Screening Assessment for the Challenge

1,4-Dioxane

Chemical Abstracts Service Registry Number
123-91-1

Environment Canada
Health Canada

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Synopsis

Pursuant to section 74 of the *Canadian Environmental Protection Act, 1999* (CEPA 1999), the Ministers of the Environment and of Health have conducted a screening assessment of 1,4-dioxane, Chemical Abstracts Service Registry Number 123-91-1. The substance 1,4-dioxane was identified in the categorization of the Domestic Substances List as a high priority for action under the Ministerial Challenge. 1,4-Dioxane was identified as a high priority as it was considered to pose greatest potential for exposure of individuals in Canada and is classified by other agencies on the basis of carcinogenicity. Although this substance met the ecological categorization criteria for persistence, it did not meet the criteria for bioaccumulation and inherent toxicity to aquatic organisms. The focus of this assessment of 1,4-dioxane relates principally to human health risks.

According to information reported under section 71 of CEPA 1999, between 10 000 and 100 000 kg of 1,4-dioxane were both imported into and manufactured in Canada in 2006. In addition, Canadian companies reported using between 10 000 and 100 000 kg in that year. In terms of environmental releases, between 10 000 and 100 000 kg of 1,4-dioxane were released into the environment in 2006, with the majority entering water and air. In Canada, 1,4-dioxane is used primarily as a solvent for pharmaceutical processing and research and development and as an analytical reagent for laboratory use. However, it is also found as an impurity in ethoxylated substances, which are used in numerous industries (manufacture of personal care products, detergents, food packaging, etc.).

Based on available information on concentrations in environmental media and results from a survey under section 71 of CEPA 1999, the general population is expected to be exposed to 1,4-dioxane from environmental media (ambient air, indoor air and drinking water), from food and during the use of consumer products (personal care and household products) containing this substance.

Based principally on the weight of evidence–based assessments of several international and other national agencies and available toxicological information, critical effects associated with exposure to 1,4-dioxane are tumorigenesis following oral and inhalation exposure, but not following dermal exposure; and other systemic effects, primarily liver and kidney damage, via all routes of exposure (i.e., oral, dermal and inhalation). The collective evidence indicates that 1,4-dioxane is not a mutagen and exhibits weak clastogenicity in some assays, but not others, at high exposure levels often associated with cytotoxicity. Consideration of the available information regarding genotoxicity, and conclusions of other agencies, indicate that 1,4-dioxane is not likely to be genotoxic. Accordingly, although the mode of induction of tumours is not fully elucidated, the tumours observed are not considered to have resulted from direct interaction with genetic material. Therefore a threshold approach is used to characterize risk to human health.

The margins between upper-bounding estimates of exposure from environmental media and use of consumer products, taking into consideration multiple product use scenarios and levels associated with effects in experimental animals are considered to be

adequately protective to account for uncertainties in the human health risk assessment for both cancer and non-cancer effects.

On the basis of the adequacy of the margins between conservative estimates of exposure to 1,4-dioxane and critical effect levels in experimental animals, it is concluded that 1,4-dioxane is not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

Based on available empirical data, 1,4-dioxane is expected to degrade only in air, but not in water, soil or sediment. It is not expected to bioaccumulate in organisms. The substance meets the persistence criteria but not the bioaccumulation criteria set out in the *Persistence and Bioaccumulation Regulations*. In addition, empirical aquatic toxicity data indicate that the substance poses a low hazard to aquatic organisms. Based on a comparison of a predicted no-effect concentration with an estimated reasonable worst-case environmental exposure concentration for Canadian surface water, it is concluded that 1,4-dioxane is not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends.

Based on the information available, it is concluded that 1,4-dioxane does not meet the criteria set out in section 64 of CEPA 1999.

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

Introduction

The *Canadian Environmental Protection Act, 1999* (CEPA 1999) (Canada 1999) requires the Minister of the Environment and the Minister of Health to conduct screening assessments of substances that have met the categorization criteria set out in the Act to determine whether these substances present or may present a risk to the environment or to human health.

Based on the information obtained through the categorization process, the Ministers identified a number of substances as high priorities for action. These include substances that

- met all of the ecological categorization criteria, including persistence (P), bioaccumulation potential (B) and inherent toxicity to aquatic organisms (iT), and were believed to be in commerce in Canada; and/or
- met the categorization criteria for greatest potential for exposure (GPE) or presented an intermediate potential for exposure (IPE) and had been identified as posing a high hazard to human health based on classifications by other national or international agencies for carcinogenicity, genotoxicity, developmental toxicity or reproductive toxicity.

The Ministers therefore published a notice of intent in the *Canada Gazette*, Part I, on December 9, 2006 (Canada 2006), which challenged industry and other interested stakeholders to submit, within specified timelines, specific information that may be used to inform risk assessment and to develop and benchmark best practices for the risk management and product stewardship of those substances identified as high priorities.

The substance 1,4-dioxane was identified as a high priority for assessment of human health risk because it was considered to present GPE and had been classified by other agencies on the basis of carcinogenicity. The Challenge for this substance was published in the *Canada Gazette* on August 30, 2008 (Canada 2008). A substance profile was released at the same time. The substance profile presented the technical information available prior to December 2005 that formed the basis for categorization of this substance. As a result of the Challenge, submissions of information were received.

Although 1,4-dioxane was determined to be a high priority for assessment with respect to human health and met the ecological categorization criteria for persistence, it did not meet the criteria for bioaccumulation potential or inherent toxicity to aquatic organisms. Therefore, this assessment focuses principally on information relevant to the evaluation of risks to human health.

Screening assessments focus on information critical to determining whether a substance meets the criteria set out in section 64 of CEPA 1999. Screening assessments examine scientific information and develop conclusions by incorporating a weight of evidence approach and precaution.

This final screening assessment includes consideration of information on substance properties, hazards, uses and exposure, including the additional information submitted under the Challenge. Data relevant to the screening assessment of this substance were identified in original literature, review and assessment documents and stakeholder research reports and from recent literature searches, up to April 2009 for both the exposure section and ecological section of the document and up to February 2009 for the health effects section. During the public comment period on the final screening assessment, a two-year inhalation study was identified and included in the toxicological dataset. Key studies were critically evaluated; modelling results may have been used to reach conclusions.

Evaluation of risk to human health involves consideration of data relevant to estimation of exposure (non-occupational) of the general population, as well as information on health hazards (based principally on the weight of evidence assessments of other agencies that were used for prioritization of the substance). Decisions for human health are based on the nature of the critical effect and/or margins between conservative effect levels and estimates of exposure, taking into account confidence in the completeness of the identified databases on both exposure and effects, within a screening context. The final screening assessment does not represent an exhaustive or critical review of all available data. Rather, it presents a summary of the critical information upon which the conclusion is based.

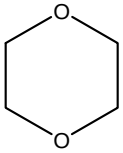
This final screening assessment was prepared by staff in the Existing Substances Programs at Environment Canada and Health Canada and incorporates input from other programs within these departments. The ecological and human health portions of this assessment have undergone external written peer review/consultation. Comments on the technical portions relevant to human health were received from scientific experts selected and directed by Toxicology Excellence for Risk Assessment (TERA), including Michael Jayjock (The Lifeline Group), Glenn Talaska (University of Cincinnati) and Chris Bevan (CJB Consulting LLC). Additionally, the draft of this screening assessment was subject to a 60-day public comment period. While external comments were taken into consideration, the final content and outcome of the screening risk assessment remain the responsibility of Health Canada and Environment Canada.

The critical information and considerations upon which the final assessment is based are summarized below.

Substance Identity

For the purposes of this document, this substance will be referred to as 1,4-dioxane. Its substance identity is summarized in Table 1.

Table 1. Substance identity for 1,4-dioxane

CAS RN	123-91-1
DSL name	1,4-Dioxane
NCI names	1,4-Diethylene dioxide (ECL, PICCS) Diethylene ether (PICCS) Diethylene oxide (PICCS) 1,4-Dioxane (English, French) (AICS, ASIA-PAC, DSL, EINECS, ENCS, NZIoC, PICCS, SWISS, TSCA) Dioxane (PICCS) Dioxane, *para*- (PICCS) *p*-Dioxane (PICCS)
Other names	Diethylene dioxide; 1,4-Diethyleneoxide; 1,4-Dioxacyclohexane; 1,4-Dioxan; Dioxan; *p*-Dioxan; Dioxane, 1,4-; Dioxano ioxyethylene ether; 1,4-Dioxin, tetrahydro-; NE 220; NSC 8728; UN 1165; UN 1165 (DOT)
Chemical group (DSL stream)	Discrete organics
Major chemical class or use	Heterocyclic organic
Major chemical subclass	Heterocyclic organic (oxygen)
Chemical formula	$C_4H_8O_2$
Chemical structure	
SMILES[1]	C1OCCOC1
Molecular mass	88.11 g/mol

Abbreviations: AICS, Australian Inventory of Chemical Substances; ASIA-PAC, Asia-Pacific Substances Lists; CAS RN, Chemical Abstracts Service Registry Number; DSL, Domestic Substances List; ECL, Korean Existing Chemicals List; EINECS, European Inventory of Existing Commercial Chemical Substances; ENCS, Japanese Existing and New Chemical Substances; NCI, National Chemical Inventories; NZIoC, New Zealand Inventory of Chemicals; PICCS, Philippine Inventory of Chemicals and Chemical Substances; SMILES, simplified molecular input line entry specification; SWISS, Swiss Giftliste 1 and Inventory of Notified New Substances; TSCA, *Toxic Substances Control Act* Chemical Substance Inventory.
Source: NCI 2006

[1] Simplified Molecular Input Line Entry Specification

Physical and Chemical Properties

Table 2 contains experimental physical and chemical properties of 1,4-dioxane that are relevant to its environmental fate.

Table 2. Experimental physical and chemical properties of 1,4-dioxane

Property	Type	Value[1]	Temperature (°C)	Reference
Melting point (°C)	Experimental	11.8		O'Neil et al. 2001
Boiling point (°C)	Experimental	101.5		PhysProp 2006
		101.1		O'Neil et al. 2001
Density (kg/m^3)	Experimental	1032.9 (1.0329 g/cm^3)	4	O'Neil et al. 2001
Vapour pressure (Pa)	Experimental	5080 (38.1 mmHg)	25	Daubert and Danner 1989
		4000 (30 mmHg)		Verschueren 1983
Henry's Law constant (Pa·m^3/mol)	Experimental	0.49 (4.80×10^{-6} atm·m^3/mol)	25	Park et al. 1987
Log K_{ow} (dimensionless)	Experimental	−0.27		Hansch et al. 1995
		−0.42		Verschueren 1983
Water solubility (mg/L)	Experimental	1 000 000 (totally miscible)	20	Riddick et al. 1986
Other solubilities (g/L)	Experimental	Miscible with most organic solvents		O'Neil et al. 2001
pK_a	Experimental	−2.92	25	Perrin 1965

Abbreviations: K_{ow}, octanol–water partition coefficient; pK_a, acid dissociation constant.
[1] Values in parentheses represent the original values reported by the authors.

Sources

No natural sources of 1,4-dioxane have been identified. However, in the case of food, there have been limited data suggesting that 1,4-dioxane is a natural constituent of the volatile fraction occurring at low concentrations in some food items (Chung et al. 1983; Hartung 1989). Although these studies point to its presence, it is not known whether this occurrence is due to natural production or contamination, as 1,4-dioxane is known to be an impurity in ethoxylated food additives and pesticides and may be found in environmental media (NICNAS 1998; 2009 personal communication from Pest

Management Regulatory Agency, Health Canada; unreferenced; 2009 personal communication from the Food Directorate, Health Canada; unreferenced).

Anthropogenic emissions of 1,4-dioxane may occur during its direct production or processing. Another main source of 1,4-dioxane in Canada is its unintentional formation as a by-product in ethoxylation reactions where ethylene oxide or ethylene glycol is condensed, during the development of ethoxylated polymers used in a variety of industrial and consumer applications (Figure 1) (Robinson and Ciurczak 1980; NICNAS 1998; Black et al. 2001). Despite vacuum stripping, trace amounts of 1,4-dioxane will remain (Robinson and Ciurczak 1980).

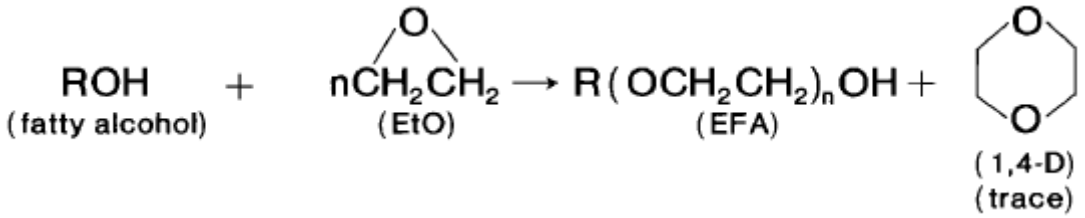


Figure 1. General mechanism for 1,4-dioxane formation through ethoxylation (Robinson and Ciurczak 1980). [EtO = ethylene oxide; EFA = ethoxylated fatty acid; 1,4-D = 1,4-dioxane]

Based on information submitted under section 71 of CEPA 1999, 10 000–100 000 kg of 1,4-dioxane were manufactured in Canada in 2006; in addition, between 10 000 and 100 000 kg were imported during the same year. Canadian companies also reported using between 10 000 and 100 000 kg of 1,4-dioxane in 2006 (Environment Canada 2008).

Uses

Historically, the use of 1,4-dioxane centred predominantly around its application as a stabilizer for 1,1,1-trichloroethane. This function has been phased out due to controls placed on 1,1,1-trichlorethane use under the Montreal Protocol (Canada 1998). Currently in Canada, 1,4-dioxane is used extensively as a solvent for pharmaceutical processing and research and development and as an analytical reagent for laboratory use (Environment Canada 2008). 1,4-Dioxane is used as a carrier solvent in the manufacture of pharmaceuticals, veterinary drugs and natural health products. In pharmaceuticals, it is classified as a Class 2 residual solvent with a concentration limit of 380 mg/kg if the maximum daily exposure of the product containing 1,4-dioxane is not greater than 10 g; for products that are administered in doses greater than 10 g/day, the permitted daily exposure should not exceed 3.8 mg/day. An identical concentration limit and permitted daily exposure has been set for residual 1,4-dioxane in both natural health products and veterinary medicinal products (2009 personal communication from Natural Health Products Directorate, Health Canada; unreferenced; 2009 personal communication from Therapeutic Products Directorate, Health Canada; unreferenced). 1,4-Dioxane is also a component of industrial agents that are used as corrosion inhibitors, antioxidants and heavy equipment degreasers (Environment Canada 2008).

As mentioned previously, residual 1,4-dioxane is formed during the production of ethoxylated substances used in a variety of applications, such as cosmetics, detergents, food packaging, agricultural products and industrial processes (EURAR 2002). In Canada, ethoxylated substances containing 1,4-dioxane as a by-product are produced and used as surfactants, emulsifiers, wetting agents and foaming agents in various industries (Environment Canada 2008). 1,4-Dioxane is also found as a formulant impurity in 168 pest control products that have both food and non-food uses (2009 personal communication from Pest Management Regulatory Agency, Health Canada; unreferenced). Furthermore, 1,4-dioxane is found as an impurity in solvents used in the formulation of ingredients and processing aids that are employed in the manufacture of food packaging materials such as paper and shrink films, although exposure to 1,4-dioxane from these materials was deduced to be minimal (2009 personal communication from Food Directorate, Health Canada; unreferenced). 1,4-Dioxane may also be present as an impurity in the food additives polysorbate 80, polysorbate 65, polysorbate 60, and polyethylene glycol, at a maximum residue limit of 10 mg/kg in accordance with the specifications for these chemicals in the *Food Chemicals Codex* (FCC, 6th Ed., 2008) (2009 personal communication from Food Directorate, Health Canada; unreferenced). The aforementioned food additives in which 1,4-dioxane may be present as an impurity, may be present only in those foods for which provision exists for the use of these additives in accordance with the *Food and Drug Regulations* (2009 personal communication from Food Directorate, Health Canada; unreferenced).

Currently, 1,4-dioxane is present on Health Canada's Cosmetic Ingredient "Hotlist", prohibiting its intentional use as an ingredient in cosmetics; however, it may be present as a manufacturing impurity (Health Canada 2007).

Releases to the Environment

Information reported under section 71 of CEPA 1999 indicated that 10 000–100 000 kg of 1,4-dioxane were released into the environment in 2006. The majority of releases were to water and air, with a small fraction released to land (Environment Canada 2008). Amounts of 1,4-dioxane released to the air and water ranged between 10 000 and 100 000 kg each in 2006. Environmental releases reported under the National Pollutant Release Inventory (NPRI) indicated that 13 800 kg of 1,4-dioxane were released to air and 6 500 kg were released to water in 2006; however, releases to land were not reported (NPRI 2006).

In 2006, the US Toxics Release Inventory (TRI) reported a total of 56 tonnes released to air, 22 tonnes released to water and 64 tonnes released by underground injection (TRI 2006). The high release quantities are likely due to greater use of 1,4-dioxane in the United States compared with Canada.

In addition to environmental releases, information submitted under section 71 of CEPA 1999 show that 100–1000 kg of 1,4-dioxane were transferred to hazardous waste

facilities. Less than 100 kg were transferred to non-hazardous waste facilities (Environment Canada 2008).

Environmental Fate

Based on its physical and chemical properties (Table 2), 1,4-dioxane is characterized by high water solubility (1 000 000 mg/L), high vapour pressure (5080 Pa), low log K_{ow} (−0.42 to −0.27) and moderate Henry's Law constant (0.49 Pa·m^3/mol). The results of Level III fugacity modelling (Table 3) suggest that significant mass fractions of the substance will reside in the compartment of release and that partitioning of 1,4-dioxane to the water compartment is potentially significant, whereas partitioning to sediments is expected to be negligible.

Table 3. Results of the Level III fugacity modelling (EQC 2003) for 1,4-dioxane

Substance released to:	Fraction of substance partitioning to each medium (%)			
	Air	Water	Soil	Sediment
Air (100%)	44.8	34.4	20.8	0.1
Water (100%)	0.1	99.6	0.1	0.2
Soil (100%)	0.4	40.4	59.2	0.1

Persistence and Bioaccumulation Potential

Environmental Persistence

Table 4a presents empirical biodegradation data that show 0 % biodegradation over 14 days in a ready biodegradation test for 1,4-dioxane (MITI 1992). This study reported a negative value for biodegradation, indicating that the substance might be lost during the test. Therefore the reported data is considered of low confidence. However EURAR (2002), based on an earlier review by BUA (1991), reported no biodegradation of 1,4-dioxane under both standardized (Organisation for Economic Co-operation and Development) and non-standardized test conditions. This result is supported by contaminated site remediation experts who report limited biodegradation of 1,4-dioxane (Steffan 2007).

The substance does not have functional groups expected to undergo hydrolysis, nor direct photolysis in aquatic media. It is also resistant to biodegradation in water under ambient conditions (CCME 2008).

Also presented in Table 4a is an empirically derived atmospheric half-life of ≤ 1 day by photooxidation with hydroxyl (OH) radicals, which is considered to be the primary loss mechanism in air (Atkinson 1989).

Table 4a. Empirical data for degradation of 1,4-dioxane

Medium	Fate process	Degradation value	Degradation endpoint / units	Reference
Water	Biodegradation	0	14-day ready biodegradation / %	MITI 1992
Air	Atmospheric oxidation	0.981	Half-life / days	Atkinson 1989

As experimental data on the degradation of 1,4-dioxane are limited, a quantitative structure–activity relationship (QSAR)-based weight of evidence approach (Environment Canada 2007) was also applied using the biodegradation models shown in Table 4b. Given the ecological importance of the water compartment, the fact that most of the available models apply to water and the fact that 1,4-dioxane is expected to be released to this compartment, biodegradation was examined primarily in water.

Table 4b. Modelled data for degradation of 1,4-dioxane

Fate process	Model and model basis	Model result and prediction	Extrapolated half-life (days)
Air			
Atmospheric oxidation	AOPWIN 2000	$t_{½}$ = 0.38 day	<2
Water			
Biodegradation (aerobic)	BIOWIN 2000 Submodel 3: Expert Survey (ultimate biodegradation)	2.99[1] "Biodegrades fast"	<182
Biodegradation (aerobic)	BIOWIN 2000 Submodel 4: Expert Survey (primary biodegradation)	3.70[1] "Biodegrades fast"	<182
Biodegradation (aerobic)	BIOWIN 2000 Submodel 5: MITI linear probability	0.53[2] "Biodegrades fast"	<182
Biodegradation (aerobic)	BIOWIN 2000 Submodel 6: MITI non-linear probability	0.67[2] "Biodegrades very fast"	<182
Biodegradation (aerobic)	TOPKAT 2004 Probability	0.0[2] "Biodegrades very slowly"	>182
Biodegradation (aerobic)	CATABOL ©2004–2008 % BOD	% BOD = 9.5 "Biodegrades very slowly"	>182

Abbreviations: BOD, biological oxygen demand; MITI, Ministry of International Trade & Industry, Japan; $t_{½}$, half-life.
[1] Output is a numerical score.
[2] Output is a probability score.

In air, a predicted atmospheric oxidation half-life of 0.38 day (see Table 4b) demonstrates that this chemical is likely to be rapidly oxidized. The compound may also be subject to photolysis and react with other photo-oxidative species in the atmosphere, such as ozone

(EURAR 2002). With an empirically derived half-life of 0.98 day and an estimated half-life of 0.38 day via reactions with hydroxyl radicals, 1,4-dioxane is considered not to be persistent in air.

In water, there are different conclusions indicated by the modelled data. Based on the modelled timeframe and probability of biodegradation values from BIOWIN (see Table 4b), the substance's degradation half-life is <182 days. However, the modelled results from TOPKAT and CATABOL indicate that the half-life in water is > 182 days (Table 4b). When concluding about persistence, greatest weight was given to the empirical data (Table 4a; EU RAR 2002; Staffen 2007; CCEM 2008) indicating that the half life of 1,4-dioxane is > 182 day, which is supported by the predictions from TOPKAT and CATABOL. Thus 1,4-dioxane is considered persistent in water.

To extrapolate a half-life in water to half-lives in soil and sediment, Boethling's factors $t_{½\ water}$:$t_{½\ soil}$:$t_{½\ sediment}$ = 1:1:4 (Boethling et al. 1995) can be used. Therefore, based on the above empirically derived half-life in water of ≥182 days and an extrapolation factor of 1, the half-life in soil is expected to be ≥182 days. For sediment, the half-life is expected to be 4 times higher (i.e., ≥728 days). It may thus be concluded that 1,4-dioxane is persistent in soil and sediment.

Based on the empirical data and model predictions, it is concluded that 1,4-dioxane meets the persistence criteria for water, soil and sediment (half-lives in soil and water ≥182 days and half-life in sediment ≥365 days), but does not meet the persistence criterion for air (half-life ≥2 days), as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

In addition, the long-range transport potential (LRTP) of 1,4-dioxane from its point of release to air is estimated to be low. The TaPL3 (2000) model was used to estimate characteristic travel distance (defined as the maximum distance travelled by 63% of the substance) of 95 km for 1,4-dioxane. Beyer et al. (2000) proposed characteristic travel distances of >2000 km as representing high LRTP, 700–2000 km as moderate LRTP and <700 km as low LRTP. Based on the model's estimate, 1,4-dioxane is expected to have a low LRTP and to remain primarily in the areas close to its emission sources.

Potential for Bioaccumulation

Experimental log K_{ow} values for 1,4-dioxane suggest that this chemical does not have the potential to bioaccumulate in organisms (see Table 2 above). An experimental bioconcentration factor (BCF) value in fish is reported to be in the range of 0.2 – 0.7 L/kg wet weight (MITI 1992).

Since few experimental data on bioaccumulation were available, a QSAR-based weight of evidence approach was also applied using the bioaccumulation factor (BAF) and BCF models. Model outcomes are presented in Table 5.

Table 5. Modelled data for bioaccumulation of 1,4-dioxane

Test organism	Endpoint	Value (L/kg wet weight)	Reference
Fish	BAF	0.96	Arnot and Gobas 2003 (Gobas BAF middle trophic level)
Fish	BCF	0.97	Arnot and Gobas 2003 (Gobas BCF middle trophic level)
Fish	BCF	10.4	OASIS Forecast 2005
Fish	BCF	3.2	BCFWIN 2000

The modified Gobas BAF middle trophic level model for fish predicted a BAF of 0.96 L/kg, indicating that 1,4-dioxane does not have the potential to bioconcentrate and biomagnify in the environment. The results of BCF model calculations also provide weight of evidence to support the low bioconcentration potential of this substance (Table 5).

Based on the available empirical and modelled data, it is concluded that 1,4-dioxane does not meet the bioaccumulation criteria (BAF or BCF ≥5000) as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

Potential to Cause Ecological Harm

The approach taken in this assessment was to examine the available scientific information and develop conclusions based on a weight of evidence approach and using precaution as required under CEPA 1999. Lines of evidence considered include results from a conservative risk quotient calculation as well as information on the persistence, bioaccumulation, toxicity, sources and fate of the substance.

There is experimental and modelled evidence that 1,4-dioxane does not cause harm to aquatic organisms at low concentrations (see Tables 6a and 6b).

Table 6a. Empirical data for aquatic toxicity of 1,4-dioxane

Test organism	Type of test	Endpoint	Value (mg/L)	Reference
Fish (*Pimephales promelas*)	Acute (96 h)	LC_{50}	9850	Geiger et al. 1990
Daphnia magna	Acute (24 h)	EC_{50}	4700	Bringmann and Kühn 1977
Ceriododaphnia dubia	Acute (48 h)	EC_{50}	163[1]	TSCATS 1989
Ceriodaphnia dubia	Chronic (7 days)	NOEC	625	Springborn Laboratories Inc. 1989
Scud (*Gammarus pseudolimnaeus*)	Acute (96 h)	LC_{50}	2274	Brooke 1987
Alga (*Microcystis aeruginosa*)	Chronic (8 days)	EC_{50}	575[2]	Bringmann and Kühn 1976

Test organism	Type of test	Endpoint	Value (mg/L)	Reference
Fathead minnow *(Pimephales promelas)*	Chronic (32 days)	MATC	145	BUA 1991
Medaka *(Oryzias latipes)*	Chronic (28 days)	LOEC	6 933	Johnson et al. 1993
Ceriodaphnia dubia	Chronic (7 days)	NOEC LOEC	635 1 250	Dow 1995

Abbreviations: EC_{50}, concentration of a substance that is estimated to cause some toxic sublethal effect on 50% of the test organisms; LC_{50}, concentration of a substance that is estimated to be lethal to 50% of the test organisms; LOEC, the lowest concentration of a substance causing an adverse effect on the test organisms; MATC, the highest concentration of a substance not exert an adverse effect on the rest organisms; NOEC, the highest concentration in a toxicity test not causing a statistically significant effect in comparison with the controls.

[1] Anomalous (questionable) result. See explanation in text.

[2] Critical toxicity value (CTV).

Table 6b. Modelled data for aquatic toxicity of 1,4-dioxane

Test organism	Type of test	Endpoint	Value (mg/L)	Reference
Fish	Acute (96 h)	LC_{50}	5 300	TOPKAT 2004
			6 750	ECOSAR 2004
			14 371	ASTER 1999
			1 397	AIES 2003–2005
Fish	Acute (14 days)	LC_{50}	6 580	ECOSAR 2004
Daphnia	Acute (48 h)	EC_{50}	2 721	ECOSAR 2004
			35.4	TOPKAT 2004
Alga	Acute (96 h)	EC_{50}	482	ECOSAR 2004

Abbreviations: EC_{50}, concentration of a substance that is estimated to cause some toxic sublethal effect on 50% of the test organisms; LC_{50}, concentration of a substance that is estimated to be lethal to 50% of the test organisms.

A more comprehensive overview of the aquatic toxicity data is available from the International Uniform Chemical Information Database (ECB 2000) and the European Union risk assessment (EURAR 2002); these data are in the same range as those presented in Table 6a. The AQUIRE database (ECOTOX 2006) reports median acute aquatic toxicity values ranging from 2274 to 12 326 mg/L.

The lowest reported experimental aquatic toxicity value was 163 mg/L, based on a 48-hour median effective concentration (EC_{50}) for *Ceriodaphnia dubia* (TSCATS 1989). This value may be questioned, however, considering the other reported levels of toxicity and also because the original study could not be obtained and evaluated for quality. When *Ceriodaphnia dubia* was tested in long term static studies, the chronic no-observed-effect concentration (NOEC) reported were much higher (~630 mg/L). (Springborn Laboratories 1989, DOW 1995). Therefore, as in the European Union assessment (EURAR 2002), this result was not used as an effect measure for risk characterization. The second lowest toxicity value (575 mg/L from Table 6a) was thus selected as the critical toxicity value (CTV), as an indicator of potential for ecological effects to sensitive species.

Table 6b contains predicted ecotoxicity values that were considered to be reliable and were used in the QSAR weight of evidence approach for aquatic toxicity (Environment Canada 2007).

The range of empirical toxicity values, as well as the aquatic toxicity predictions obtained from the various QSAR models considered, indicates that the substance is not highly hazardous to aquatic organisms (i.e., acute LC_{50} or EC_{50} >> 1.0 mg/L).

A conservative predicted no-effect concentration (PNEC) is derived from the CTV identified from the empirical data. An assessment factor of 10 has been applied to the empirical chronic effect value of 575 mg/L—to account for inter- and intraspecies variations in sensitivity and to extrapolate from a laboratory-based endpoint to a chronic effect value in the field. This results in a PNEC of 57.5 mg/L.

Based on the section 71 survey, the majority of releases of 1,4-dioxane are to water and air. If released to water, the substance is expected to remain mainly within water (see Table 3). This substance has not been detected in surface water in eastern Canada (CCME 2008). For the purpose of the ecological assessment, two conservative aquatic exposure scenarios were developed to estimate the release into the aquatic environment from industrial operations and consumer uses, with the corresponding resulting aquatic concentrations.

The scenario to estimate the release from industrial operations is based on the largest total amount manufactured at a single facility, assuming that a conservative fraction is released to water (5%), no removal in a sewage treatment plant and discharge to a relatively small receiving water body. The predicted environmental concentration (PEC) is estimated to be 0.39 mg/L for this conservative scenario using Environment Canada's Industrial Generic Exposure Tool – Aquatic. The resulting risk quotient (PEC/PNEC) is 0.007; indicating that the release of 1,4-dioxane from industrial operations is unlikely to cause ecological harm in Canada (Environment Canada 2009a).

As 1,4-dioxane can be found in consumer products, Mega Flush – Environment Canada's spreadsheet model – was used for estimating down-the-drain releases from consumer products. Mega Flush provides an estimate of the maximum PEC as 0.004 mg/L and the resulting risk quotient (PEC/PNEC) is 0.0008; indicating that the release of 1,4-dioxane from consumer uses is unlikely to cause ecological harm in Canada (Environment Canada 2009b).

Although no suitable ecological effect studies were found for this compound in media other than water, given the low aquatic toxicity of this substance and its short half-life in air, effects from exposure to 1,4-dioxane in air (the other main receiving medium) are unlikely.

It should be noted that this conclusion was reached despite the conservative assumptions that were made in response to uncertainties encountered in the assessment. Some

uncertainty relates to the fact that 1,4-dioxane has not been detected in the aquatic environment in Canada, and to address this an exposure model was used to predict a worst case concentration of this substance in water. There is also some uncertainty associated with the choice of critical toxicity value and the associated PNEC used in the risk quotient calculation. This was addressed by dividing the empirical chronic critical toxicity value by an assessment factor of 10.

Potential to Cause Harm to Human Health

Exposure Assessment

Environmental Media

The upper-bounding estimates of daily intake of 1,4-dioxane from ambient air, indoor air, soil and drinking water were calculated. These are summarized for all age groups in Appendix 1. The total upper-bounding estimates range from 0.19 µg/kg body weight (kg-bw) per day for breast-fed infants <6 months old to 1.26 µg/kg-bw per day for formula-fed infants <6 months old. Breast-fed infants and formula-fed infants were exposed to 1,4-dioxane mainly through air and drinking water (experimental detection of 1,4-dioxane has not been reported in breast milk or baby formula). Drinking water represented the predominant contribution to the total estimated daily intake for all age groups, followed by indoor air.

Several studies have been conducted in the United States and Canada that show detectable levels of 1,4-dioxane in ambient air (maximum 0.646 µg/m^3) and indoor air (maximum 0.85 µg/m^3), respectively from all collected data(Harkov 1984; Brown et al. 1994; Fellin and Otson 1997; Singhvi 1999). Canadian data were used in estimating the intake from both air sources: 0.646 µg/m^3 (ambient air) and 0.685 µg/m^3 (indoor air) (Fellin and Otson 1997). Soil studies are limited, with only a few studies from Japan and Canada comprising the data set (Golder Associates 1987; Government of Japan 2004). 1,4-Dioxane was not detected in soils in Canada, with the detection limit (100 µg/kg) used for estimating exposure (Golder Associates 1987).

Several recent studies from Japan show the presence of detectable levels of 1,4-dioxane (maximum 16 µg/L) in surface water (Abe 1999; Kawata et al. 2003; Simazaki et al. 2006). However, 1,4-dioxane has not been detected in surface water in eastern Canada (CCME 2008). Canadian drinking water data were used for intake estimation. Otson (1987) showed that 1,4-dioxane was not detected in 42 raw and 42 treated samples of water from a municipal water treatment plant in the Great Lakes region. In this instance, the detection limit (10 µg/L) was used for estimating exposure.

Although no studies pertaining to 1,4-dioxane in food in Canada were found, data from Japan suggested that 1,4-dioxane was present in several food groups (Nishimura et al. 2004). However, after consultation with the Food Directorate of Health Canada, reanalysis of the published concentrations indicated that they were all below the detection

limit of 2 µg/kg cited in the study (2009 personal communication from Food Directorate, Health Canada; unreferenced). A subsequent study by the same research group, sampling meals as prepared and consumed, detected 1,4-dioxane in 1 out of 27 samples (Nishimura et al. 2005). Taking into account that dietary intakes of certain foods differ between Canada and Japan, potential differences in 1,4-dioxane content of water between Canada and Japan (used in food processing and cooking), differing food additive provisions between these two countries at the time these Japanese studies were conducted, and the limitations in those two studies, the food data from these studies were not included in estimates of daily intake from environmental media (Appendix 1).

One study examined the likelihood of the presence of 1,4-dioxane in breast milk (using a physiologically-based pharmacokinetic model (PBPK)), particularly in regards to women with potential occupational exposure to this substance (Fisher et al. 1997). In this study an experimental milk/blood partition coefficient of 0.89 was observed, indicating that more 1,4-dioxane would be expected to be present in blood than milk. A Center for Disease Control and Prevention (CDC) study examined blood samples from more than 2000 individuals from the NHANES US biomonitoring study (2008-2008) and 1,4-dioxane was not detected in any of the blood samples taken (Blount et al. 2008). Therefore, based on this PBPK model, negligible amounts would be expected in milk. This is supported by the physical-chemical properties of 1,4-dioxane. Typically, lipophilic chemicals are preferentially taken up in breast milk due to their higher fat content. However, 1,4-dioxane is very hydrophilic (log KOW -0.27 to -0.42). Accordingly, exposure to 1,4-dioxane from breast milk is not expected.

1,4-Dioxane may be present as an impurity in the food additives polysorbate 80, polysorbate 65, polysorbate 60, and polyethylene glycol. Therefore, trace amounts of 1,4-dioxane could potentially be carried over into foods that are permitted to contain these additives or into processing aid formulations that might contain these additives (2009 personal communication from Food Directorate, Health Canada; unreferenced). Estimated intakes of 1,4-dioxane resulting from its potential presence as an impurity in permitted food additives (polysorbates 80, 65 and 60 and polyethylene glycol) were calculated based on the maximum residue limit (not more than 10 mg/kg) of 1,4-dioxane in these additives according to the Food Chemicals Codex (FCC, 6th Ed., 2008) and the maximum level of use for those foods in which these additives are permitted according to Tables IV and VIII of the *Food and Drug Regulations* (see Appendix 2). These estimates are considered very conservative, in that food additives (polysorbates and polyethylene glycol) and 1,4-dioxane residues were assumed to be present at their maximum permitted levels in foods (*Food and Drug Regulations*) and residual level in the food additives (Food Chemicals Codex), respectively (Canada [1978]) (2009 personal communication from Food Directorate, Health Canada; unreferenced). Furthermore, residual levels of 1,4-dioxane in foods are expected to be very low due to its volatility and, as such, even lower in foods processed and cooked at high temperatures (2009 personal communication from Food Directorate, Health Canada; unreferenced). Further details on the conservative nature of these estimates are provided in Appendix 2. It should be noted, however, that the estimated intakes of 1,4-dioxane from food additive use are lower than the total intake from environment media, with the highest estimate of 0.335 µg/kg-bw per day for

children aged 1–4 years and if added to environmental media intake, do not impact the lowest or highest estimated intake from the environmental media.

Consumer Products

1,4-Dioxane has been found in various personal care products in varying concentrations (see Table 7a).

Table 7a. Concentration of 1,4-dioxane in various personal care products

Product type	1,4-Dioxane concentration (mg/kg)	Reference
Hair shampoo	ND–45.5 11.5–41.1 5.5–41 0.05–33 1.2–10	Scalia 1991 Fuh et al. 2005 Makino et al. 2006 Tanabe and Kawata 2008 OCA 2009
Hair shampoo (baby)	1.6–10 0.69–1.1	OCA 2009 CSC 2009
Hair conditioner	0.05–0.14	Tanabe and Kawata 2008
Liquid hand soap	0.2–18 0.05–7.2 ND–7.5 <5	OCA 2009 Tanabe and Kawata 2008 Scalia 1991 Makino et al. 2006
Body wash	0.9–23 15.7 0.05–1.5	OCA 2009 Scalia 1991 Tanabe and Kawata 2008
Body wash (baby)	0.63–5.3 0.6–4.6	OCA 2009 CSC 2009
Skin Moisturizer	ND–75	VCCEP 2007
Lotion (baby)	11	Scalia et al. 1992
Multiple Products Survey (1981–1997)	2–279 (1981) 6–34 (1997)	Black et al. 2001

* Note - some products may not be available in Canada

Maximum concentrations reported in products (see Table 7a) were used to derive exposure estimates from use of personal care products (see Table 7b). In the absence of Canadian specific concentrations in products, non-Canadian data was used to estimate exposures. There is no indication that the ethoxylation process (source of 1,4-dioxane residual levels in products) is different in countries from which the data was chosen. A number of the products in these surveys may also be available on the Canadian market. Exposure was modelled using ConsExpo 4.1 software (ConsExpo 2006). Health Canada's Cosmetic Notification System (CNS) was used to determine if product types other than those summarized in Table 7b contained ingredients which could contain 1,4-dioxane as a by-product. Hair dye was identified as a product type containing ethoxylated alkyl sulphates. As measured concentrations of 1,4-dioxane in hair dye were not available, measurements of 1,4-dioxane in cosmetic ingredients from a recent FDA survey were used to estimate exposure from use of that product type. Black et al 2001

reported concentrations of 45-1102 ppm in ethoxylated alkyl sulphates and multiplying this maximum by-product concentration by the composition value reported in CNS for hair dye gives an estimation of 1,4-dioxane concentration.

1,4-Dioxane has been shown to have low skin absorption (3.4%: skin lotion vehicle) in monkey forearms (Marzulli et al. 1981), which is thought to be a function of evaporation (NICNAS 1998; EURAR 2002; VCCEP 2007). Also, when added to lotion-like material, 1,4-dioxane has been known to evaporate rapidly (90% in 15 minutes) in unoccluded conditions (Bronaugh 1982). Therefore when characterizing exposure it was assumed that 90% of 1,4-dioxane is available for inhalation, whereas 10% is available for dermal exposure. A distinction between rinse-off and stay-on products was also made; thus, various skin retention factors were used in the analysis (see Appendix 3).

Table 7b. Estimated adult female[1] exposure to 1,4-dioxane from personal care products

Personal care product	Concentration (%) (reference)	Mean event concentration (mg/m^3)	Inhalation (mg/kg-bw per day)	Dermal (mg/kg-bw per day)
Hair conditioner	0.000 014 (Tanabe and Kawata 2008)	1.7×10^{-5}	6.9×10^{-9}	7.9×10^{-8}
Hair shampoo	0.004 55 (Scalia et al. 1992)	0.0078	8.0×10^{-6}	9.1×10^{-5}
Skin moisturizer (body cream)	0.0075 (VCCEP 2007)	0.0023	0.0012	0.0017
Body wash	0.0023 (OCA 2009)	0.0017	2.2×10^{-6}	2.5×10^{-6}
Total		**0.0118**	**0.0012**	**0.0018**
Total absorbed		–	**0.0012[2]**	**6.1×10^{-5} [3]**
Hair Dye (mg/kg - bw)	0.033 - 0.11 (CNS 2009)	1.39 – 4.64	0.0200 – 0.0667[2]	0.0002 - 0.0005[3]

[1] Body weight assumed to be 70.9 kg.
[2] 100 % inhalation absorption
[3] 3.4% dermal absorption (Marzulli et al. 1981).

Exposure from personal care products was estimated for women, men and children (body weight 7.5 kg). However, after analysis, it was apparent that because of factors such as frequency of use, women were the most exposed group. In the interests of clarity, only the exposure estimates for women and children are presented in this report.

Using finished product concentrations of 1,4-dioxane in children's products (see table 7a) exposure was estimated for 0- to 6-month-old children (Table 7c). Aggregate exposure for three products was estimated to be 4.2×10^{-5} mg/kg/day.

Table 7c. Estimated Children's[1] exposure to 1,4-dioxane in personal care products

Personal care product	Concentration (%)	Mean event concentration (mg/m^3)	Inhalation (mg/kg-bw per day)	Dermal (mg/kg-bw per day)
Skin moisturizer	0.0011 % (Scalia 1992)	5.8×10^{-5}	2.7×10^{-5}	4.1×10^{-4}
Hair Shampoo	0.001 % (CSC 2009)	1.0×10^{-4}	1.4×10^{-7}	2.9×10^{-6}
Body Wash	0.00053 % (OCA 2009)	1.5×10^{-4}	4.9×10^{-7}	9.9×10^{-7}
Total		**3.1×10^{-4}**	**2.8×10^{-5}**	**4.1×10^{-4}**
Total absorbed		**---**	**2.8×10^{-5} [2]**	**1.4×10^{-5} [3]**

[1] Body weight of children 0–6 months of age assumed to be 7.5 kg.
[2] 100 % inhalation absorption
[3] 3.4% dermal absorption (Marzulli et al. 1981).

1,4-Dioxane has also been detected in dishwashing liquid, detergents and other household products (Fuh et al. 2005; Tanabe and Kawata 2008). Due to the potential for daily exposure to hand dishwashing detergent, exposure was estimated for a 70.9-kg adult. Using CNS consumer product data (maximum concentration: 0.033%; CNS 2009), exposure was analysed using ConsExpo 4.1 software and found to be minimal (inhalation: 2.2×10^{-4} mg/kg-bw per day; dermal: 6.5×10^{-5} mg/kg-bw per day).

Confidence in the exposure estimates for environmental media and consumer products is high. All soil, air and water data are Canadian and considered adequate to permit quantification of exposure to 1,4-dioxane. The confidence in estimates of intake from food sources is low, as Canadian data on the levels of 1,4-dioxane in foods were unavailable and therefore not included in the intake estimate. However, as discussed previously, the estimated exposures from certain food additives are considered to be an overestimate, with actual levels in food expected to the very low (2009 personal communication from Food Directorate, Health Canada; unreferenced). Although there is some uncertainty associated with concentration of 1,4-dioxane in some product types available in Canada, due to the limited information on the presence or concentrations of the substance in consumer products available in Canada, the estimates of exposure from the use of products containing 1,4-dioxane were based on conservative assumptions; therefore, there is confidence that the exposure estimates are conservative upper-bounding estimates.

Health Effects Assessment

The available health effects information for 1,4-dioxane is summarized in Appendix 4.

On the basis of investigations in experimental animals, 1,4-dioxane has been classified by the International Agency for Research on Cancer in Group 2B – "possibly carcinogenic to humans" (IARC 1999), by the European Commission as a Category 3 Carcinogen – "cause concern for humans owing to possible carcinogenic effects but in respect of which the available information is not adequate for making a satisfactory assessment" (European Commission 2000; ESIS 2009), by the US Environmental Protection Agency

as Group B2 – "probable human carcinogen" (US EPA 1990) and by the US National Toxicology Program as "reasonably anticipated to be a human carcinogen" (NTP 2005). These classifications were based on observed hepatocellular adenomas and carcinomas in mice, tumours of the nasal cavity, liver subcutaneous tissues, mammary gland and peritoneal mesotheliomas in rats and tumours of the liver and gallbladder in guinea pigs orally exposed to 1,4-dioxane (US EPA 1990; IARC 1999).

When 1,4-dioxane was administered in drinking water, a significantly increased incidence of hepatocellular adenomas and carcinomas was consistently observed in both sexes of various strains of rats and mice and in male guinea pigs (female guinea pigs were not tested) in chronic carcinogenicity bioassays, whereas nasal cavity tumours were observed only in rats of both sexes exposed to 0.5% (240 –398 mg/kg-bw per day) 1,4-dioxane and above dose levels via drinking water for 2 years. Significantly increased incidences of liver tumours in both mice and rats were observed in animals administered 0.05–1% (77–1070 mg/kg-bw per day 1,4-dioxane and above dose levels in drinking water for 2 years (Kociba et al. 1974; National Cancer Institute 1978; Yamazaki et al. 1994; JBRC 1998c), whereas significantly increased incidences of peritoneal mesotheliomas in male rats and mammary gland tumours in female rats were observed in rats exposed to 0.5% (398–514 mg/kg-bw per day) 1,4-dioxane in drinking water for 2 years (Yamazaki et al. 1994; JBRC 1998c). At a dose of 0.01% (9.6–19 mg/kg-bw per day) 1,4-dioxane in drinking water for 2 years, no tumour formation or any exposure-related effects were observed in exposed rats (Kociba et al. 1974). Additionally, liver and/or nasal tumours were observed in rats exposed to 0.75–1.8% (approximately equivalent to 770–1850 mg/kg-bw per day) 1,4-dioxane in drinking water for relatively shorter periods—that is, 13 months (Hoch-Ligeti et al. 1970; Argus et al. 1973) or 63 weeks (Argus et al. 1965); no statistical analyses were provided. In a carcinogenicity bioassay conducted in male guinea pigs, 22 animals were exposed to varied doses of 0.5–2% (1200–4800 mg/kg-bw per day) 1,4-dioxane via drinking water for 23 months; three animals developed hepatomas, one developed kidney adenoma and two developed gallbladder carcinomas (Hoch-Ligeti and Argus 1970).

An older chronic inhalation study did not provide any evidence of carcinogenicity or other adverse effects in rats of both sexes exposed to 1,4-dioxane at 400 mg/m^3 for 2 years (Torkelson et al. 1974). However, during the public comment period for this assessment, a two-year inhalation study in male F344 rats was published (Kasai et al., 2009). Dose-dependent and significant increases in incidences of nasal squamous cell carcinomas and hepatocellular adenomas were observed primarily in the 1250 ppm (4500 mg/m^3) exposed rats and a significantly increased incidence of peritoneal mesotheliomas was observed at 250 ppm (900 mg/m^3) and above. The incidences of renal cell carcinomas, fibroadenomas in the mammary gland and adenomas in the Zymbal gland also increased with dose, but were not statistically significant. Preneoplastic lesions in the nasal cavity, including significantly increased incidences of nuclear enlargement, atrophy and respiratory metaplasia, were observed at 50 ppm (180 mg/m^3) and above.

Dermal application of 1,4-dioxane alone did not induce exposure-related skin or other types of tumours in mice (King et al. 1973; Perone et al. 1976). However, in the dermal

tumour promoting assay, when mice were administered dimethylbenzanthracene prior to 1,4-dioxane application, skin tumours as well as tumours in lung, kidney, spleen and liver were observed (King et al. 1973). 1,4-Dioxane-induced lung tumours were also observed in male but not female mice when the substance was administered by intraperitoneal injection (Maronpot et al. 1986; Stoner et al. 1986).

In humans, although several epidemiological investigations did not provide evidence of 1,4-dioxane-induced tumour formation in occupational environments (Thiess et al. 1976, 1981; NIOSH 1977; Buffler et al. 1978; Kramer et al. 1978), a large comparative mortality study based on data in the Danish Cancer Registry showed that the standardized proportionate incidence ratios for liver tumours in male workers exposed to 1,4-dioxane and other chemicals in occupational settings were significantly higher than the expected rates, and an increase in liver cancer incidence of 50% was identified in one workplace where only 1,4-dioxane was used (Hansen 1993; Hansen et al. 1993).

The genotoxicity of 1,4-dioxane has been assessed in a range of *in vitro* and *in vivo* assays. All tests for mutagenicity were negative, including those in a variety of bacterial, yeast and mammalian cells, dominant lethal mutation assay in mice and recessive lethal mutation assay in *Drosophila* (BASF 1977, 1979a, b, c, 1991; Stott et al. 1981; Haworth et al. 1983; Nestmann et al. 1984; Yoon et al. 1985; Khudoley et al. 1987; Kwan et al. 1990; McGregor et al. 1991; Morita and Hayashi 1998). In addition, all mutagenicity tests with one of the metabolites of 1,4-dioxane, 1,4-dioxan-2-one, were also negative (Goldsworthy et al. 1991; EURAR 2002). For clastogenicity investigations, including chromosomal aberration, micronuclei induction and sister chromatid exchange, the *in vitro* results were principally negative, with one weak positive result, and the results of the *in vivo* micronuclei induction assays in both CD-1 and C57BL/6 mice were mixed (Galloway et al. 1987; McFee et al. 1994; Mirkova 1994; Tinwell and Ashby 1994; Morita and Hayashi 1998; Roy et al. 2005). Significantly increased chromosomal aberrations were observed in 11 workers exposed to alkylene oxides, including 1,4-dioxane, for more than 20 years in the occupational environment. However, workers were also exposed to known mutagens, such as ethylene oxide and propylene oxide (Thiess et al. 1981). Significantly increased aneuploidy was observed in *Drosophila* (Muñoz and Barnett 2002), but not in yeast (Zimmermann et al. 1985), exposed to 1,4-dioxane. 1,4-Dioxane was positive in some assays, but not in others, for effects on deoxyribonucleic acid (DNA), such as DNA strand breaks, enhanced DNA repair processes or cell proliferation (measured as replicative DNA synthesis) and cell transformation. However, these were typically significant only at higher doses or following prolonged exposure and often in the presence of cytotoxicity (Stott et al. 1981; Heidelberger et al. 1983; Sina et al. 1983; Sheu et al. 1988; Sai et al. 1989; Kitchin and Brown 1990; Goldsworthy et al. 1991; Hellmer and Bolcsfoldi 1992; Uno et al. 1994; Miyagawa et al. 1999; Sasaki et al. 2000). The European Commission has concluded that the total weight of evidence indicates that 1,4-dioxane is a non-genotoxic compound (EURAR 2002). The Agency for Toxic Substances and Disease Registry (ATSDR) has indicated that "collectively, the information available suggests that 1,4-dioxane is a non-genotoxic compound, or at best, a weakly genotoxic compound" (ATSDR 2007). The Government of Australia has

concluded that "overall, the weight of evidence from *in vitro* and *in vivo* tests indicates that 1,4-dioxane is unlikely to be a mutagen" (NICNAS 1998).

Although potential modes of action of carcinogenicity of 1,4-dioxane have been examined by other agencies, these have not been fully elucidated, as data on dose–response and temporal progression with which to characterize and/or identify the key events in the processes of 1,4-dioxane-induced tumour formation and thus support any of the hypothesized carcinogenic modes of action are insufficient, inconsistent or not available. However, the collective evidence indicates that 1,4-dioxane is not genotoxic. Accordingly although the mode of induction of tumours in not fully elucidated, the tumours observed are not considered to have resulted from direct interaction with genetic material (NICNAS 1998; EURAR 2002; ATSDR 2007; VCCEP 2007). It has been suggested that the nasal tumour formation in rats exposed to 1,4-dioxane via drinking water may be due to the inspiration of water into the nasal cavity during drinking from sipper bottles, resulting in high doses applied directly to nasal tissue and subsequent cytotoxicity (Reitz et al. 1990; Goldsworthy et al. 1991; Stickney et al. 2003; Sweeney et al. 2008), which is related to the rat nasal anatomy and experimental methodology. However, in a recent two-year study in rats (Kasai et al., 2009), preneoplastic and neoplastic lesions in rat nasal cavity were observed via inhalation exposure, therefore the human relevance of nasal tumour induced by 1,4-dioxane can not be excluded. Additionally, 1,4-dioxane is not a complete carcinogen, as it exhibited only tumour promotion activity, not tumour initiation activity (Pereira et al. 1982; Bull et al. 1986; Stoner et al. 1986; Lundberg et al. 1987).

Several investigations indicated that 1,4-dioxane was absorbed rapidly and completely following oral and inhalation exposure in both humans and rats, whereas much less absorption (overall 3.4%) occurred via dermal application in monkeys or through human skin *in vitro* (Young et al. 1976, 1977, 1978; Marzulli et al. 1981; Bronaugh 1982). Following intraperitoneal injection, 1,4-dioxane was distributed rapidly to, and has been detected in, all the tissues examined (Woo et al. 1977; Mikheev et al. 1990). In both rats and humans, the primary metabolite of 1,4-dioxane is β-hydroxyethoxyacetic acid (HEAA), which has been detected in the urine (Young et al. 1976, 1977, 1978; Braun and Young 1977). 1,4-Dioxan-2-one has also been detected in rat urine, depending on the pH and temperature of the analytical conditions. At high pH, HEAA will be detected, and at low pH, HEAA will be converted to 1,4-dioxan-2-one (Braun and Young 1977; Woo et al. 1977, 1978). At lower doses, rapid elimination (half-life of approximately 1 hour) of 1,4-dioxane has been observed in humans exposed to 50 ppm (180 mg/m^3) 1,4-dioxane by inhalation. A similar half-life was observed in rats given low oral or intravenous doses of 1,4-dioxane (less than 10 mg/kg-bw). However, non-linear toxicokinetics of 1,4-dioxane has been demonstrated in rats when the oxidation of 1,4-dioxane to its primary metabolites, HEAA and 1,4-dioxan-2-one, was saturated at doses greater than 50 ppm (180 mg/m^3) in air or 10 mg/kg-bw orally and the accumulation of 1,4-dioxane was observed (Young et al. 1976, 1977, 1978; Stickney et al. 2003). In addition, enhanced metabolism in the liver did not increase the liver toxicity of 1,4-dioxane measured by hepatic glutathione content or serum alanine transaminase activity, indicating that the metabolites did not play a major role in the liver toxicity of 1,4-dioxane (Nannelli et al.

2005). Collectively, the evidence suggests that a threshold for toxicity and carcinogenicity may exist at doses at which 1,4-dioxane metabolism becomes saturated (VCCEP 2007).

Several physiologically based pharmacokinetic/pharmacodynamic models have been developed to predict the absorption, distribution, metabolism and elimination of 1,4-dioxane in rodents and humans (Young et al. 1977, 1978; Leung and Paustenbach 1990; Reitz et al. 1990; Fisher et al. 1997; Sweeney et al. 2008). The Fisher et al. (1997) model indicates that 1,4-dioxane may also be excreted into human milk, as discussed in the exposure assessment section. However, excretion of 1,4-dioxane into breast milk has not been assessed or predicted by other PBPK models. As described in the exposure assessment section, exposure to 1,4-dioxane from breast milk is not expected.

The primary non-neoplastic effects induced by 1,4-dioxane were liver and kidney damage, typically associated with high-dose exposure and cytotoxicity, which were observed by all routes of exposure that have been tested and in both humans and experimental animals. Other adverse effects induced by 1,4-dioxane include effects on central nervous, respiratory and blood systems and stomach. The oral lowest-observed-adverse-effect level (LOAEL) for non-neoplastic effects, based on liver lesions, was observed at 16 mg/kg-bw per day in rats exposed to 1,4-dioxane via drinking water for 2 years (Yamazaki et al. 1994; JBRC 1998c). In addition, the lowest oral LO(A)ELs identified from subchronic, short-term and acute studies are 130 mg/kg-bw per day (Kano et al. 2008), 400 mg/kg-bw per day (the only dose tested; Nannelli et al. 2005) and 1050 mg/kg-bw per day (Kanada et al. 1994), respectively (Appendix 4). A practical no-observed-adverse-effect level (NOAEL) for chronic effects and the lowest level in available studies in which tumours were not observed in rats is 0.01% in drinking water (equivalent to 9.6 and 19 mg/kg-bw per day for males and females, respectively), based on the aforementioned 2-year study (Kociba et al. 1974).

1,4-Dioxane vapour–induced non-neoplastic effects, such as histopathological changes in nasal cavity and liver, were observed in rats at 100 ppm (360 mg/m^3) and above in a 13-week study (Kasai et al. 2008) and at 50 ppm (180 mg/m^3) and above in a two-year study (Kasai et al., 2009), although in another inhalation study, no treatment-related effects were observed in rats exposed to 111 ppm (400 mg/m^3) 1,4-dioxane vapour for 2 years (Torkelson et al. 1974). In addition, glutathione peroxidase activation in both brain and ovaries was observed in female rats exposed to 1,4-dioxane vapour at 100 mg/m^3 for a month (Burmistrov et al. 2001). Irritation of the eye and respiratory system was observed following acute inhalation exposure in both humans and experimental animals. Central nervous system depression as well as liver and kidney lesions were observed in animals exposed to high concentrations of 1,4-dioxane vapour. In terms of acute exposure, a no-observed-adverse-effect concentration (NOAEC) of 20 ppm (72 mg/m^3) was identified in humans (Ernstgård et al. 2006), whereas mild effects, such as serum enzyme activity changes, were observed in rats exposed to 1000 ppm (3600 mg/m^3, the lowest acute lowest-observed-effect concentration [LOEC]) 1,4-dioxane vapour for 4 hours (Drew et al. 1978).

In regards to dermal exposure, although significant lesions were not observed in mice topically exposed to 0.2 mL 1,4-dioxane 3 times/week for 60 weeks or to 0.05 mL 1,4-dioxane 3 times/week for 78 weeks (King et al. 1973; Perone et al. 1976), these data are considered unsuitable to derive a NOAEL, as the dermal application in these studies might not have occurred under occlusion, and therefore the dermal applied dose levels might be greatly reduced by the fast evaporation of 1,4-dioxane.

1,4-Dioxane-induced reproductive and developmental effects were observed only at higher doses, based on limited available data. Effects on male reproductive organs, such as reduced mineralization of testis, were observed in mice exposed to 1,4-dioxane at 2000 mg/L via drinking water (250 mg/kg-bw per day) for 2 years, but not in rats exposed to 1,4-dioxane at doses up to 1015 mg/kg-bw per day orally or at 400 mg/m^3 in air for 2 years (Kociba et al. 1974; Torkelson et al. 1974; Yamazaki et al. 1994; JBRC 1998c). Significantly reduced average live fetus weights and delayed fetal ossification in sternebrae were observed in rats exposed to 1035 mg 1,4-dioxane/kg-bw per day during the organogenesis period (Giavini et al. 1985). In addition, immunological effects such as suppressed T-cell responses and augmented B-cell responses were observed in murine and human lymphocytes *in vitro* and slightly lower lymphocyte response to mitogens was observed in mice exposed to 1,4-dioxane by intraperitoneal injection (1670 mg/kg-bw per day for 7 days) (Thurman et al. 1978).

The confidence in the toxicity database is high, as data on acute toxicity, carcinogenicity, repeated-dose toxicity, genotoxicity and developmental toxicity are available, although data on reproductive and dermal effects are limited, and there is uncertainty in the mechanism of tumorigenesis.

Characterization of Risk to Human Health

Based principally on the weight of evidence–based assessments of several international agencies (International Agency for Research on Cancer, European Union, US Environmental Protection Agency and US National Toxicology Program) and available information, critical effects associated with exposure to 1,4-dioxane are tumorigenesis following oral and inhalation exposure, but not dermal exposure, and other systemic effects, primarily liver and kidney damage, via all routes of exposure (i.e., oral, dermal and inhalation). The collective evidence indicates that 1,4-dioxane is not a mutagen and exhibits weak clastogenicity in some assays, but not others, at high exposure levels often associated with cytotoxicity. Consideration of the available information regarding genotoxicity, and conclusions of other agencies, indicate that 1,4-dioxane is not likely to be genotoxic. Non-linear toxicokinetics of 1,4-dioxane have been observed in rats, and the existence of a threshold dose for toxicity and carcinogenicity was suggested, at which 1,4-dioxane metabolism becomes saturated. Although the available epidemiological data did not provide adequate evidence regarding 1,4-dioxane carcinogenicity in humans, the human relevance of 1,4-dioxane carcinogenicity, especially liver tumour induction, cannot be discounted, as similar metabolic pathways for 1,4-dioxane in humans and experimental animals have been found. Although the mode of induction of tumours is not fully elucidated, the tumours observed are not considered to have resulted from direct

interaction with genetic material. Therefore a threshold approach is used to characterize risk to human health. Margins of exposure are derived between the level at which no tumours or any adverse effects were observed in experimental studies, as well as the lowest exposure level associated with adverse effects induced by 1,4-dioxane, and conservative estimates of the general population exposure to 1,4-dioxane (Appendix 5).

The principal routes of exposure to 1,4-dioxane for the general population are expected to be from the general environment (i.e., ambient air, indoor air and drinking water), from food and during the use of consumer products containing the substance. Comparison of the upper-bounding estimates of total daily intake from the general environment to both the level at which no tumours or any adverse effects were observed and the LOAEL for non-cancer effects result in margins of exposure ranging from 7000 to 84 000. Furthermore, pertaining to inhalation exposure, a comparison of the highest concentration of 1,4-dioxane in indoor air measured in the greater Toronto area over a period of 3 months in 1996 with the lowest-observed-adverse-effect concentration (LOAEC) for non-cancer effects in rats, or eye irritation in humans, results in margins of exposure of 5 orders of magnitude. These margins are considered adequately protective to account for the uncertainties in the dataset for the human health risk assessment for both cancer and non-cancer effects.

In addition, a range of personal care products containing 1,4-dioxane as a manufacturing by-product have been identified. Inhalation and dermal contact through use of these products would contribute to exposure to 1,4-dioxane. For those products that may be used collectively by consumers on a daily basis, the aggregate upper-bounding estimates of daily exposure to 1,4-dioxane in various personal care products (i.e., shampoo, conditioner, shower gel and skin moisturizer) by the dermal and inhalation routes combined are compared to both the level at which no tumours or any adverse effects were observed and the LOAEL for non-cancer effects. This approach is considered conservative, as the available dermal data are inadequate to derive a dermal LOAEL, and the low dermal absorption rate has been taken into consideration in the dermal exposure estimates. The resulting margins of exposure are approximately 8000–13300. Use of a single personal care product or dishwashing liquid or children products (Table 7c) generates lower exposure levels and would result in even larger margins of exposure. For the less frequently used products, such as hair dye, the upper-bounding dermal and inhalation exposure estimates are compared with the lowest oral acute LOEL, based on the effects on the central nervous system in rats, resulting in a margin of exposure of 15 630– 52 000. Therefore, the margins of exposure for intake from combined inhalation and dermal exposure during use of consumer products are considered adequately protective.

In terms of inhalation exposure from products, a comparison of the conservative sum of upper-bounding estimates of inhalation exposure from personal care products used in close succession on a daily basis, with the LOAEC, based on nasal effects in rats, results in a margin of exposure of 15250. The upper-bounding estimate from use of hair dye compared with the acute LOEC, based on altered serum enzyme activity in rats, and with eye irritation in humans results in margins of exposure are approximately 40 (irritation in

humans) to 780 (rats). As these estimates for hair dye are for a very conservative upper bound (i.e., 100% ethoxylated alkyl sulphate in final product, which is certainly an overestimate), the margins of exposure during use of consumer products are considered adequately protective.

Uncertainties in Evaluation of Risk to Human Health

There is a high rating of confidence pertaining to data on concentrations of 1,4-dioxane in environmental media in Canada. All soil, air and water data are Canadian and considered adequate to permit quantification of exposure to 1,4-dioxane. There is some uncertainty in estimating intake from food sources, as Canadian data on the levels of 1,4-dioxane in foods were unavailable and as a result were not included in the overall intake estimate. The exposures from certain food additives are considered to be an overestimate, with actual levels in food expected to be very low (2009 personal communication from Food Directorate, Health Canada; unreferenced). Although there is some uncertainty due to the limited information on the presence or concentrations of the substance in consumer products available in Canada, the estimates of exposure from the use of products containing 1,4-dioxane were based on conservative assumptions.

There is uncertainty around the extent to which 1,4-dioxane, present as a residual solvent in health products (phamaceuticals, veterinary drugs and natural health products), may contribute to general population exposure (see Uses section).

There is uncertainty regarding the mechanism of 1,4-dioxane-induced tumorigenesis, as data on dose–response and temporal progression with which to characterize and/or identify the key events in the processes of 1,4-dioxane-induced tumour formation of different types and thus support any of the hypothesized carcinogenic modes of action are insufficient, inconsistent or not available. However, the collective evidence indicates that this substance may not directly interact with genetic material, and therefore a margin of exposure approach is used within the screening assessment to characterize risk. As well, there is uncertainty with respect to the human relevance of 1,4-dioxane carcinogenicity, as epidemiological investigations have not provided conclusive evidence; however, the potential relevancy was not discounted in the assessment. As to the non-neoplastic effects, there are some uncertainties concerning the critical exposure levels associated with these effects via dermal exposure to 1,4-dioxane, as the dermal dataset is limited. As well, data on reproductive toxicity associated with 1,4-dioxane exposure are limited, as there is no multigenerational study available.

Conclusion

Based on the information presented in this final screening assessment, it is concluded that 1,4-dioxane is not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends.

On the basis of the adequacy of the margins between conservative estimates of exposure to 1,4-dioxane and critical effect levels, it is concluded that 1,4-dioxane is not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

It is concluded that 1,4-dioxane does not meet any of the criteria in section 64 of CEPA 1999. While 1,4-dioxane does meet the criteria for persistence, it does not meet the criteria for bioaccumulation potential as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

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

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Appendix 1: Upper-bounding estimates of daily intake of 1,4-dioxane by the general population in Canada

Route of exposure	Estimated intake (µg/kg-bw per day) of 1,4-dioxane by various age groups							
	0–6 months[1]			0.5–4 years[4]	5–11 years[5]	12–19 years[6]	20–59 years[7]	60+ years[8]
	Breast fed[2]	Formula fed[3]	Not formula fed					
Ambient air[9]	0.2			0.05	0.04	0.02	0.02	0.02
Indoor air[10]	0.17			0.36	0.28	0.16	0.14	0.12
Drinking water[11]	0.00	1.07	0.40	0.45	0.35	0.20	0.21	0.22
Food and beverages[12]	–	–	–	–	–	–	–	–
Soil[13]	0.0			0.0	0.0	0.0	0.0	0.0
Total intake	**0.19**	**1.26**	**0.59**	**0.86**	**0.67**	**0.38**	**0.37**	**0.36**

[1] Assumed to weigh 7.5 kg, to breathe 2.1 m^3 of air per day, to drink 0.8 L of water per day (formula fed) or 0.3 L/day (not formula fed) and to ingest 30 mg of soil per day (Health Canada 1998).

[2] No data on detectable levels of 1,4-dioxane in breast milk were located.

[3] For exclusively formula-fed infants, intake from water is that amount required to reconstitute formula. No data on 1,4-dioxane levels in formula were found; however, the detection limit for 1,4-dioxane in drinking water was used in this model (Otson 1987). Approximately 50% of non-formula-fed infants are introduced to solid foods by 4 months of age and 90% by 6 months of age (NHW 1990).

[4] Assumed to weigh 15.5 kg, to breathe 9.3 m^3 of air per day, to drink 0.7 L of water per day and to ingest 100 mg of soil per day (Health Canada 1998).

[5] Assumed to weigh 31.0 kg, to breathe 14.5 m^3 of air per day, to drink 1.1 L of water per day and to ingest 65 mg of soil per day (Health Canada 1998).

[6] Assumed to weigh 59.4 kg, to breathe 15.8 m^3 of air per day, to drink 1.2 L of water per day and to ingest 30 mg of soil per day (Health Canada 1998).

[7] Assumed to weigh 70.9 kg, to breathe 16.2 m^3 of air per day, to drink 1.5 L of water per day and to ingest 30 mg of soil per day (Health Canada 1998).

[8] Assumed to weigh 72.0 kg, to breathe 14.3 m^3 of air per day, to drink 1.6 L of water per day and to ingest 30 mg of soil per day (Health Canada 1998).

[9] 1,4-Dioxane has been measured in ambient air in Canada and the United States near point sources. The highest measured concentration not near a point source, 0.646 µg/m^3 in the greater Toronto area over a period of 3 months, was used for the level of 1,4-dioxane in ambient air (Fellin and Otson 1997). Canadians are assumed to spend 3 h/day outside (Health Canada 1998).

[10] 1,4-Dioxane has been measured in homes in both Canada and the United States. It was detected in 44 homes in the greater Toronto area during 3 months in 1996. The maximum concentration of 1,4-dioxane was 0.685 µg/m^3 (Fellin and Otson 1997). Canadians are assumed to spend 21 h indoors each day (Health Canada 1998).

[11] 1,4-Dioxane was detected in drinking water sources in 10 different communities in the Great Lakes region of Canada. The concentration from drinking water for 1,4-dioxane taken from 42 raw and 42 exposed municipal drinking water sources was below the detection limit of 10 µg/L which was used as the most conservative approach (Otson 1987).

[12] No data were identified on the concentration of 1,4-dioxane in foods in Canada. Studies from Japan had reported levels of 1,4-dioxane in food; however, inclusion of those data was considered unnecessary, in consideration of differences between Canada and Japan in dietary intakes, food additive provisions at the time, and potential concentrations of 1,4-dioxane content in water, as well as limitations in these studies. 1,4-Dioxane may potentially be present in food items as a result of its presence as an impurity in certain food additives (see Appendix 2), but was not included in the intake calculation here.

[13] Trace concentrations of 1,4-dioxane in soil have been measured in Canada and Japan. Concentrations from 30 samples in southeastern Ontario were below the detection limit, therefore the detection limit of 100 µg/kg was used for the calculation of soil intake (Golder Associates 1987).

Appendix 2: Estimated intake of 1,4-dioxane from food additive uses[1,2]

Age group (years)	Sex	Mean all-person estimated intake of 1,4-dioxane (µg/kg-bw per day)[3,4,5,6]
1–4	–	0.335
5–11	–	0.287
12–18	Males	0.162
12–18	Females	0.125
31–50	Males	0.105
31–50	Females	0.088

[1] 2009 personal communication from Food Directorate, Health Canada; unreferenced.

[2] Estimated intakes are for 1,4-dioxane as an impurity in permitted food additives (polysorbates 80, 65 and 60 and polyethylene glycol) for which provision currently exists in the Canadian *Food and Drug Regulations* (Canada [1978]), based on the maximum residue limit (not more than 10 mg/kg) of 1,4-dioxane in these additives according to the Food Chemicals Codex (FCC, 6th Ed., 2008) and food consumption data from the Continuing Survey of Food Intakes by Individuals (CSFII, 1994–1996, 1998). CSFII data were utilized since food categories corresponding to the foods in which the polysorbates and polyethylene glycol are permitted as food additives according to the *Food and Drug Regulations* were readily available, allowing estimation of potential exposures to 1,4-dioxane from its potential carry-over into foods as a result of its presence as an impurity in these food additives. These data are considered relevant for estimating intakes from the Canadian diet.

[3] Industry was not approached in order to determine actual use levels of the polysorbates or polyethylene glycol in foods for which provision currently exists in the *Food and Drug Regulations*. Consequently, all estimates are based on the maximum permitted level of use for the polysorbates and polyethylene glycol in each of the food items and/or food categories in which they are permitted according to the *Food and Drug Regulations*.

[4] The *Food and Drug Regulations* list possible alternative food additives (stabilizers) for use in some of those foods for which provision exists for the use of polysorbates. In addition, food additives, such as the polysorbates and polyethylene glycol will not always be used in those foods for which they are permitted (i.e., technological need and manufacturers' discretion).

[5] Polysorbates could potentially be used in antifoam formulations that might serve food additive or processing aid applications. However, the contribution to the dietary intake of 1,4-dioxane from these uses is considered significantly lower, and nearly negligible, relative to that of the conservative estimated intake that was made for polysorbate uses listed in Division 16 of the *Food and Drug Regulations*.

[6] The intakes of 1,4-dioxane estimated from food do not account for losses due to the volatility and the lower boiling point of 1,4-dioxane. For instance, some processed foods are exposed to higher temperatures during food processing; as a result, residual 1,4-dioxane levels would be expected to be lower in these commodities.

Appendix 3: ConsExpo sample calculation scenarios

Personal care product scenarios for adult women

Consumer product scenario	Assumptions	Estimated exposure
Hair conditioner	**Inhalation (constant rate)** - Concentration: 0.000014% (Tanabe and Kawata 2008) - Used ConsExpo model version 4.1, exposure to vapour, constant release mode[1] - Frequency: 104 times/year[1] - Body weight: 70.9 kg, adult - Limited air concentration to vapour pressure of pure substance - Exposure duration: 4 min[1] - Room volume: 10 m^3 [1] - Ventilation rate: 2/h[1] - Applied amount: 14 g (90% partitioning, model input 12.6 g)[1] - Release duration: 20 min[2] - Inhalation rate: 36.7 m^3/day[1] - Uptake fraction: 1	Mean event concentration = 1.68×10^{-5} mg/m^3 Chronic dose = 6.90×10^{-9} mg/kg-bw per day
	Dermal (direct dermal contact with product: instant application) - Concentration: 0.000014% (Tanabe and Kawata 2008) - Exposed area: 1.44×10^3 cm^2 [1] - Applied amount: 14 g (10% partitioning, model input 1.4 g)[1] - Uptake fraction: 1 - Skin retention factor: 0.1	Chronic dose = 7.87×10^{-8} mg/kg bw per day
Hair shampoo	**Inhalation (constant rate)** - Concentration: 0.00455% (Scalia 1991) - Used ConsExpo model version 4.1, exposure to vapour, constant release mode[1] - Frequency: 260 times/year[1] - Body weight: 70.9 kg, adult - Limited air concentration to vapour pressure of pure substance - Exposure duration: 4 min[1] - Room volume: 10 m^3 [1] - Ventilation rate: 2/h[1] - Applied amount: 20 g (90% partitioning, model input 18 g)[1] - Release duration: 20 min[2] - Inhalation rate: 36.7 m^3/day[1] - Uptake fraction: 1	Mean event concentration = 0.00782 mg/m^3 Chronic dose = 8.01×10^{-6} mg/kg-bw per day
	Dermal (direct dermal contact with product: instant application) - Concentration: 0.00455% (Scalia 1991) - Exposed area: 1.44×10^3 cm^2 [1] - Applied amount: 20 g (10% partitioning, model input 2 g)[1] - Uptake fraction: 1 - Skin retention factor: 0.1	Chronic dose = 9.14×10^{-5} mg/kg bw per day

Consumer product scenario	**Assumptions**	**Estimated exposure**
Skin moisturizer (body cream)	**Inhalation (constant rate)** - Concentration: 0.0075 % (VCCEP 2007) - Used ConsExpo model version 4.1, exposure to vapour, constant release mode[1] - Frequency: 730 times/year[1] - Body weight: 70.9 kg, adult - Limited air concentration to vapour pressure of pure substance - Exposure duration: 12 h[1] - Room volume: 20 m^3 [1] - Ventilation rate: 1/h[1] - Applied amount: 8 g (90% partitioning, model input 7.2 g)[1] - Release duration: 20 min[2] - Inhalation rate: 36.7 m^3/day[1] - Uptake fraction: 1	Mean event concentration = 2.25×10^{-3} mg/m^3 Chronic dose = 1.16×10^{-3} mg/kg-bw per day
	Dermal (direct dermal contact with product: instant application) - Concentration: 0.0075 % (VCCEP 2007) - Exposed area: 1.63×10^4 cm^2 [1] - Applied amount: 8 g (10% partitioning, model input 0.8 g)[1] - Uptake fraction: 1 - Skin retention factor: 1	Chronic dose = 1.69×10^{-3} mg/kg-bw per day
Shower gel	**Inhalation (constant rate)** - Concentration: 0.0023 % (OCA 2009) - Used ConsExpo model version 4.1, exposure to vapour, constant release mode[1] - Frequency: 329 times/year[1] - Body weight: 70.9 kg, adult - Limited air concentration to vapour pressure of pure substance - Exposure duration: 4 min[1] - Room volume: 10 m^3 [1] - Ventilation rate: 2/h[1] - Applied amount: 8.7 g (90% partitioning, model input 7.83 g)[1] - Release duration: 20 min[2] - Inhalation rate: 36.7 m^3/day[1] - Uptake fraction: 1	Mean event concentration = 1.72×10^{-3} mg/m^3 Chronic dose = 2.23×10^{-6} mg/kg-bw per day
	Dermal (direct dermal contact with product: instant application) - Concentration: 0.0023 % (OCA 2009) - Exposed area: 1.75×10^4 cm^2 [1] Applied amount: 8.7 g (10% partitioning, model input 0.87 g)[1] - Uptake fraction: 1 - Skin retention factor: 0.01	Chronic dose = 2.54×10^{-6} mg/kg bw per day

Consumer product scenario	Assumptions	Estimated exposure
Hair dye	**Inhalation (constant rate)** - Ethoxylated alkyl sulphate composition in Hair Dye: 30 – 100 % (CNS 2009) - Maximum ethoxylated alkyl sulphate concentration: 1100 ppm (Black 2001) - Concentration Range: 0.033 - 0.11 % - Used ConsExpo model version 4.1, exposure to vapour, constant release mode[1] - Frequency: 10 times/year[1] - Body weight: 70.9 kg, adult - Limited air concentration to vapour pressure of pure substance - Exposure duration: 40 min[1] - Room volume: 10 m^3 [1] - Ventilation rate: 2/h[1] - Applied amount: 100 g (90% partitioning, model input 90 g)[1] - Release duration: 20 min[2] - Inhalation rate 36.7 m^3/day[1] - Uptake fraction: 1	Mean event concentration = 1.39 – 4.64 mg/m^3 Acute dose = 2.00×10^{-2} – 6.67×10^{-2} mg/kg-bw
	Dermal (direct dermal contact with product: instant application) - Concentration Range: 0.033 - 0.11 % (CNS 2009) - Exposed area: 580 cm^2 [1] - Applied amount: 100 g (10% partitioning, model input 10 g)[1] - Uptake fraction: 1 - Skin retention factor: 0.1	Acute dose = 4.65×10^{-3} – 1.55×10^{-2} mg/kg-bw

Household product scenarios

Consumer product scenarios	Assumptions	Estimated exposure
Dishwashing liquid	**Inhalation (exposure to vapour: evaporation)** - Weight fraction: 0.033% (CNS 2009) - Used ConsExpo model version 4.1, exposure to vapour, constant release mode[1] - Frequency: 426 times/year[1] - Body weight: 70.9 kg, adult - Limited air concentration to vapour pressure of pure substance - Exposure duration: 60 min[1] - Room volume: 15 m^3 [1] - Ventilation rate: 2.5/h[1] - Applied amount: 1.5×10^4 g (Applied amount takes into account the weight of the water in a diluted dishwashing solution in a sink of specified dimensions) [1] - Application duration: 16 min[1] - Molecular weight matrix: 18 g/mol[1] - Mass transfer rate: 4.14×10^3 [1] - Inhalation rate: 36.7 m^3/day[1] - Uptake fraction: 1	Mean event concentration = 8.89×10^{-3} mg/m^3 Chronic dose = 2.24×10^{-4} mg/kg-bw per day
	Dermal (direct dermal contact with product: instant application) - Weight fraction: 0.033% (CNS 2009) - Exposed area: 860 cm^2 [1] - Applied amount: 8.6 g[1] - Uptake fraction: 1	Chronic dose = 6.51×10^{-5} mg/kg-bw per day

[1] RIVM (2006)
[2] Based on Bronaugh (1982)

Appendix 4: Summary of effects information for 1,4-dioxane

Endpoint	Lowest effect levels[1]/Results
Laboratory animals and *in vitro*	
Acute toxicity	**Lowest oral LD_{50}** (rabbits, cats) = 2000 mg/kg-bw (NICNAS 1998). [additional studies: Argus et al. 1973; Pawar and Mungikar 1978; NICNAS 1998; EURAR 2002; ATSDR 2007] **Lowest oral LOEL** (rats) = 1050 mg/kg-bw, based on subtle effects on central nervous system by perturbation of certain neurotransmitters in male rats (Kanada et al. 1994). [additional studies: Kitchin and Brown 1990; DeRosa et al. 1996] **Lowest dermal LD_{50}** (rabbits) = 7600 mg/kg-bw (NICNAS 1998). [additional studies: NICNAS 1998] **Lowest inhalation LC_{50}** (mice, 2 h) = 37 000 mg/m^3 (EURAR 2002). [additional studies: NICNAS 1998; EURAR 2002] **Lowest inhalation LOEC** (rats, 4 h) = 1000 ppm (36 000 mg/m^3), based on significantly increased serum enzyme activities, such as glutamic oxaloacetic transaminase, glutamic pyruvic transaminase and ornithine carbamyl transferase in male CD rats (Drew et al. 1978). [additional studies: Frantík et al. 1994]
Short-term repeated-dose toxicity	**Lowest oral LOEL** = 400 mg/kg-bw per day (the only dose tested); based on increased cytochrome P450 enzyme activities in liver, kidney and nasal mucosa of male SD rats receiving 1,4-dioxane in drinking water at 1.5% v/v, corresponding to 400 mg/kg-bw per day for 10 days (Nannelli et al. 2005). [additional studies: Fairley et al. 1934; Mungikar and Pawar 1978; Pawar and Mungikar 1978; Lundberg et al. 1987; Goldsworthy et al. 1991; JBRC 1998a; NICNAS 1998; Roy et al. 2005] In a limited dermal study, four rabbits and four guinea pigs were exposed to 1,4-dioxane–water solutions applied to shaved skin on the nape. Each rabbit received 10 drops and each guinea pig received 5 drops of 1,4-dioxane solution, 11 times/week, for 48, 65, 76 or 100 days (one animal per group). Kidney cortex lesions, kidney medulla hemorrhages and liver degeneration were observed in both species (Fairley et al. 1934). A dermal LOAEL cannot be established due to the limited experimental design and reporting. [no additional dermal studies identified] **Lowest inhalation LOEC** = 100 mg/m^3 (female rats, 4 h/day, 5 days/week, 4 weeks), based on significantly increased glutathione peroxidase activation in both brain and ovaries; NO(A)EC = 10 mg/m^3 (Burmistrov et al. 2001). [additional studies: Fairley et al. 1934; Goldberg et al. 1964; NICNAS 1998]
Subchronic toxicity	**Lowest oral LOAEL (rats)** = 1600 mg/L in drinking water (equivalent to 130 mg/kg-bw per day), based on significantly increased relative liver and kidney weights and histopathological alterations observed in nasal cavity (respiratory epithelium nuclear enlargement) and liver (centrilobular swelling). In this study, F344/DuCrj rats and Crj:BDF1 mice, 10 per sex per group, were administered 1,4-dioxane at 0, 640, 1600, 4000, 10 000 or 25 000 mg/L in drinking water (0, 52, 130, 325, 813 and 2031 mg/kg-bw per day in rats and 0, 170, 425, 1063, 2656 and 6641 mg/kg-bw per day in mice) for 13 weeks. Histological lesions in the upper and lower respiratory tract, liver, kidneys and brain were observed in exposed rats, whereas only the former two organs were affected in exposed mice. Altered

Endpoint	Lowest effect levels[1]/Results
	hepatocellular foci stained positively with the anti-glutathione S-transferase placental form antibody (a preneoplastic lesion) were observed in exposed rats (mice were not examined). A dose-dependent increase in the relative weights of kidney and lung was observed in exposed rats and mice, whereas the relative liver weights were increased only in exposed rats. Hematological parameter changes were observed in exposed rats and mice only at the highest dose level. A NOAEL was determined at 640 mg/L for both rats and mice (equivalent to 52 mg/kg-bw per day in rats and 170 mg/kg-bw per day in mice) (JBRC 1998b; Kano et al. 2008). [additional studies: King et al. 1973; Stott et al. 1981; Stoner et al. 1986] In the aforementioned limited dermal study (under "Short-term repeated-dose toxicity"), kidney cortex lesions, kidney medulla hemorrhages and liver degeneration were observed in rabbits and guinea pigs exposed to 1,4-dioxane topically for up to 100 days (Fairley et al. 1934). A dermal LOAEL cannot be established due to the limited experimental design and reporting. [no additional dermal studies identified] **Lowest inhalation concentration LOAEC** (rats) = 100 ppm (lowest concentration tested, equivalent to 360 mg/m^3), based on nuclear enlargement of nasal respiratory epithelial cells in both male and female rats exposed to 1,4-dioxane vapour. In this study, F344 rats were exposed to 0, 100, 200, 400, 800, 1600, 3200 or 6400 ppm 1,4-dioxane vapour, 6 h/day, 5 days/week, for 13 weeks. Decreased body weights and increased relative liver, kidney and lung weights were observed at 200 ppm and above. Altered hematological parameters and lesions in liver (single cell necrosis and centrilobular swelling) and kidney (hydropic changes in renal proximal tubules) were observed at 3200 ppm. Nuclear enlargement and vacuolic changes occurred in olfactory epithelium at 200 ppm and above and in bronchus at 1600 ppm and above in males and at 3200 ppm in females. Nuclear enlargement in trachea occurred at 1600 ppm and above. Glutathione S-transferase placental form positive liver foci were observed at 1600 ppm and above in exposed females and at 3200 ppm in exposed males. All rats exposed to 6400 ppm 1,4-dioxane vapour died in the first week of exposure (Kasai et al. 2008). [additional studies: Fairley et al. 1934; Torkelson et al. 1974]
Chronic toxicity/ carcinogenicity	**Oral (drinking water) carcinogenicity bioassay in rats** Male Wistar rats, 26 per test group and 9 controls, were exposed to 1% 1,4-dioxane via drinking water daily (equivalent to 860 mg/kg-bw per day) for 63 weeks. Six of 26 exposed animals developed liver tumours, of which 1 also had a renal pelvic carcinoma and myeloid leukemia. One control animal developed lymphosarcoma. Severe kidney damage, including effects on tubular and glomerular epithelium as well as liver histological changes, such as groups of cells with enlarged hyperchromic hepatic nuclei and groups of large cells with reduced cytoplasmic basophilia, were also observed in the exposed animals (Argus et al. 1965). Charles River CD rats, 30 males per group, were exposed to 0, 0.75, 1.0, 1.4 or 1.8% 1,4-dioxane via drinking water (approximately equivalent to 0, 770, 1000, 1430 and 1850 mg/kg-bw per day) for 13 months. Two of the animals in each of the 1.4% and 1.8% groups developed hepatocellular carcinoma. There was a dose-related increase in the incidence of liver nodules (0 in the controls, 4, 9, 13 and 11 in the 0.75%, 1.0%, 1.4% and 1.8% groups, respectively). Hepatomas were seen in three rats given 1.4% and in 12 receiving 1.8% 1,4-dioxane. Tumours of the nasal cavity were not found in the 30 controls but were present in 1/30, 1/30, 2/30 and 2/30 of the rats receiving 0.75%, 1.0%, 1.4% or 1.8% 1,4-dioxane,

Endpoint	Lowest effect levels[1]/Results
	respectively. Marked kidney damage was observed in all exposed animals (Hoch-Ligeti et al. 1970; Argus et al. 1973). Sherman rats, 60 per sex per group, were exposed to 0%, 0.01%, 0.1% or 1.0% 1,4-dioxane via drinking water (equivalent to 0, 9.6, 94 and 1015 mg/kg-bw per day in the males and 0, 19, 148 and 1599 mg/kg-bw per day in the females) for 716 days. Liver carcinomas (10/66) and cholangiomas (2/66) and squamous cell carcinomas of the nasal cavities (3/66) were found in the highest dose group (males and females combined).. Hepatic carcinomas were also found in controls (1/106) and in the 0.1% groups (1/106). Hepatic degeneration and necrosis and kidney damage were observed in the two highest dose groups. Significantly reduced survival rates and body weights and increased absolute and relative liver weights were observed in the highest dose group. No effects on hematology or reproductive organs were observed in exposed animals. At the 0.01% dose level (equivalent to 9.6 and 19 mg/kg-bw per day for males and females, respectively), there was no evidence of tumour formation or other toxic effects considered to be exposure related (NOAEL) (Kociba et al. 1974). Osborne-Mendel rats, 35 per sex per group, were exposed to 0%, 0.5% or 1.0% 1,4-dioxane via drinking water for 110 weeks (equivalent to 0, 240 and 530 mg/kg-bw per day for males and 0, 350 and 640 mg/kg-bw per day for females, respectively). The survival rates of both dose groups were significantly reduced, but sufficient animals of each sex were alive at 52 weeks. A significantly increased incidence of hepatocellular adenomas was observed in females (0/31, 10/33 and 11/32 for 0%, 0.5% and 1.0% dose levels, respectively), but not in males. A significantly increased incidence of squamous cell carcinomas in the nasal cavities was observed in both sexes (0/33, 12/33 and 16/34 for 0%, 0.5% and 1.0% dosed males and 0/34, 10/35 and 8/35 for 0%, 0.5% and 1.0% dosed females, respectively). In addition, testicular mesotheliomas were observed more frequently in dosed animals (2/33, 4/33 and 5/34 for 0%, 0.5% and 1.0% dosed males, respectively). Non-neoplastic effects were observed in the kidney (tubular degeneration), liver (cytomegaly) and stomach (ulceration). Additionally, dosed rats exhibited higher incidences of pneumonia (National Cancer Institute 1978). F344/DuCrj rats, 50 per sex per group, were exposed to 0, 200, 1000 or 5000 mg 1,4-dioxane/L (equivalent to 0, 16, 81 and 398 mg/kg-bw per day for males and 0, 21, 103 and 514 mg/kg-bw per day for females, respectively) in the drinking water for 104 weeks. Significantly reduced survival in both sexes was observed in the highest dose group. The combined incidence of hepatocellular adenoma and carcinoma in males was 0/50, 2/50, 4/50 and 38/50 for the 0, 200, 1000 and 5000 mg/L groups, respectively. The corresponding incidences in females were 1/50, 0/50, 5/50 and 48/50. In both sexes, the incidences of liver tumours in the 5000 mg/L groups were significantly increased. Malignant neoplasms of the nasal cavity occurred only in the 5000 mg/L group, including squamous cell carcinomas (3/50 and 7/50 for males and females, respectively), sarcomas (2/50 in males only), esthesioneuroepithelioma (1/50 in both males and females) and rhabdomyosarcoma (1/50 in males only). In addition, in the 5000 mg/L group, the incidences of mesothelioma of the peritoneum, fibroma of the subcutis and fibroadenoma of the mammary gland were significantly increased in the males, and the incidences of adenoma of the mammary gland were significantly increased in the females. Non-neoplastic lesions were observed in the nasal cavity (nuclear enlargement and atrophy of the olfactory epithelium, squamous cell metaplasia and hyperplasia of the respiratory epithelium, hydropic change and sclerosis in the lamina propria, adhesion, inflammation and/or proliferation of the nasal gland), liver (spongiosis and hyperplasia) and kidney (nuclear enlargement

Endpoint	Lowest effect levels[1]/Results
	of the proximal tubule) in males at 200 mg/L and above and in females at 1000 mg/L and above (Yamazaki et al. 1994; JBRC 1998c). **Oral (drinking water) carcinogenicity bioassay in mice** B6C3F1 mice, 50 per sex per group, were exposed to 0%, 0.5% or 1.0% 1,4-dioxane via drinking water (equivalent to 0, 720 and 830 mg/kg-bw per day for males and 0, 380 and 860 mg/kg-bw per day for females, respectively) for 90 weeks. A significantly increased incidence of hepatocellular carcinomas was observed in both sexes of exposed animals (2/49, 18/50 and 24/47 for males and 0/50, 12/48 and 29/37 for females exposed to 0%, 0.5% and 1.0% 1,4-dioxane, respectively). The combined incidence of hepatocellular adenomas and carcinomas was also increased (8/49, 19/50 and 28/47 for males and 0/50, 21/48 and 35/37 for females exposed to 0%, 0.5% and 1.0% 1,4-dioxane, respectively). Of the non-neoplastic lesions, the increased incidence of pneumonia and rhinitis was significant. Hepatic cytomegaly was commonly observed in the exposed animals (National Cancer Institute 1978). Crj:BDF1 mice, 50 per sex per group, were exposed to 0, 500, 2000 or 8000 mg 1,4-dioxane/L via drinking water (equivalent to 0, 66, 250 and 770 mg/kg-bw per day for males and 0, 77, 320 and 1070 mg/kg-bw per day for females, respectively) for 104 weeks. The survival rates of females exposed to 2000 and 8000 mg/L and males exposed to 8000 mg/L were decreased due to liver tumours. Significantly increased incidences of hepatocellular carcinomas were observed in males exposed to 8000 mg/L (15/50, 20/50, 23/50 and 36/50 for 0, 500, 2000 and 8000 mg/L groups, respectively) and in all exposed females (0/50, 6/50, 30/50 and 45/50 for 0, 500, 2000 and 8000 mg/L groups, respectively). An increased incidence of hepatocellular adenomas was observed at 500 and 2000 mg/L in both sexes (7/50, 16/50, 22/50 and 8/50 in males and 4/50, 30/50, 20/50 and 2/50 in females for 0, 500, 2000 and 8000 mg/L groups, respectively). Non-neoplastic lesions in the nasal cavity, trachea, lung and kidney were observed at 2000 mg/L and above in both sexes. In males, lesions were also observed in liver (angiectasis) at 8000 mg/L and in testis (decreased mineralization) at 2000 mg/L and above. Body weight reduction and effects on hematological, biochemistry and urinalysis parameters were observed at 2000 mg/L and above (Yamazaki et al. 1994; JBRC 1998c). **Oral (drinking water) carcinogenicity bioassay in guinea pigs** Male guinea pigs, 22 per test group and 10 controls, were exposed to varied dose level of 0.5–2% 1,4-dioxane via drinking water (1200–4800 mg/kg-bw per day) for 23 months. In the exposed animals, three developed hepatomas, one kidney adenoma and two gallbladder carcinomas. In addition, 9/22 exposed animals developed bronchial epithelial hyperplasia (1/10 in controls) and infiltration of mononuclear cells in the lung (4/10 in controls) (Hoch-Ligeti and Argus 1970). **Inhalation carcinogenicity bioassay in rats** Wistar rats, 288 per sex in test group and 192 per sex in control group, were exposed to air containing 400 mg/m^3 (111 ppm) 1,4-dioxane (of 99.9% purity), 7 h/day, 5 days/week, for 2 years. A control group was exposed to filtered room air. Fifty percent of the animals survived 20–24 months. There were no statistically significant increases in the incidence of tumours in the 525 exposed rats that were examined compared with 347 controls. No exposure-related effects on hematological and clinical chemistry values, growth, mortality rate, demeanour, organ weight changes, including liver, kidney and spleen, or histopathological alterations of organs, including liver, kidneys, spleen, lungs, trachea, thoracic lymph nodes, heart, pancreas, stomach, intestine, thyroid,

Endpoint	Lowest effect levels[1]/Results
	mesenteric lymph nodes, urinary bladder, pituitary, adrenals, testes, ovaries, oviduct, uterus, mammary gland, lachrymal gland, lymph nodes, brain, vagina and bone marrow, were observed (Torkelson et al. 1974). Male F344/DuCrj (SPF) rats, 50 males/group were exposed to 0, 50, 250 or 1250 ppm (equivalent to 0, 180, 900, 4500 mg/m^3) 1,4-dioxane for 6 h/day, 5 days/wk for 104 weeks. Dose-dependent and statistically significant increases in incidences of nasal squamous cell carcinomas and hepatocellular adenomas were found primarily in the 1250 ppm exposed group. A significantly increased incidence of peritoneal mesotheliomas was observed in the 250 and 1250 ppm exposed groups. The incidences of renal cell carcinomas, fibroadenomas in the mammary gland and adenomas in the Zymbal gland were also increased, although not significantly, in a dose-dependent manner. Preneoplastic lesions occurred in the nasal cavity and liver of the 1,4-dioxane-exposed groups. Significantly increased incidences of nuclear enlargement, atrophy and respiratory metaplasia in the nasal cavity were noted at 50 ppm and above. In addition, a statistically significant but marginal decrement of terminal body weights, significantly increased plasma levels of AST, ALT, ALP and γ-GTP, significantly decreased haemoglobin levels, mean corpuscular volume and mean corpuscular haemoglobin and significantly increased relative liver weights were found in the 1250 ppm exposed group. Although significant increases in relative lung weights were also observed in the 1250 ppm exposed group, the authors claimed that this change was not biologically meaningful as there was no dose-response relationship for relative lung weight. A non-cancer LOAEC was established at 50 ppm (180 mg/m^3) for effects on the nasal cavity (Kasai et al., 2009). **Dermal carcinogenicity bioassay and tumour-promoting assay in mice** 1,4-Dioxane dissolved in acetone (0.2 mL, unspecified concentration) was applied to the shaved back skin of Swiss-Webster mice, 30 per sex per group, 3 times/week for 60 weeks. One of 25 exposed females developed skin carcinoma, and 1/22 exposed males developed subcutaneous tumours. No significant lesions were observed. No tumours were observed in acetone-treated animals. A tumour-promoting assay was also conducted, in which 50 µg of dimethylbenzanthracene was applied to mice 1 week prior to the application of 1,4-dioxane. Two of four, 3/4 and 2/4 exposed males developed skin papillomas, carcinomas and subcutaneous tumours, respectively, and 2/5 and 3/5 exposed females developed skin papillomas and carcinomas, respectively, in the tumour-promoting test. Squamous cell carcinoma of the nasal septum was observed in one animal with skin papilloma. Nine exposed animals developed lung tumours. Tumours in kidney, spleen and liver and non-neoplastic lesions of liver and skin were also observed in exposed animals (King et al. 1973). 1,4-Dioxane (0.05 mL; four grades in four groups, respectively) was topically applied to C3h/HeJAgouti mice, 30 males per group, 3 times/week for 78 weeks. The controls were exposed to ethanol. Five hepatic and one pulmonary neoplasm were observed in exposed animals; these neoplasms were reported to be within normal limits. No clinical effects were observed. Only 40/120 test animals survived to the end of the study. It was not reported whether the dermal application was under occlusion (Perone et al. 1976). **Other carcinogenicity bioassays** *Tumour induction by intraperitoneal injection* A/J mice, 30 males per group, were administered 1,4-dioxane (purity unspecified) by intraperitoneal injection 3 times/week for 8 weeks for total doses of 0, 400, 1000 and 2000 mg/kg-bw. The high dose increased the multiplicity of lung

Endpoint	Lowest effect levels[1]/Results
	tumours to 0.97 per mouse ($p < 0.05$) compared with 0.28 per mouse in controls given vehicle alone (Maronpot et al. 1986). In a mouse lung adenoma assay in A/J mice, 16 per sex per test group, 1,4-dioxane produced a significant increase in the incidence of lung tumours in the males given an intermediate intraperitoneal dose (total dose of 12 000 mg/kg-bw, eight injections in 24 weeks). No such increase was noted in males given a lower or higher intraperitoneal dose (total dose of 4800 or 24 000 mg/kg-bw, respectively, eight injections in 24 weeks); in females given the same intraperitoneal doses; or in either males or females given 1,4-dioxane orally (total dose of 24 000 mg/kg-bw, 8 times in 24 weeks) (Stoner et al. 1986). *Oral (gavage) tumour-promoting assay* SD rats, 8–11 males per group and 19 controls, were partially hepatectomized and intraperitoneally injected with a single dose of 30 mg N-nitrosodiethylamine (NDEA)/kg-bw to initiate hepatocarcinogenesis. Five days later, these animals were administered 1,4-dioxane (purity 99.5%) at 0, 100 or 1000 mg/kg-bw per day by gavage once a day, 5 days/week, for 7 weeks. The 1000 mg 1,4-dioxane/kg-bw per day dose significantly increased the number and total volume of γ-glutamyltranspeptidase (GGT) positive foci in the liver. In two other groups of rats without NDEA initiation, 100 or 1000 mg 1,4-dioxane/kg-bw per day alone did not induce the GGT positive foci (Lundberg et al. 1987). *Tumour initiation assay* Female SENCAR mice, 20–40 per group, were exposed to 1000 mg 1,4-dioxane/kg-bw orally, subcutaneously or topically as a tumour initiator, followed by administration of 1 µg 12-O-tetradecanoylphorbol-13-acetate (TPA) topically as a tumour promoter, 3 times/week, for 20 weeks. The experiment was terminated at 52 weeks. No significant increases in the formation of papillomas were observed compared with the controls that were exposed to acetone as the initiator and TPA as the promoter (Bull et al. 1986). Male SD rats, 10 per group, received an intraperitoneal dose of 881 mg 1,4-dioxane/kg-bw followed by administration of 500 mg sodium phenobarbital/L in the drinking water for 49 days. No increase in the number of GGT positive foci in the liver was found, indicating a lack of initiation activity (Pereira et al. 1982). **Non-neoplastic endpoints** Lowest oral **LOAEL** for non-neoplastic effects = 200 mg/L (16 mg/kg-bw per day), based on an effect on the liver (spongiosis) in male rats exposed to 200 mg 1,4-dioxane/L via drinking water for 2 years (Yamazaki et al. 1994; JBRC 1998c). EURAR has stated, although the increased incidence of liver spongiosis was not statistically significant at 200 ppm, there was a dose-related trend associated with this effect and therefore 200 ppm can be established as the LOAEL (EURAR 2002). Oral **NOAEL** = 9.6 mg/kg-bw per day in male rats or 19 mg/kg-bw per day in female rats exposed to 0.01% 1,4-dioxane via drinking water for 2 years (Kociba et al. 1974). Inhalation LOAEC = 50 ppm (180 mg/m^3) (male F344/DuCrj (SPF) rats exposed to 0, 50, 250 or 1250 ppm (equivalent to 0, 180, 900, 4500 mg/m^3) 1,4-dioxane for 6 h/day, 5 days/wk for 104 weeks) for effects on the nasal cavity (Kasai et al., 2009) (see above for details). Inhalation **NOAEC** = 400 mg/m^3 (rats, exposed to 1,4-dioxane 7 h/day, 5 days/week, for 2 years), no exposure-related non-neoplastic effects were observed

Endpoint	Lowest effect levels[1]/Results
	(Torkelson et al. 1974). Dermal application of 0.05 mL 1,4-dioxane, equivalent to 700–1000 mg/kg-bw per day (mice, exposed to 1,4-dioxane 3 times/week for 78 weeks), no exposure-related non-neoplastic effects were observed (Perone et al. 1976).
Reproductive toxicity	**Lowest oral LOAEL** (mice, exposed to 1,4-dioxane via drinking water for 2 years) = 2000 mg/L (250 mg/kg-bw per day), based on reduced mineralization of testis observed in above-mentioned long-term study (JBRC 1998c). However, this effect was not reported in Yamazaki et al. (1994). [additional study: Lane et al. 1982 (this is a three-generation study in which mice were exposed to 0.58–5.83 mg 1,1,1-trichloroethane/mL containing 3% 1,4-dioxane in drinking water , one control group was exposed to 1,4-dioxane/emulphor; no effects on adult reproductive parameters, litter survival, growth, development or general pathology were observed)] No dermal or inhalation reproductive studies were identified. Other route of exposure: No effects on rate of conception, mean number of implantations, percentage of living fetuses or mutagenicity index in male mice following single intraperitoneal injection of 2500 mg/kg-bw (BASF 1977).
Developmental toxicity	**Lowest oral (gavage) LOAEL** (pregnant SD rats, 17–20 per group, exposed to 0, 0.25, 0.5 or 1.0 mL 1,4-dioxane/kg-bw per day by gavage on gestation days 6–15) = 1.0 mL/kg-bw per day (1035 mg/kg-bw per day), based on significantly reduced average live fetus weights and delayed fetal ossification in sternebrae. Reduced maternal weight gain was also observed at 1.0 mL 1,4-dioxane/kg-bw per day. **NOAEL** = 0.5 mL/kg-bw per day (517 mg/kg-bw per day) (Giavini et al. 1985). [no additional oral studies identified] No dermal or inhalation developmental studies were identified. Other route of exposure: No effects on rate of conception, mean number of implantations, percentage of living fetuses or mutagenicity index in male mice following single intraperitoneal injection of 2500 mg/kg-bw (BASF 1977).
Genotoxicity and related endpoints: *in vivo*	**Dominant lethal mutation assay** *Negative results:* No effects on rate of conception, mean number of implantations, percentage of living fetuses or mutagenicity index were observed in male mice following single intraperitoneal injection of 2500 mg/kg-bw (BASF 1977). **Sex-linked recessive lethal mutation assay** *Negative results:* *Drosophila melanogaster* exposed to 1,4-dioxane by feeding (35 000 ppm) or injection (50 000 ppm) (Yoon et al. 1985). **Micronuclei induction** *Positive results:* Significant dose-related increases in micronuclei induction were observed in the bone marrow and liver of young CD-1 mice following oral (gavage) administration of 1500, 2500 or 3500 mg 1,4-dioxane/kg-bw per day for 5 days; the micronuclei originated primarily from chromosome breakage (Roy et al. 2005). Evidence of exposure-related increases in micronuclei induction was observed in

Endpoint	Lowest effect levels[1]/Results
	male and female C57BL/6 mice following single oral (gavage) administration of 1,4-dioxane at 900 mg/kg-bw and above (Mirkova 1994). Significant increase in micronuclei numbers was observed in hepatocytes of male CD-1 mice following single gavage administration at 2000 mg/kg-bw and above (Morita and Hayashi 1998). *Negative results:* No evidence of exposure-related increase in micronuclei was observed in the bone marrow in male BALB/c mice following single oral (gavage) administration (5000 mg/kg bw) (Mirkova 1994). No significant dose-dependent increase in micronuclei was observed in the bone marrow of male B6C3F1 mice following single or three daily intraperitoneal injections of 1,4-dioxane (up to 4000 mg/kg bw) (McFee et al. 1994). No significant increase in micronuclei was observed in the bone marrow of CBA mice (1800 mg/kg bw) or C57BL/6 mice (up to 3600 mg/kg bw) following single oral administration (Tinwell and Ashby 1994). No increase in micronuclei numbers was observed in peripheral red blood cells of male CD-1 mice following single gavage doses of up to 3000 mg/kg-bw (Morita and Hayashi 1998). **DNA repair assay (unscheduled DNA synthesis)** *Positive results:* Significantly increased hepatic DNA synthesis was observed in male SD rats exposed to 1000 mg 1,4-dioxane/kg-bw per day in drinking water for 11 weeks, along with significantly increased relative liver weight (Stott et al. 1981). *Negative results:* No significantly increased DNA repair was observed in the hepatocytes of male F344 rats following single oral administration of 1000 mg 1,4-dioxane/kg-bw by gavage for 2 or 12 h or exposed to 1% or 2% 1,4-dioxane in drinking water for 1 week (Goldsworthy et al. 1991). No significantly increased unscheduled DNA synthesis was observed in the nasal epithelial cells of male F344 rats exposed to 1% 1,4-dioxane in drinking water for 8 days followed by a single gavage dose at up to 1000 mg/kg-bw (Goldsworthy et al. 1991). No significantly increased hepatic DNA repair was observed in male Sprague-Dawley (SD) rats administered a single dose of 1000 mg 1,4-dioxane/kg-bw by gavage (Stott et al. 1981). **Cell proliferation assay (replicative DNA synthesis)** *Positive results:* Significantly increased hepatic cell proliferation (measured as hepatic labelling index) was observed in male F344 rats exposed to 1% 1,4-dioxane in drinking water for 2 weeks (Goldsworthy et al. 1991). Significantly increased replicative DNA synthesis was observed in male F344 rats 24 h after a single dose administration of 2000 mg 1,4-dioxane/kg-bw; no histopathological changes were noted (exposure route was not reported) (Miyagawa et al. 1999).

Endpoint	Lowest effect levels[1]/Results
	Equivocal results: Induction of replicative DNA synthesis was observed in hepatocytes of male F344 rats 24 h after administration of a single dose of 1,4-dioxane by gavage at 2000 mg/kg-bw, but not at 39 or 48 h after administration; the 24-h results were not reproducible in the same study; therefore, the overall results were rated as negative (Uno et al. 1994). *Negative results:* The hepatic labelling index did not increase at either 24 or 48 h following a single dose of 1000 mg 1,4-dioxane/kg-bw in male F344 rats (Goldsworthy et al. 1991). No increase in cell proliferation in the nasal epithelial cells (measured as unit length labelling index) was observed in male F344 rats exposed to 1% 1,4-dioxane in drinking water for 2 weeks (Goldsworthy et al. 1991). **DNA damage assay** *Positive results:* A dose-related increase in hepatic DNA damage was observed in female SD rats following oral administration of 1,4-dioxane (168–4200 mg/kg bw) at 21 and 4 h before sacrifice. The results were significant at 2550 mg/kg-bw and above (Kitchin and Brown 1990). *Negative results:* No indication of DNA damage (comet assay) was observed in the stomach, colon, liver, kidney, urinary bladder, lung, brain or bone marrow in male ddY mice following single gavage doses of up to 3200 mg/kg-bw (Sasaki et al. 2000). No increased 8-hydroxydeoxyguanosine level in DNA was observed in F344 rats administered a single gavage dose of 1,4-dioxane at half of the LD_{50} level (Sai et al. 1989). **DNA alkylation assay** *Negative results:* No significantly increased hepatic DNA alkylation was observed in male Sprague-Dawley (SD) rats administered a single dose of 1000 mg 1,4-dioxane/kg-bw by gavage (Stott et al. 1981). **Aneuploidy assay** *Positive results:* Significantly increased induction of meiotic non-disjunction was observed in mature oocytes of female *Drosophila melanogaster* orally administered 1,4-dioxane at 2% and above, and in immature oocytes at 1% and above, prior to mating (Muñoz and Barnett 2002). **Ribonucleic acid (RNA) synthesis assay** Decreased RNA polymerase A and B activities were observed in male SD rats intravenously administered 1,4-dioxane at 10 mg per rat (29 mg/kg bw) and above (Kurl et al. 1981).
Genotoxicity and related endpoints: *in vitro*	**Mutagenicity assay** *Negative results:* Ames tests in *Salmonella typhimurium* TA98, TA100, TA102, TA1530, TA1535, TA15337 and TA1538, with and without metabolic activation (BASF 1979a, b, c; Stott et al. 1981; Haworth et al. 1983; Nestmann et al. 1984; Khudoley et al. 1987).

Endpoint	Lowest effect levels[1]/Results
	Point mutation assays in *Saccharomyces cerevisiae* D61M (Zimmermann et al. 1985); in *Photobacterium phosphoreum*, without metabolic activation (Kwan et al. 1990); in Chinese hamster ovary (CHO) cells, with and without metabolic activation (BASF 1991); and in mouse lymphoma cells, with and without metabolic activation (McGregor et al. 1991; Morita and Hayashi 1998). **Chromosomal aberration assay** *Negative results:* CHO W-B1 cells (Galloway et al. 1987) and K1 cells (Morita and Hayashi 1998), with and without metabolic activation. **Micronuclei induction assay** *Negative results:* CHO K1 cells, with and without metabolic activation (Morita and Hayashi 1998). **Sister chromatid exchange assay** *Negative results:* CHO W-B1 cells, with metabolic activation (Galloway et al. 1987), and K1 cells, with and without metabolic activation (Morita and Hayashi 1998). *Weak positive results:* CHO W-B1 cells, without metabolic activation (Galloway et al. 1987). **Aneuploidy assay** *Negative results:* *Saccharomyces cerevisiae* D61M (Zimmermann et al. 1985). **DNA damage assay** *Positive results:* Induction of DNA single strand breaks in rat hepatocytes; cytotoxicity was observed (Sina et al. 1983). *Negative results:* Differential DNA repair assay in *Escherichia coli* K-12, with and without metabolic activation (Hellmer and Bolcsfoldi 1992). DNA repair in primary hepatocytes freshly isolated from male F344 rats with or without activation of 1,4-dioxane metabolism. In order to activate 1,4-dioxane metabolism, prior to the cell isolation, some of the rats were exposed to 1% 1,4-dioxane in drinking water for 1 week (Goldsworthy et al. 1991). **Covent DNA binding assay** Microsome-catalysed binding of 1,4-dioxane to DNA was not detected *in vitro* (Woo et al. 1977). **Cell transformation** *Positive results:* 1,4-Dioxane-induced cell transformation in BALB/3T3 cells; cytotoxicity was observed (Sheu et al. 1988). *Negative results:* 1,4-Dioxane did not increase adenovirus SA7-induced transformation of Syrian hamster embryo cells (SA7/SHE system) (Heidelberger et al. 1983).

Endpoint	Lowest effect levels[1]/Results
	DNA synthesis inhibition assay *Positive results:* HeLaS3 cells (many other non-genotoxic chemicals gave positive results in this assay) (Heil and Reifferscheid 1992).
Immunotoxicity	1,4-Dioxane suppressed T-cell responses (on lymphocytes) while augmenting B-cell responses (in both spleen and lymph node cells) were observed in murine and human lymphocytes *in vitro* and slightly lower lymphocyte response to mitogens was observed in CBA/J mice exposed to 1,4-dioxane by intraperitoneal injection (1670 mg/kg-bw per day). CBA/J mice were administered 0.5 mL of 0.1–10% 1,4-dioxane daily by intraperitoneal injection for 7 days (Thurman et al. 1978).
Sensitization	Negative to skin of guinea pigs (BASF 1993).
Irritation	**Skin irritation** Undiluted 1,4-dioxane was applied to the shaven back of rabbits (1 per sex per group) for 1, 5 and 15 min and 20 h and to the ear for 20 h under occlusion conditions. Very slight erythema was observed 24 h after 1- to 15-min applications, and slight scale formation was observed after 8 days. Slight erythema and slight edema were observed on the back of one animal 24 h after the 20-h application. Slight erythema was observed on the ear 24 h after the 20-h application (BASF 1973). Skin irritation was observed in Wister rats (three per sex per group), ddY mice (three per sex per group) and Hartley guinea pigs (three females) under unoccluded conditions 1 week after application of 1,4-dioxane at 20 mg/kg-bw and above (Sekizawa et al. 1994).
	Eye irritation Two male White Vienna rabbits received an instillation of 0.05 mL undiluted 1,4-dioxane. Slight corneal opacity and conjunctival redness and slight to severe chemosis were observed after 24 h, and smeary depositions were observed after 8 days of application (BASF 1973). Significantly increased opacity and thickness of isolated bovine cornea and stroma were observed after 30 min or 4.5 h of incubation with 5–10% (v/v) 1,4-dioxane (Igarashi and Northover 1987).
	Respiratory tract Irritation of the respiratory tract was observed in rats, mice, guinea pigs and rabbits exposed to 1,4-dioxane vapour (Yant et al. 1930; Gross 1938; BASF 1980).
Humans	
Acute toxicity	In an acute inhalation study, six female and six male healthy volunteers were exposed to 1,4-dioxane at 0 (control) or 20 ppm (72 mg/m^3) for 2 h at rest. No significant differences were observed in the symptoms rating on a visual analogue scale, blink frequency, pulmonary function, nasal swelling or inflammatory markers in the plasma between the exposed volunteers and the controls. The authors suggested 20 ppm to be a NOAEC, although they stated that subclinical central nervous system effects cannot be entirely excluded at this exposure concentration (Ernstgård et al. 2006).
Chronic toxicity/ carcinogenicity	Several case studies reported fatalities among workers exposed to 1,4-dioxane in occupational environments. Clinical signs of toxicity included severe epigastric pain, convulsions and coma. Histology revealed hepatic and renal lesions. Hemorrhagic nephritis as well as demyelination and loss of nerve fibre in the central nervous system were also observed (Barber 1934; Johnstone 1959; Sullivan 1994). The exposure levels could not be defined precisely and co-exposure to other chemicals and alcohol consumption were potential confounding factors.

Endpoint	Lowest effect levels[1]/Results
	In a cross-sectional study, 74 workers were exposed to 1,4-dioxane at estimated concentrations of 0.02–48 mg/m^3 for an average of almost 25 years. Twelve deaths were reported, including two from cancer. The group showed no evidence of liver or kidney damage and did not have a higher incidence of cancer deaths compared with the population at large. In six active workers, no increased rate of chromosomal aberrations in lymphocytes was noted (Thiess et al. 1976). In a further study, a significant increase in mean lymphocyte chromosomal aberration frequency was found in 11 workers exposed to alkylene oxides, including 1,4-dioxane, for more than 20 years. Exposure to known mutagens such as ethylene oxide and propylene oxide confounds any conclusions with regard to causation (Thiess et al. 1981). In a cohort study, 80 workers were potentially exposed to 0.18–184 mg 1,4-dioxane/m^3. Four cancers were reported: colonic cancer, pulmonary carcinoma, lymphosarcoma and glioblastoma. Observed deaths were not significantly different from expected cancer deaths (NIOSH 1977). In a retrospective cohort study, 165 workers (Texas) were exposed to 1,4-dioxane (<25 ppm, equivalent to 90 mg/m^3) from 1954 to 1975 for 1 month to 10 years or more. Three cancer deaths were reported: carcinomas of stomach and alveolar cell. There was no significant difference between the observed total mortality or deaths due to malignant neoplasms and the expected mortality rates of white Texan males. However, the authors stated that as the size of the cohort was small, with all workers apparently being exposed to low levels of 1,4-dioxane for relatively short durations, these results were not conclusive (Buffler et al. 1978). In a matched-pair study of 151 workers exposed for 1–6 years to the atmospheric concentrations up to 1.4 mg/L of 1,1,1-trichloroethane blended with 4% 1,4-dioxane stabilizer, no adverse effects on the liver or heart were reported (Kramer et al. 1978). In a comparative mortality study of over 19 000 cases in the Danish Cancer Registry, a standardized proportionate incidence ratio (SPIR) of 1.64 was determined for liver cancer in male workers employed in companies using 1,4-dioxane between 1970 and 1984. This SPIR is significantly higher than the expected rate (p = 0.04), and the confounding factors, particularly alcohol consumption, could not account for this. However, when a latency period (minimum 10 years) is incorporated in the analysis, the SPIR is reduced to 1.15. Statistically, the confidence interval (1.03–2.48) indicates the possibility of a real effect; however, uncontrolled factors, such as the potential for exposure to other carcinogenic chemicals, particularly 1,1,1-trichloroethane, and the lack of quantitative exposure data for 1,4-dioxane confound any conclusions regarding a causal association with liver cancer in this study. An increase in liver cancer incidence of 50% was identified in one workplace where only 1,4-dioxane was used. Alcohol consumption alone could not account for this increase. The same authors carried out a workplace exposure survey (1983–1991) and reported that the majority of 1,4-dioxane levels measured were less than 10 mg/m^3. However, these data were insufficient to allow the authors to speculate on workplace exposure levels in the comparative mortality study (Hansen 1993; Hansen et al. 1993).
Genotoxicity and related endpoints	In the aforementioned cross-sectional study, 74 workers were exposed to 1,4-dioxane concentrations estimated at 0.02–48 mg/m^3 for an average of almost 25 years. In six active workers, no increased rate of chromosomal aberrations in lymphocytes was noted (Thiess et al. 1976). In a further study, a significant

Endpoint	Lowest effect levels[1]/Results
	increase in mean lymphocyte chromosomal aberration frequency was found in 11 workers exposed to alkylene oxides, including 1,4-dioxane, for more than 20 years. Workers were also exposed to other known mutagens, such as ethylene oxide and propylene oxide (Thiess et al. 1981).
Reproductive/ developmental toxicity	In the electronics industry, increased incidences of miscarriages, premature births, maternal toxicosis, fetal ossifications and decreased birth weights as well as gonadotoxic effects on offspring were observed in female workers exposed to chemicals including 1,4-dioxane in the occupational environment (Mikheev et al. 1979; Ailamazian 1990).
Irritation	Irritation of eyes, nose and throat was reported in volunteers exposed to 1,4-dioxane vapour at 50 ppm (180 mg/m^3) for 6 h or at higher concentrations for shorter periods of time (Yant et al. 1930; Wirth and Klimmer 1937; Silverman et al. 1946; Young et al. 1977; Gingell et al. 1994). In addition, 1,2-dioxane is a fat solvent, skin irritation was observed after long or repeated exposure (Sonneck 1964; Adams 1983). The European Commission has concluded that 1,4-dioxane is irritating to the eye and the respiratory tract, but not to the skin (EURAR 2002).

[1] LC_{50}, median lethal concentration; LD_{50}, median lethal dose; LO(A)EC, lowest-observed-(adverse-)effect concentration; LO(A)EL, lowest-observed-(adverse-)effect level; NO(A)EC, no-observed-(adverse-)effect concentration; NO(A)EL, no-observed-(adverse-)effect level.

Appendix 5: Summary of margins of exposure for 1,4-dioxane

Exposure scenario	Concentration or intake	Critical effect level	Critical effect	Margin of exposure
Environmental media				
Estimated daily intake from environmental media[1]	0.19 –1.26 µg/kg-bw per day	9.6 mg/kg-bw per day	Oral exposure level at which no tumour formation was observed and oral NOAEL for non-neoplastic effects (liver toxicity) in experimental animals (Kociba et al. 1974).	7 620 – 50530
		16 mg/kg-bw per day	Oral LOAEL for liver lesions in experimental animals (Yamazaki et al. 1994; JBRC 1998c).	12 700 – 84200
Estimated inhalation exposure from indoor air	0.685 µg/m^3 (Fellin and Otson 1997)	180 mg/m^3	Nasal chronic toxicity in rats (2 years) (Kasai et al. 2009)	262 770
		180 mg/m^3	Irritation of eyes, nose and throat (6 h, humans) (Young et al. 1977).	
Estimated intake from daily-use personal care products				
Actual finished product data: Aggregate estimated intake range from daily-use products (intake from dermal and inhalation exposure)[2]	0.0012 mg/kg-bw per day	9.6 mg/kg-bw per day	Oral exposure level at which no tumour formation was observed and oral NOAEL for non-neoplastic effects (liver toxicity) in experimental animals (Kociba et al. 1974).	8 000
		16 mg/kg-bw per day	Oral LOAEL for liver lesions in experimental animals (Yamazaki et al. 1994; JBRC 1998c).	13 300
Estimated inhalation exposure from daily-use personal care products				
Actual finished product data: Aggregate estimated mean event air concentrations for daily-use products[2]	0.0118 mg/m^3	180 mg/m^3	Nasal chronic toxicity in rats (2 years) (Kasai et al. 2009).	15 250
Estimated exposure from acute-use personal care product				
Hair dye: Estimated intake range from CNS-derived data (intake from dermal and inhalation exposure)[2]	0.0202–0.0672 mg/kg-bw	1050 mg/kg-bw	Acute oral LOEL based on subtle effects on central nervous system (neurotransmitters) in rats (Kanada et al. 1994).	15 630 –52 000
Hair dye: Estimated mean event air concentration from CNS-derived data[2]	1.39–4.64 mg/m^3	180 mg/m^3	Irritation of eyes, nose and throat (6 h, humans) (Young et al. 1977).	40–130
		3600 mg/m^3	Acute LOEC based on altered serum enzyme activity (4 h, rats) (Drew et al. 1978).	780–2 590

[1] See Appendix 1 for details on environmental concentrations.
[2] See Table 7b for details on actual personal care products exposure estimates and CNS derived product exposure estimates.