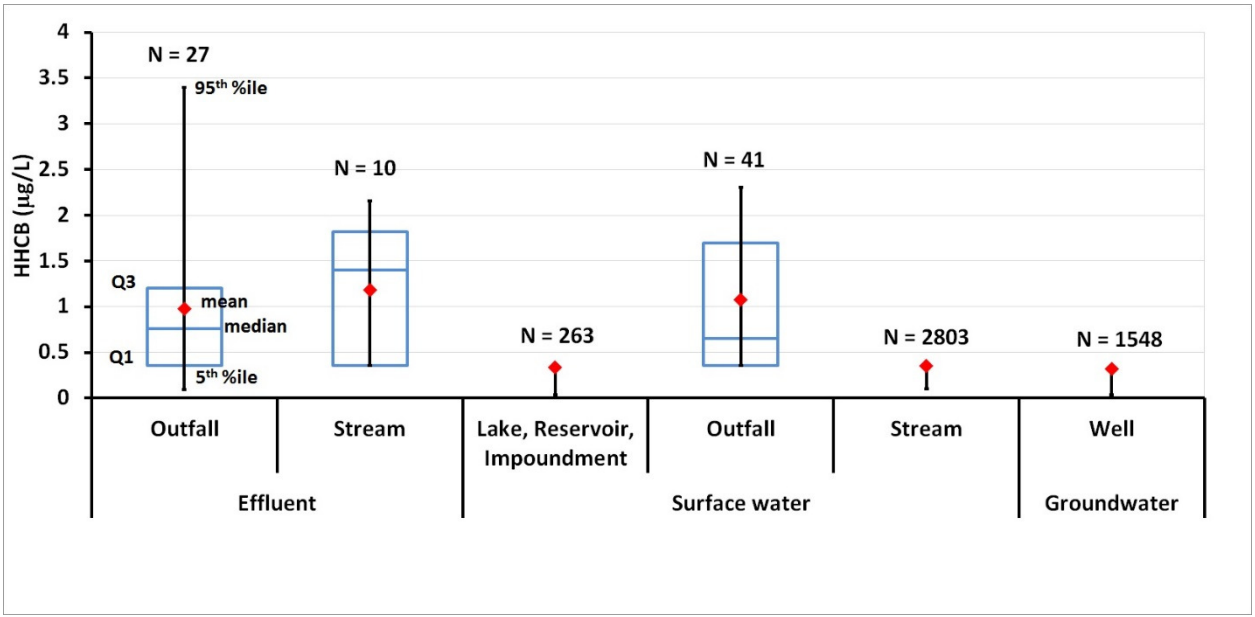


Figure Apx G-1. Monitoring Data Summary from USGS NWIS for HHCB Concentrations in Water, Filtered

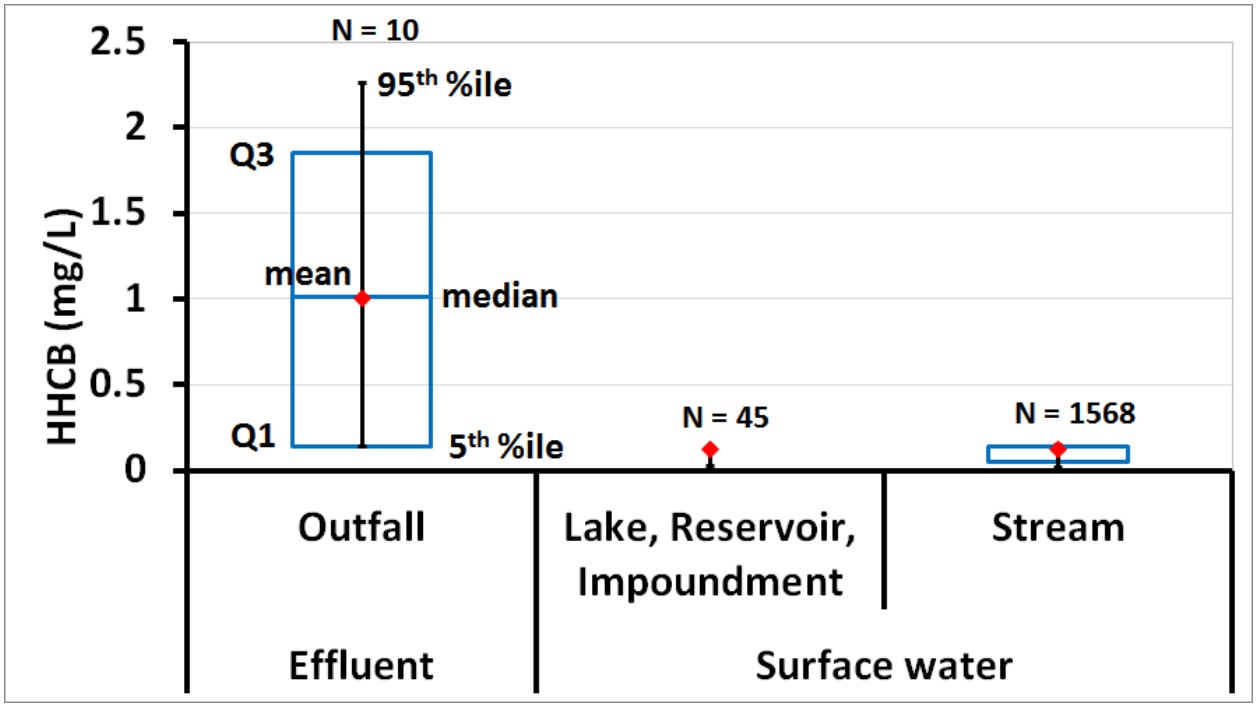


Figure Apx G-2. Monitoring Data Summary from USGS NWIS for HHCB Concentrations in Water, Unfiltered

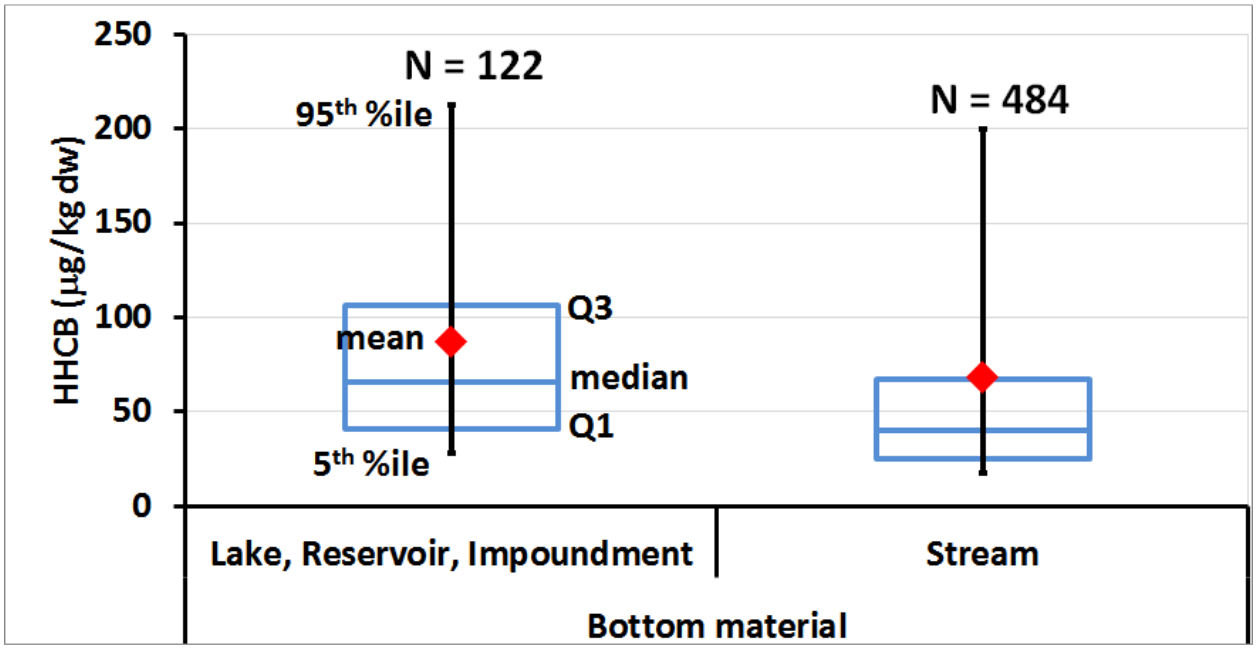


Figure Apx G-3. Monitoring Data Summary from USGS NWIS for HHCB Concentrations in Solids

The majority of the data available from the USGS NWIS data set for solids was from bottom material. Bottom material data were predominantly divided between two types of sites: lake/reservoir/impoundment and streams (Figure G-3). The 95th percentile concentration range at lake/reservoir/impoundment sites was 213 µg/kg, and the mean concentration was 87 µg/kg. The 95th percentile concentration at stream sites was 200 µg/kg and the mean concentration was 68 µg/kg. The range of data was similar for both site types, with the Q3 value for lake/reservoir/impoundment sites slightly higher than that observed for stream sites, though it is unclear what this may be attributed to.