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Canadian Council of Ministers of the Environment | Le Conseil canadien des ministres de l'environnement

SCIENTIFIC CRITERIA DOCUMENT FOR THE DEVELOPMENT OF THE CANADIAN WATER QUALITY GUIDELINE FOR CARBAMAZEPINE

Protection of Aquatic Life

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NOTE TO READERS

The Canadian Council of Ministers of the Environment (CCME) is the primary minister-led intergovernmental forum for collective action on environmental issues of national and international concern.

This document was developed by Water Management Committee's Guidelines Project Team, based on initial work by the Ontario Ministry of the Environment and Climate Change. It provides the background information and rationale for the development of the Canadian Water Quality Guidelines for carbamazepine.

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LIST OF ABBREVIATIONS

AA-EQS	Annual Average Environmental Quality Standard (chronic criterion)
BCF	Bioconcentration Factor
CAS	Conventional Activated Sludge
CAS-N	Conventional Activated Sludge with Nitrification
CAS-BNR	Conventional Activated Sludge with Biological Nutrient Removal
CBZ	Carbamazepine
CBZ-DiOH	10,11-dihydro-10,11-*trans*-dihydroxycarbamazepine
CCME	Canadian Council of Ministers of the Environment
CCME BTG	CCME Biosolids Task Group
CF	Condition Factor
CP	Carbonyl Protein
C_w	Mean Concentration in Water During Course of Study
CWQG	Canadian Water Quality Guideline
D_{lipw}	pH-Corrected Liposome Water Partioning Coefficient
DMSO	Dimethylsulfoxide solvent
D_{ow}	pH-Corrected Octanol Water Partioning Coefficient
DO	Dissolved Oxygen
DT50	Mean Dissipation Half-Life (time for concentration to be reduced by 50%)
DT_{LOQ}	Dissipation Time for concentration to be reduced to Limit of Quantification
DT90	Dissipation Time for concentration to be reduced by 90%
EC10	The concentration that causes 10% of the experimental biota to show an observed adverse effect. The adverse effect may be immobilization, changes in reproductive potential, growth, or some other ecologically relevant endpoint. Even though some effects do occur, they are not considered to be adverse (i.e. 10% effect is considered to be no different from natural background).
EC50	The concentration that causes 50% of the experimental biota to show an observed adverse effect. The adverse effect may be immobilization, changes in reproductive potential, growth, or some other ecologically relevant endpoint.
EDC	Endocrine Disrupting Compound
EROD	Ethoxyresorufin-*O*-deethylase, a highly sensitive indicator of contaminant uptake in fish, providing evidence of receptor-mediated induction of cytochrome P450-dependant monooxygenases (the CYP1A subfamily specifically) by xenobiotic chemicals.
FETAX	Frog Embryo Teratogenesis Assay-Xenopus
GABA	Gamma-Aminobutyric Acid
GUDI	Groundwater Under the Direct Influence of Surface Water
HC5	Hazardous Concentration for 5% of species, or the 95% protection level calculated from an SSD plotted using mostly NOEC data (for derivation of a chronic HC5) or acute LC/EC50 data (for derivation of an acute HC5).
HRT	Hydraulic Retention Time
HSI	Hepatosomatic Index

IUPAC	International Union of Pure and Applied Chemistry
K_d	Solid Liquid Partioning Coefficient
$K_{i,boil}$	Specific Biological Degradation Rate Constant of Pharmaceutical i (L/gTS/hr or L/gTS/d)
K_{in}	Carbamazepine Uptake Rate Constant
K_{out}	Carbamazepine Depuration Rate Constant
K_{O3}	Second Order Rate Constant of Ozonation Process
K_{oc}	Sedimet Water Partioning Coefficient
K_{ow}/K_{oe}	Octanol Water Partioning Coefficient/Facteur de partition octanol-eau
LC10	The concentration which is lethal to 10% of the experimental biota. Even though some effects do occur, they are not considered to be adverse (i.e. 10% lethality is considered to be no different from natural background mortality).
LC50	The concentration which is lethal to 50% of the experimental biota
LC-ES-MS/MS	Liquid Chromatography-Electrospray Tandem Mass Spectrometry
LC-IDMS/MS	Liquid Chromatography/Isotope Dilution Tandem Mass Spectrometry
LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometry
LOD	Limit of Detection
LOEC	Lowest Observed Effect Concentration
LOQ	Limit of Quantification
LPO	Lipid Peroxidation
MAC-EQS	Maximum Allowable Concentration Environmental Quality Standard (acute criterion)
MATC	Maximum Acceptable Toxicant Concentration (geometric mean of the NOEC and LOEC)
MDL	Method Detection Limit
ML	Minimum Levels of Quantitation
NOEC	No Observed Effect Concentration
OECD	Organisation for Economic Co-operation and Development
pH	The negative log of the hydrogen ion activity, $-\log[H^+]$
P_{ka}	Acid Dissociation Constant
PLE	Pressurized Liquid Extraction
PLHC-1	Topminnow, *Poeciliopsis lucida*, hepatocellular carcinoma cell line
PNEC	Predicted No-Effect Concentration
POCIS	Polar Organic Chemical Integrative Sampler
PPCP	Pharmaceuticals and Personal Care Products
PRTH	The Rainbow trout, *Onchorynchus mykiss*, hepatocyte cell line
QSAR	Quantitative Structure Activity Relationship
RTG-2 cell line	The Rainbow trout, *Onchorynchus mykiss*, gonad cell line
SMILES	Simplified Molecular-Input Line-Entry System
SPE	Solid Phase Extraction
SRT	Solids Retention Time
SSD	Species Sensitivity Distribution
STP	Sewage Treatment Plant
Temp	Temperature
UGT	Uridine Diphosphate Glucuronosyltransferase

US EPA	United States Environmental Protection Agency
USGS	United States Geological Survey
V_D	Volume of Distribution

EXECUTIVE SUMMARY

Carbamazepine (CBZ) is a drug commonly prescribed as an antiepileptic (Miao and Metcalfe 2003), but is also used for the treatment of pain associated with trigeminal neuralgia and as a psychotropic agent (Albani *et al.* 1995; Deleu *et al.* 2001; Duche and Loiseau 1995), in the treatment of schizophrenia (Emrich *et al.* 1993; Okuma 1993), and bipolar disorder (Dilsaver *et al.* 1996; Weissman *et al.* 1988). It has been suggested that CBZ, in combination with other drugs, is effective at treating symptoms of alcohol withdrawal (Franz *et al.* 2001).

The annual quantity of CBZ used in Canada in 2012 was approximately 21,448 kg (IMS AG 2013). The vast majority (>20,000 kg yr) is used in the private home, and the remainder is used in hospital. The main route of entry of carbamazepine into the aquatic environment is via municipal sewage treatment effluent, with other possible sources being combined sewer overflows during rain events, leakage in sewage systems, introduction into septic systems, application of biosolids onto agricultural land (Bonvin 2013), and irrigation with reclaimed water (York Region 2014). While disposal into landfills may be a source of concern (Bonvin 2013), monitoring has not identified CBZ in landfill leachate in Ontario to date (Stafford 2008). Further investigation may be required to identify landfills as a potential source. An examination of CBZ (and other contaminants of emerging concern) in landfill leachate was conducted in the United States (Masoner *et al.* 2014). CBZ was detected in 75% of leachate samples, with a maximum measured concentration of 2.59 µg/L. There is some manufacturing of CBZ in Canada (e.g. Sandoz®-CBZ chewable tablets and Sandoz®-CBZ controlled release tablets are manufactured in Québec) (Sandoz 2005). It is expected that manufacturing releases are very low (0.2 - 0.5% of product volume) (EAU-NSACB 2014).

Carbamazepine has been found to be recalcitrant to many conventional wastewater treatment processes (Bonvin 2013), and as a result has been detected in municipal sewage treatment plant effluents (Brun *et al.* 2006; Ferrari *et al.* 2003; Metcalfe *et al.* 2003a; Metcalfe *et al.* 2003b), biosolids (Smyth 2011), surface water (Hua *et al.* 2006; Kleywegt *et al.* 2011; Metcalfe *et al.* 2003a; Tabe *et al.* 2009), groundwater (Kormos 2007; Stafford 2008) and treated drinking water (Kleywegt *et al.* 2011). Detections have also been reported in fish fillet and liver tissue (Ramirez *et al.* 2009) as well as crops grown on biosolids amended soils (Holling *et al.* 2012).

The highest concentration of CBZ detected in a Canadian surface water was in a river system in southern Ontario (downstream from 11 sewage treatment plants), where the reported concentration was approximately 1 µg/L (988 ng/L) (Kormos 2007). CBZ concentrations detected in European surface waters have also been in the µg/L range (Oetken *et al.* 2005). The highest reported CBZ concentration in a Canadian municipal sewage treatment effluent is 2.3 µg/L (Metcalfe *et al.*, 2003b). The main transformation product 10,11-dihydro-10,11-*trans*-dihydroxycarbamazepine (CBZ-DiOH) has also been detected in Canadian surface waters (Ottonabee River in Peterborough, Ontario) (Miao and Metcalfe 2003), often at higher concentrations than CBZ itself (Kase *et al.* 2011; Miao and Metcalfe 2003). However, this transformation product (CBZ-DiOH) has been identified as biologically inactive (Leclercq *et al.* 2009; Miao and Metcalfe 2003). CBZ is designed to have pharmacodynamic effects and be bioactive, which is of concern for aquatic receptors (Kim *et al.* 2007; Laville *et al.* 2004), especially when biochemical pathways are evolutionarily conserved among organisms

(Gunnarsson *et al.* 2008). The mode of action in humans is believed to primarily occur via a use-dependent blockage of voltage-gated Na^+ channels (Galus *et al.* 2013a), and it has also been shown that CBZ can interact with GABA (gamma-aminobutyric acid) receptors (Kim *et al.* 2009), in both cases reducing neuronal excitability throughout the nervous system. GABA receptor homology has been shown to exist between humans, fish (Zebrafish, Japanese medaka, Fathead minnow), and amphibians (Western Clawed frog) (Christen *et al.* 2010). Homology does not imply that the receptor specific physiological function is identical in mammals and non-mammals, so the overall effects of CBZ to aquatic organisms may differ from mammals (Christen *et al.* 2010). Another suggested site of therapeutic action of CBZ in humans includes interaction with the adenylyl cyclase system and the consequent reduction of intracellular cyclic adenosine monophosphate (cAMP) levels (Chen *et al.* 1996; In: Martin-Diaz *et al.*, Montezinho *et al.* 2007). A study with molluscs conducted by Martin-Diaz *et al.* (2009) showed effects of CBZ on the cAMP pathway, indicating that the specific molecular target of the drug found in mammals is conserved also in mussels.

CBZ is somewhat lipophilic in nature (with a log Kow of 2.25 to 2.45, CBZ expresses both lipophilic and hydrophylic tendancies), and once it enters into surface waters, it may interfere with molecules, cells and organs of aquatic organisms, which may lead to biological effects, especially if the receptor in the target organism (human) is similar to target receptors found in aquatic organisms. Based on available (primary or secondary) data (summarized in the appendix), the sensitivities of invertebrates and fish to short-term CBZ exposures overlap, however invertebrates have the lowest effect concentrations. The sensitivities of invertebrates, fish and aquatic plants/algae to long-term CBZ exposures also overlap, with invertebrates again showing the highest sensitivity, followed by fish and then aquatic plants/algae. Fish have been shown to have a better ability to metabolize CBZ compared with invertebrates, thereby potentially explaining the increased sensitivity of invertebrates (Huggett *et al.* 2004).

The Canadian Council of Ministers of the Environment (CCME) 2007 protocol provides methods for the derivation of both a short-term benchmark concentration and a long-term water quality guideline. In the case of CBZ, there was insufficient primary data available to derive a short-term freshwater benchmark concentration. Most published short-term toxicity studies with CBZ utilized solvent to keep CBZ dissolved in solution, resulting in effect concentrations that were above the compound's natural solubility in water. Based on this, CBZ is unlikely to have acute toxic effects on aquatic organisms. In addition, due to the exceedence of solubility limit, these data could not be considered for the derivation of a short-term benchmark concentration.

A long-term freshwater Canadian Water Quality Guideline (CWQG) for CBZ for the protection of aquatic life was developed. The data requirements were not satisfied to derive a long-term freshwater CWQG using the species sensitivity distribution (SSD) approach. The reason for this was that most types of long-term toxicity studies conducted with CBZ resulted in endpoints (e.g., molecular markers, *in vitro* assays) not traditionally used in the development of CWQGs. Therefore, following the tiered approach, a long-term freshwater (Type B2) CWQG was developed. The long-term freshwater guideline value is summarized in the table below. The lowest acceptable endpoint from a long-term exposure was the critical study used in the derivation of the Type B2 long-term exposure guideline, to which a safety factor of 10 was applied (CCME 2007). The seven-day exposure with *C. dubia* by Ferrari *et al.* (2003, 2004) was

identified as the critical study, with a seven-day NOEC and LOEC of 25 and 100 µg/L for significant decrease in reproduction compared to controls. As per CCME (2007) protocol, the LOEC is to be used as the preferred endpoint. Applying a safety factor of 10 to the LOEC results in an CWQG of 10 µg/L.

Standard chronic toxicity tests may not utilize endpoints adequate for assessing specific effects associated with low-level exposure to pharmaceuticals such as CBZ. Standard tests assess impacts on apical endpoints such as mortality, growth and reproduction. Pharmaceuticals can elicit low dose effects due to being designed for biological activity. Perhaps more sensitive and specific endpoints would be more useful, such as developmental abnormalities, sex ratios or metabolic perturbations (e.g. biomarkers), where testing may cover multiple generations, but the ecological relevance of these would need to be established and validated (CCME 2007; Ferrari *et al.* 2003). Other sublethal endpoints to consider include behavior, immune function and fecundity (MacLeod *et al.* 2007).

Detection of the (biologically inactive) transformation product 10,11-dihydro-10,11-*trans*-dihydroxycarbamazepine (CBZ-DiOH) in surface waters has often been at higher concentrations than CBZ itself. However, concentrations of transformation products will not be incorporated into the CWQG value for CBZ. The toxicity of CBZ-DiOH to aquatic organisms has not been well established. In addition, analytical methods for quantification of CBZ-DiOH have been developed but are not yet adopted by regulatory agencies into accredited methods. Not including concentrations of transformation products into final guideline values is the same approach that is used for pesticides.

Type B2 Canadian water quality guideline for CBZ (µg/L) for the protection of aquatic life, developed using the 2007 CCME derivation protocol.

	Long-Term Exposure	Short-Term Exposure
Freshwater	10[a]	NRG
Marine	NRG	NRG

NRG = no recommended guideline

[a] = Type B2 guideline – The minimum toxicological data to derive a Type A or Type B1 guideline were not available. The lowest acceptable endpoint, e.g. the most sensitive preferred low-effects endpoint, from a long-term exposure study is the critical study used in the derivation of the Type B2 long-term exposure guideline. The endpoint concentration from this critical study is divided by a safety factor of ten to derive the long-term exposure guideline value. When more toxicity data become available, the Type B2 guideline can be upgraded to a Type B1 or Type A SSD-based value – CCME 2007).

There were insufficient data to develop either a short- or long-term marine CWQG for CBZ.

The highest CBZ concentration reported in Canadian surface waters was 988 ng/L (0.988 µg/L), measured in a river source water sample in southern Ontario (Kormos 2007 – Section 6.3). The highest CBZ concentration reported in Canadian effluent was 2,300 ng/L (2.3 µg/L) (Metcalfe *et al.* 2003b), well below the long-term guideline.

RÉSUMÉ

La carbamazépine (CBZ) est un médicament couramment prescrit comme antiépileptique (Miao et Metcalfe 2003), mais qui est aussi utilisé pour le traitement de la douleur associée à la névralgie faciale et comme agent psychotrope (Albani et coll. 1995, Deleu et coll. 2001, Duche et Loiseau 1995), pour le traitement de la schizophrénie (Emrich et coll. 1993; Okuma 1993) et de la bipolarité (Dilsaver et coll. 1996; Weissman et coll. 1988). Il a été suggéré que la CBZ, en combinaison avec d'autres médicaments, soit efficace pour traiter des symptômes du sevrage alcoolique (Franz et coll. 2001).

La quantité annuelle de CBZ utilisée au Canada en 2012 était d'environ 21 448 kg (IMS AG 2013). Pour une très grande part (> 20 000 kg/an) elle est utilisée par des particuliers, et le reste dans les hôpitaux. Les effluents des stations d'épuration des eaux d'égouts municipales constituent la principale voie de pénétration de la carbamazépine dans l'environnement aquatique, les autres voies possibles étant les débordements d'égouts pendant des événements pluvieux, les fuites des réseaux d'égouts, l'introduction dans des systèmes septiques, l'application de biosolides sur des terres agricoles (Bonvin 2013) et l'irrigation avec de l'eau recyclée (York Region 2014). Bien que l'élimination dans des lieux d'enfouissement puisse être une source d'inquiétude (Bonvin 2013), la surveillance de tels lieux en Ontario n'a pas permis de détecter de CBZ dans leur lixiviat (Stafford 2008). D'autres études pourraient être requises pour identifier les lieux d'enfouissement en tant que source potentielle. Une étude de la présence de la CBZ (et d'autres contaminants d'intérêt émergents) dans le lixiviat de lieux d'enfouissement a d'ailleurs été réalisée aux États-Unis (Masoner et coll. 2014). De la CBZ a été détectée dans 75 % des échantillons de lixiviat, avec une concentration maximale mesurée de 2,59 µg/L. Il y a une certaine production de CBZ au Canada (p. ex., des comprimés à croquer Sandoz®-CBZ et des comprimés à libération prolongée Sandoz®-CBZ sont produits au Québec) (Sandoz 2005). On prévoit que les rejets résultant de cette production seront très faibles (0,2 à 0,5 % du volume produit) (UEE-BECSN 2014).

Il a été montré que la carbamazépine est réfractaire à de nombreux procédés classiques d'épuration des eaux usées (Bonvin 2013), avec comme résultat qu'elle a été détectée dans les effluents de stations d'épuration des eaux d'égouts municipales (Brun et coll. 2006; Ferrari et coll. 2003; Metcalfe et coll. 2003a; Metcalfe et coll. 2003b), dans des biosolides (Smyth 2011), dans des eaux de surface (Hua et coll. 2006; Kleywegt et coll. 2011; Metcalfe et coll. 2003a; Tabe et coll. 2009), dans des eaux souterraines (Kormos 2007; Stafford 2008) et dans de l'eau potable (Kleywegt et coll. 2011). On en a aussi détecté dans des filets et du foie de poissons (Ramirez et coll. 2009), ainsi que dans des plantes cultivées dans des sols amendés avec des biosolides (Holling et coll. 2012).

La plus forte concentration de CBZ mesurée dans les eaux de surface au Canada était dans un réseau fluvial dans le sud de l'Ontario (en aval de 11 stations d'épuration des eaux d'égouts), pour lesquelles la concentration rapportée était d'environ 1 µg/L (988 ng/L; Kormos 2007). En Europe, on a aussi mesuré des concentrations de CBZ dans des eaux de surface dans la gamme du µg/L (Oetken et coll. 2005). La plus forte concentration de CBZ rapportée dans l'effluent d'une stations d'épuration des eaux d'égouts municipales au Canada est de 2,3 µg/L (Metcalfe et coll. 2003b). Le principal produit de transformation de la CBZ est la *trans*-10,11-dihydro-10,11-

dihydroxycarbamazépine (CBZ-DiOH), qui a aussi été détectée dans des eaux de surface au Canada (rivière Ottonabee à Peterborough, Ontario) (Miao et Metcalfe 2003), souvent à des concentrations plus élevées que celle de la CBZ (Kase et coll. 2011; Miao et Metcalfe 2003). Toutefois, ce produit de transformation (CBZ-DiOH) a été décrit comme biologiquement inactif (Leclercq et coll. 2009; Miao et Metcalfe 2003). La CBZ est conçue pour avoir des effets pharmacodynamiques et être bioactive, ce qui est inquiétant pour les récepteurs aquatiques (Kim et coll. 2007; Laville et coll. 2004), en particulier quand les voies biochimiques sont conservées d'un organisme à l'autre (Gunnarsson et coll. 2008). On pense que le mode d'action chez les humains a principalement lieu au moyen d'un blocage des canaux sodium voltage-dépendants activés (Galus et coll. 2013a), et il a aussi été montré que la CBZ peut interagir avec les récepteurs du GABA (acide gamma-aminobutyrique) (Kim et coll. 2009). Dans les deux cas il y a réduction de l'excitabilité neuronale dans l'ensemble du système nerveux. Il a été montré qu'il existe une homologie des récepteurs du GABA chez les humains, des poissons (poisson zèbre, medaka, tête-de-boule) et des amphibiens (dactylèthre) (Christen et coll. 2010). L'homologie n'implique cependant pas que la fonction physiologique spécifique du récepteur est identique chez les mammifères et les non-mammifères. Les effets globaux de la CBZ chez les organismes aquatiques pourraient être différents de ceux chez les mammifères (Christen et coll. 2010). Un autre site d'action thérapeutique de la CBZ chez les humains comprend l'interaction avec le système de l'adénylylcyclase et la réduction subséquente des niveaux d'adénosine monophosphate cyclique (AMPc) intracellulaire (Chen et coll. 1996; Montezinho et coll. 2007). Une étude sur des mollusques réalisée par Martin-Diaz et coll. (2009) a permis de démontrer les effets de la CBZ sur la voie de l'AMPc, indiquant ainsi que la cible moléculaire spécifique de ce médicament mise en évidence chez les mammifères était la même chez les moules.

La CBZ est de nature quelque peu lipophile (avec un log K_{oe} de 2,25 à 2,45, la CBZ a des tendances lipophiles et hydrophiles) et, une fois qu'elle pénètre dans des eaux de surface, elle peut interférer avec des molécules, des cellules et des organes d'organismes aquatiques. Ceci peut conduire à des effets biologiques, en particulier si le récepteur de l'organisme cible (humain) est similaire aux récepteurs cibles rencontrés dans des organismes aquatiques. D'après les données disponibles (primaires et secondaires résumées dans l'appendice), les sensibilités des invertébrés et des poissons aux expositions à court terme à la CBZ se chevauchent, les concentrations avec effet les plus faibles étant liées aux invertébrés. Les sensibilités des invertébrés, des poissons et des plantes aquatiques ou des algues à des expositions à long terme à la CBZ se chevauchent également, les invertébrés exhibant de nouveau la plus forte sensibilité, suivis des poissons et des plantes aquatiques ou des algues. Il a été montré que les poissons ont une meilleure capacité de métaboliser la CBZ, comparativement à celle des invertébrés, ce qui peut potentiellement expliquer la sensibilité plus grande des invertébrés (Huggett et coll. 2004).

Le protocole de 2007 du Conseil canadien des ministres de l'environnement (CCME) fournit des méthodes pour le calcul de la concentration de base à court terme et d'une recommandation pour la qualité des eaux à long terme. Dans le cas de la CBZ, il n'y avait pas assez de données primaires disponibles pour calculer une concentration de base pour l'eau douce à court terme. La plupart des études publiées sur la toxicité à court terme de la CBZ ont été réalisées avec un solvant afin que la CBZ demeure en solution, ce qui a entraîné des concentrations produisant un effet qui étaient supérieures à l'hydrosolubilité naturelle de ce composé. À partir de ces données, il est improbable que la CBZ ait des effets toxiques aigus sur les organismes aquatiques. De plus,

en raison du dépassement de la limite de solubilité, ces données ne pourraient pas être prises en compte pour le calcul d'une concentration de base à court terme.

Une recommandation canadienne pour la qualité des eaux douces (RCQE) à long terme en vue de la protection de la vie aquatique a été établie pour la CBZ. Les exigences sur les données n'étaient pas satisfaites pour pouvoir calculer une RCQE pour l'eau douce à long terme en suivant une approche de distribution de sensibilité des espèces (DSE), car dans la plupart des types d'études de toxicité à long terme réalisées sur la CBZ on a mesuré des indicateurs (p. ex., marqueurs moléculaire, épreuves *in vitro*) qui ne sont pas habituellement utilisés pour l'élaboration des RCQE. En conséquence, en suivant une approche par étapes, une RCQE provisoire (de type B2) pour l'eau douce à long terme a été calculée. La valeur de cette recommandation pour l'eau douce à long terme est donnée dans le tableau ci-après. L'indicateur d'effet acceptable le plus faible pour une exposition à long terme provenait de l'étude critique utilisée pour le calcul de la recommandation pour l'exposition à long terme de type B2, auquel un facteur de sécurité de 10 a été appliqué (CCME, 2007). L'étude de Ferrari et coll. (2003, 2004) sur une exposition de 7 jours de *C. dubia* a été déterminée comme l'étude critique, au cours de laquelle une CSEO et une CMEO à 7 jours de 25 et 100 µg/L respectivement ont été déterminées pour une diminution significative de la reproduction en comparaison de celle de témoins. Conformément au protocole du CCME (2007), la CMEO doit être utilisée comme indicateur de préférence. En appliquant un facteur de sécurité de 10 à la CMEO, nous avons calculé une RCQE de 10 µg/L.

Il se peut que les indicateurs utilisés dans les épreuves standards de toxicité chronique ne soient pas pertinents pour l'évaluation d'effets spécifiques associés à une exposition à faible niveau à des produits pharmaceutiques comme la carbamazépine. Les épreuves standards servent à évaluer les incidences sur des indicateurs apicaux comme la mortalité, la croissance et la reproduction. Les composés pharmaceutiques, étant spécifiquement conçus pour une activité biologique particulière, peuvent déclencher des effets à faible dose. Des indicateurs spécifiques et plus sensibles seraient peut-être plus utiles, tels les anomalies du développement, les rapports entre les sexes et les perturbations métaboliques (p. ex., bioindicateurs), lorsque les tests couvrent plusieurs générations, mais la pertinence écologique de ceux-ci devrait être établie et validée (CCME 2007; Ferrari et coll. 2003). Parmi d'autres indicateurs sublétaux à prendre en compte, il y a le comportement, la fonction immunitaire et la fécondité (MacLeod et coll. 2007).

On a souvent détecté le produit de transformation (biologiquement inactif), la *trans*-10,11-dihydro-10,11-dihydroxycarbamazépine (CBZ-DiOH), dans des eaux de surface à des concentrations supérieures à celles de la CBZ. Toutefois, les concentrations des produits de transformation ne seront pas incluses dans la valeur de la RQEC provisoire pour la CBZ. La toxicité de la CBZ-DiOH pour les organismes aquatiques n'est pas encore bien établie. De plus, bien que des méthodes d'analyse pour la quantification de la CBZ-DiOH aient été élaborées, elles ne sont pas encore adoptées par les organismes de réglementation comme méthodes accréditées. La non-intégration des concentrations des produits de transformation dans la recommandation finale suit une approche similaire à celle suivie pour les pesticides.

Recommandation canadienne pour la qualité des eaux de type B2 pour la CBZ (µg/L) en vue de la protection de la vie aquatique, élaborée en suivant le protocole de calcul de 2007 du CCME.

	Exposition à long terme	Exposition à court terme
Eau douce	10[a]	PR
Eau marine	PR	PR

PR = pas de recommandation

[a] = Recommandation de type B2 – Les données toxicologiques minimales pour pouvoir calculer une recommandation de type A ou de type B1 n'étaient pas disponibles. L'indicateur acceptable le plus faible, à savoir l'indicateur pour effets faibles préféré le plus sensible, tiré d'une étude d'exposition à long terme, est l'étude critique utilisée pour le calcul de la recommandation de type B2 pour l'exposition à long terme. La concentration d'indicateur dérivée de cette étude a été divisée par un facteur de sécurité de 10 pour calculer la valeur de la recommandation pour l'exposition à long terme. Quand plus de données sur la toxicité seront disponibles, la recommandation de type B2 sera remplacée par une recommandation de type B1 ou de type A basée sur la DSE (CCME 2007).

Il n'y avait pas assez de données pour élaborer une RCQE marine à court ou long terme pour la carbamazépine.

La plus forte concentration de CBZ rapportée pour des eaux de surface au Canada était de 988 ng/L (0,988 µg/L), mesurée dans un échantillon d'eau de rivière servant de source d'alimentation en eau potable dans le sud de l'Ontario (Kormos 2007 – section 6.3). La plus forte concentration de CBZ rapportée dans des effluents au Canada était de 2 300 ng/L (2,3 µg/L) (Metcalfe et coll. 2003b).

1.0 INTRODUCTION

The Canadian Water Quality Guidelines (CWQG) for the Protection of Aquatic Life are developed through compilation and interpretation of aquatic toxicity data, thereby providing an important tool in the evaluation of ambient water quality. CBZ concentrations monitored in the environment can be compared to the guideline value to help predict whether sensitive species will be impacted in the ecosystem. Exceedance of the guideline values does not denote definite negative impacts to the environment, but rather that further investigation is necessary, for example site-specific analysis of water chemistry parameters and sensitive species residing in the ecosystem.

In 2007, CCME revised the Protocol for the Derivation of Water Quality Guidelines for the Protection of Aquatic Life. The goals of the revised protocol include: (i) accounting for the unique properties of contaminants which influence their toxicity; and (ii) incorporating the species sensitivity distribution (SSD) method, which uses acceptable data as outlined in the protocol (provided these data pass quality control criteria) in a more flexible approach.

The structure of this document follows specifications of the CCME 2007 protocol. It includes sections related to physical and chemical properties, production and uses, environmental fate and behaviour, environmental concentrations, toxicity data, bioaccumulation/bioconcentration, and toxicity modifying factors. It is the first CWQG derived for a pharmaceutical.

2.0 PHYSICAL AND CHEMICAL PROPERTIES

2.1 Identity

Carbamazepine (CAS No. 298-46-4) or 5H-Dibenzo[b,f]azepine-5-carboxamide (IUPAC name) is a tricyclic neutral lipophilic compound, which can cross membranes easily (Myllynen 2003). CBZ is commonly prescribed as an antiepileptic (Miao and Metcalfe 2003), but is also used for the treatment of pain associated with trigeminal neuralgia and as a psychotropic agent (Albani *et al.* 1995; Deleu *et al.* 2001; Duche and Loiseau 1995), in the treatment of schizophrenia (Emrich *et al.* 1993; Okuma 1993), and bipolar disorder (Dilsaver *et al.* 1996; Weissman *et al.* 1988). It has been suggested that CBZ, in combination with other drugs, is effective at treating symptoms of alcohol withdrawal (Franz *et al.* 2001).

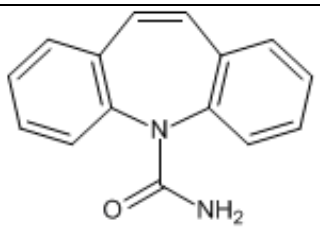
As of 2007, 33 products containing CBZ molecule were in use in Canada, of which 85% was dispensed through retail pharmacy, with the remainder used in hospitals (EAU-NSACB 2014). Approximately 28,000 kg of CBZ was sold for therapeutic use in Canada in 2001 (Miao *et al.* 2005). Total sales in Canada for a) 2007 were 24,818 kg (21,095 kg to pharmacies and 3,723 kg to hospitals), b) 2011 were 21,350 kg (20,624 kg to pharmacies and 726 kg to hospitals), and c) 2012 were 21,448 kg (20,773 kg to pharmacies and 675 kg to hospitals) (IMS AG 2013b). As of 2007, seven products on the market containing the CBZ transformation product Oxcarbazepine were in use in Canada (1415.5 kg), of which 92% was dispensed through retail pharmacy and the remainder used in hopitals (EAU-NSACB 2014).

CBZ is a white to off-white crystalline powder. It has a fairly low solubility in water (Table 2.1) and is highly soluble in alcohol (1:10), chloroform (1:10) and acetone (MSDS Santa Cruz Biotech 2010). CBZ has an estimated melting point of 190.2 °C (SMDS 2010). It has a Henry's law constant reported as ranging from $1.09x10^{-5}$ Pa m^3 mol^{-1} (Zhang *et al.* 2008) to $1.57x10^{-4}$ Pa m^3 mol^{-1} (SMDS 2010) and a vapour pressure of $1.17x10^{-5}$ (SMDS 2010). These values indicate that it has low potential for volatilization. The low K_d (solid liquid partitioning coefficient) values for CBZ indicate that it will preferentially stay in solution, where some partitioning to soil/sediment may be observed (Ternes *et al.* 2004). The same can be concluded from the low to moderate K_{oc} (sediment-water partition coefficient) value (SMDS 2010).

The toxicity of organic compounds to aquatic receptors is assumed to be related, in part, to their hydrophobicity (as represented by the log Kow value). Compounds with a log Kow of greater than 1.72 are associated with higher bioavailability, having greater capability to penetrate fatty acid membranes (Kim *et al.* 2009). However, the log Kow value for CBZ (2.25-2.45) is still considered to be low, which indicates that this compound will likely partition to an aqueous phase more so than an organics phase (Zhang *et al.* 2008). What has been proposed is a pH-corrected octanol-water partition coefficient (Dow) that may be a better estimate for uptake potential of pharmaceuticals (Meredith-Williams *et al.* 2012). Ionisable pharmaceuticals have been shown to be very sensitive to changes in pH in the environment, affecting uptake and resulting toxicity. However based on the high pKa value of CBZ, and under the pH range of most surface waters (pH 6-8), CBZ is expected to exist in neutral form (will not ionize) (Lahti 2012).

CBZ has a biological pseudo first order degradation rate constant (kbiol) of <0.01 L/gSS-1 d-1. Substances with a kbiol of <0.1 L/gSS-1 d-1 are not expected to undergo any significant degradation in typical municipal wastewater treatment plants (Joss *et al.* 2005). CBZ has a kO3 value greater than 5x104 M-1s-1, indicating that ozonation will be efficient at transforming this compound (CBZ will easily be oxidized by ozone) (Huber *et al.* 2003). The nonaromatic double bonds in CBZ are responsible for the high reactivity with ozone. (Tabe *et al.* 2009). A study by Tootchi *et al.* (2013) demonstrated that 100% of CBZ was oxidized by two major degradation pathways employed at drinking water treatment facilities. Oxidation occurred through both direct reaction with ozone and reaction with hydroxyl radicals formed during ozone decomposition. Direct reaction with ozone produced two transformation products, BQM (1-(2-benzaldehyde)-4-hydro-(1H,3H)-quinazoline-2-one) and BQD (1-(2-benzaldehyde)-(1H,3H)-quinazoline-2,4-dione) (Figure 2.1). These two transformation products were either at very low levels or were non-detectable following CBZ reaction with hydroxyl radicals, indicating that BQM and BQD are susceptible to degradation (Tootchi *et al.* 2013).

Table 2.1. Physical and chemical properties of CBZ.

Property	Description[2]		Reference
Synonyms	Tegretol® and multiple generic names (Apo-carbamazepine, Dom-carbamazepine, Mazepine, Mylan-carbamazepine, PMS-carbamazepine, Sandoz-carbamazepine, Taro-carbamazepine, Teva-carbamazepine)		Health Canada 2013
IUPAC name	5H-dibenzo[b,f]azepine-5-carboxamide		Miao and Metcalfe 2003
Structure			PhACT® 2009
Molecular Formula	$C_{15}H_{12}N_2O$		PhACT® 2009
SMILES code	NC(=O)N2c1ccccc1C=Cc3ccccc23		RIVM 2014
Molecular Weight	236.27 g mol^{-1}		PhACT® 2009
Water Solubility	17.7 mg L^{-1} (25°C) (estimated); 112 mg L^{-1} (exp);		Zhang *et al.* 2008; RIVM 2014
Melting point	190.2 °C (est)		RIVM 2014
Boiling point	410.02 °C (est)		RIVM 2014
Vapour Pressure (Pa)	$1.17x10^{-5}$ (est) (25°C)		RIVM 2014
Henry's Law Constant[7] ($Pa/m^3.mol$)	$1.09x10^{-5}$(25°C); $1.10x10^{-5}$ (est) to $1.57x10^{-4}$ (est)		Zhang *et al.* 2008; RIVM 2014
Kinetic constant for pseudo first order degradation ($k_{i,biol}$) (L/gSS^{-1} D^{-1})	<0.01		Ternes *et al.* 2005
Octanol-Water partition coefficient (log K_{ow})	2.25 (est using EPI Suite 4.0) & 2.45 (exp)		RIVM 2014
BCF (measured)	61.3 (pH=1) 63.2 (pH=4-10) 0.8-4.2		ACS Daten Bank 2004 ACS Daten Bank 2004 Fick 2010
Volume of Distribution (V_D)	1.5±0.3 (l kg^{-1})[d]		Meredith-Williams *et al.* 2012
Sediment-Water partition coefficient (log K_{oc})	2.227 (est) to 3.123 (est)		RIVM 2014
Distribution coefficient (K_D)	5.32 (sandy sediment); 19.8 (biosolid amended soil); <20 (primary sludge); 1.2±0.5 (secondary sludge)		Sheytt *et al.* 2005; Williams *et al.* 2006; Ternes *et al.* 2004; Ternes *et al.* 2004
Second order rate constant (k_{O3}) of ozonation process	$3x10^5$		Huber *et al.* 2003

(T=20ºC) ($M^{-1}s^{-1}$)			
p*Ka*	Neutral (at environmentally relevant pH conditions); 15.37 (est), 13.94		Zhang *et al.* 2008; RIVM 2014
Elimination half-life (human)	25-65 h		Zhang *et al.* 2008
Excretion (human)	Percent of oral dosage excreted in urine (72%) -less than 1% is excreted as parent CBZ compound in urine Percent of oral dosage excreted in faeces (28%) -unabsorbed parent CBZ compound makes up approximately 13% of the total dose in faeces		Bahlmann *et al.* 2014
Metabolites[1] in urine (% of oral dosage) (human)	CBZ[5] = 0.8% CBZ-epoxide[5,6] = 1.4% CBZ-DiOH[5] = 32% CBZ-N-glucuronide = 11% 1-OH-CBZ = 2-10% 2-OH-CBZ = 4.3% 3-OH-CBZ = 5.1% 4-OH-CBZ = <2% 9-HMCA = 5.2% Hydroxymethoxy-CBZ [sum] = <3% Methylsulfinyl/-sulfonyl-CBZ [sum] = <5.6% 10-OH-CBZ = <0.1% Other = <2%		Bahlmann *et al.* 2014
Metabolites[1] in faeces (% of oral dose) (human)	CBZ = 13% Other <2% Unknown = 15%		Bahlmann *et al.* 2014
Dosage (human)	Maintenance usually 800-1,200 mg daily		Zhang *et al.* 2008

[1]CBZ (carbamazepine) (CAS No. 298-46-4)
CBZ-epoxide (10,11-dihydro-10,11- epoxycarbamazepine) (CAS No. 36507-30-9)
CBZ-DiOH (*trans*-10,11-dihyrdo-10,11-dihydroxycarbamazepine) (CAS No. 58955-93-4)
2-OH-CBZ (2-hydroxy-carbamazepine) (CAS No. 68011-66-5)
3-OH-CBZ (3-hydroxy-carbamazepine) (CAS No. 68011-67-6)
4-OH-CBZ (4-hydroxy-carbamazepine)
9-HMCA (9-hydroxymethyl-10-carbamoylacridan)
10-OH-CBZ (10,11-dihydro-10-hydroxy-CBZ)

[2] est = estimated, exp = experimentally derived

[3] *SS* suspended solids concentration [gSS/L]

[4] The geometric mean of all valid water solubility values will be used to compare toxicity data effect concentrations.

[5] These are the only unconjugated metabolites found in human urine. All others are conjugated to glucuronide (Maggs *et al.* 1997).

[6]The only transformation product known to be bioactive (Zhang *et al.* 2008)

[7] The Henry's constant typically increases with increasing temperature until a maximum is reached, then the value decreases with a further increase in temperature. See "Avoid common pitfalls when using Henry's Law" (Smith and Harvey 2007).

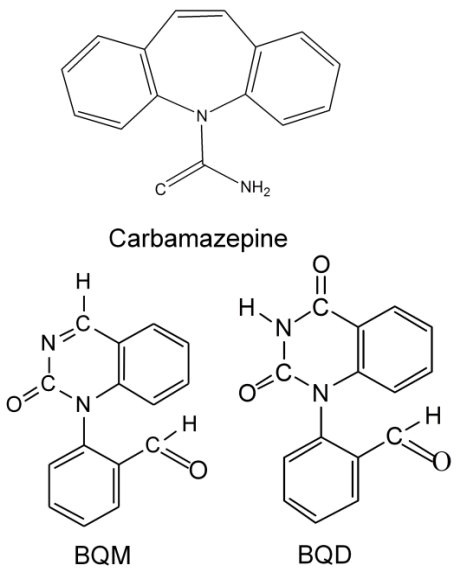


Figure 2.1. Structures of two major CBZ transformation products, BQM and BQD, following oxidation (Tootchi *et al.* 2013).

2.2 Analytical Methods

A standardized method (1694) developed by the US Environmental Protection Agency (US EPA 2007) is used to detect CBZ (and other pharmaceuticals and personal care products) in aqueous, soil, sediment and biosolids materials. The method employs high performance liquid chromatography combined with tandem mass spectrometry (HPLC/MS/MS) using isotope dilution and internal standard quantitation techniques (US EPA 2007). The method detection limits (MDL) and minimum levels of quantitation (ML) are presented as concentrations at which CBZ can be detected in the absence of interferences. The MDL and ML for water are 1.4 and 5 ng/L, respectively. For other samples (soil, sediment and biosolids) the MDL and ML are 1.6 and 5 ng/g, respectively. The MDL and ML for extract are 0.4 and 1.25 ng/L, respectively.

The Ontario Ministry of the Environment and Climate Change Laboratory Services Branch (LaSB) method E3454 is used for analyzing trace concentrations of pharmaceuticals and personal care products (PPCPs), and endocrine disruptors (EDCs) in aqueous samples (MOECC 2012). This method uses solid phase extraction (SPE) to extract the target compounds (analytes) from a sample (e.g., water, effluent) and analyses them using high performance liquid chromatography tandem mass spectrometry (LC-MS/MS) technology. The method detection limit for CBZ is 2 ng/L.

The Water Quality Centre of Trent University in Peterborough, ON developed a method for detection of CBZ in biosolids. Analytes are extracted by pressurized liquid extraction (PLE) followed by solid-phase extraction (SPE) cartridges for cleanup of the extracts. Liquid chromatography-electrospray tandem mass spectrometry (LC-ES-MS/MS) is used to analyze the extracts (Miao and Metcalfe 2003). For raw biosolids, the limit of detection (LOD) and limit of

quantification (LOQ) are 0.15 ug/kg wet weight (ww) and 0.50 ug/kg ww, respectively. For treated biosolids, the LOD and LOQ are 0.17 ug/kg ww and 0.58 ug/kg ww, respectively.

Lajeunesse *et al.* (2010) developed a method for detection of CBZ in small aquatic tissue samples (specifically developed for the crustacean *Thamnocephalus platyurus* and the algae *Pseudokirchneriella subcapitatata*) to the ng/L level (LOD = 0.2 ng/L). The method involves liquid chromatography-tandem mass spectrometry (LC-MS/MS), and has been fully validated.

Dussault *et al.* (2009) derived an analytical method for the extraction and determination of CBZ (and three other pharmaceuticals and personal care products) from samples of water, sediment and biological tissue. Water samples are analyzed using SPE, sediment are extracted using PLE, and biota are extracted by liquid extraction. Analysis is conducted using tandem LC-MS in electro-spray ionization mode. Since tandem LC-MS is susceptible to matrix effects, the impact of water (Milli-Q, reconstituted water), sediment and biological tissue (*Chironomus tentans, Hyalella azteca*) on analysis was assessed. The limit of detection (LOD; µg/L) and limit of quantification (LOQ; µg/L) for CBZ in these various matrices was presented as: Milli-Q water (1.13, 3.76), reconstituted water A (0.82, 2.74), reconstituted water B (1.01, 3.35), sediment (7.99, 26.63), *C. tentans* (0.86, 2.87) and *H. azteca* (1.10, 3.66).

A method for the detection of five CBZ transformation products (in addition to CBZ) was developed by Miao and Metcalfe (2003) in aqueous samples (influent, effluent, surface water) using SPE followed by LC-ES-MS/MS. The transformation products include 10,11-dihydro-10,11-epoxycarbamazepine, 10,-11-dihydro-10,11-dihydroxycarbamazepine, 2-hydroxycarbamazepine, 3-hydroxycarbamazepine, and 10,11-dihydro-10-hydroxycarbamazepine. Leclercq *et al.* (2009) applied the liquid chromatography–electrospray ionization mass spectrometry method, described in Breton *et al.* (2005), to detect the presence of CBZ, oxcarbazepine, and seven of their transformation products (carbamazepine-10,11-epoxide, 10-hydroxy-10,11-dihydrocarbamazepine, 10,11-dihydro-10,11-trans-dihydroxycarbamazepine, 2-hydroxycarbamazepine, iminostilbene, acridine, and acridone) in both wastewater influent and effluent. Writer *et al.* (2013) used LC-MS/MS to determine concentration of parent compound CBZ and LC–TOF-MS (liquid chromatography time of flight mass spectrometry) to identify concentrations of transformation products (DiOH-CBZ, 10-OH-CBZ, oxcarbazepine) in municipal wastewater effluent and associated receiving waters.

3.0 PRODUCTION AND USES

CBZ has been in use as an anticonvulsant for more than thirty years (Bernus *et al.* 1996). CBZ was synthesized in Switzerland in 1953, approved in the United Kingdon in 1965, in the United States in 1974, and has likely been in use in Canada since the 1970s (EAU-NSACB 2014). Approximately 28,000 kg of CBZ was sold for therapeutic use in Canada in 2001 (Miao *et al.* 2005), with the majority (>20,000 kg/yr) being used in the private home and the remainder in hospital (IMS AG 2013a). Total sales in Canada for 2007 was 24,818 kg (21,095 kg to pharmacies and 3,723 kg to hospitals), for 2011 was 21,350 kg (20,624 kg to pharmacies and 726 kg to hospitals) and for 2012 was 21,448 kg (20,773 kg to pharmacies and 675 kg to hospitals) (IMS AG 2013b). No survey data on the manufacture or import of CBZ in Canada was located

(Marthaler 2013). There is some manufacturing of CBZ in Québec, Canada (e.g., Sandoz®-CBZ chewable tablets and Sandoz®-CBZ controlled release tablets) (Sandoz 2005). Finer scale information related to sales data on a provincial or municipal level is available from the firm IMS Health at a cost (Lehner *et al.* 2013).

4.0 SOURCES TO THE ENVIRONMENT

CBZ is a pharmaceutical prescribed to humans. Following therapeutic use, CBZ is metabolized predominantly in the liver, undergoing hepatic metabolism by the cytochrome P450 system (Miao and Metcalfe 2003), referred to as Phase I transformation (Bonvin 2013). An additional Phase II transformation process can occur, known as conjugation (addition of glucuronic acid to a susceptible functional group) (Bonvin 2013). At least 30 metabolites have been identified in humans, including some pharmacologically active (carbamazepine-10,11-epoxide, Cbz-Ep) and genotoxic substances (acridine and acridone) (Leclercq *et al.* 2009). The metabolite CBZ-DiOH has been identified as being essentially biologically inactive (Leclercq *et al.* 2009; Miao and Metcalfe 2003). Concentrations of CBZ-DiOH an order of magnitude higher than the CBZ effective dose were required to produce an effect in rats and mice (reduction in high-frequency repetitive neuronal firing) (McDonald 2002). Not all metabolites are excreted; many are intermediates between phase I and phase II metabolism (McKague 2014). These (and whatever remains of the parent compound) are excreted in urine or feces, flushed down the toilet, and enter wastewater treatment plants from residential dwellings and institutional sources (e.g., human waste from hospitals, long-term care facilities and veterinary clinics) (Kleywegt 2013). Disposal of excess or expired medication in the sink or toilet is another way in which CBZ enters wastewater treatment plants. In Ontario pharmaceutical waste, including sharps, from the residential sector have been accepted by pharmacies since the year 2000. These wastes are collected and sent off-site to commercial incineration facilities.

Once at the wastewater treatment plant, a variety of treatment processes are employed at individual facilities to remove contaminants. Not all of these treatment processes are successful at removing compounds such as CBZ. This results in the introduction of CBZ and transformation products (both biotically and abiotically derived) into lakes and rivers following incomplete removal by wastewater treatment. CBZ can also be introduced to surface waters through combined sewer overflows during rain events during which treated, partially treated, and/or raw sewage is released to lakes and rivers (Sauvé *et al.* 2012). Leaking sewage systems can also be a source of CBZ introduction into ground and eventally surface waters (Osenbrück *et al.* 2007). Use of reclaimed water for irrigation purposes on agricultural (non-human consumption) cropland, golf courses and for landscape irrigation can also be a source of CBZ introduction into the environment (York Region 2014).

CBZ and transformation products can also be introduced in groundwater. A study by Godfrey *et al.* (2007) detected CBZ in septic effluent and in two aquifers overlain by septic systems in western Montana. Another study assessing concentrations of CBZ (and other organic wastewater contaminants) in a shallow sand and gravel aquifer showed detection (maximum measured CBZ concentration of 72 ng/L) with septic systems identified as the primary sources (Schaider *et al.* 2014). A reconnaissance study was conducted in the United States by Focazio *et al.* (2008),

where 25 groundwater (as well as 49 surface water) sources of drinking water were sampled and analyzed for pharmaceuticals and other wastewater organic contaminants. CBZ was among the most frequently detected compounds in groundwater (20%) and surface water (22%) sites. The transport of effluent-derived pharmaceuticals from a creek (during effluent dominant flow conditions) to shallow groundwater was examined by Bradley *et al.* (2014). The results of this study showed that infiltration of effluent contaminated surface water (Fourmile Creek in Iowa, United States) resulted in CBZ detection in groundwater at a concentration greater than 0.02 µg/L at distances of up to 6 m from the stream bank.

There is also potential for CBZ to enter the environment via biosolids that are disposed of in landfills, or via biosolids that are applied onto agricultural land. Disposal of excess or expired CBZ medication in household waste will make its way to landfill sites (solid waste disposal), with potential subsequent runoff to surface waters, or leaching to ground water (and potential introduction to surface water) (Ternes 1998) (see Section 6.0 for monitoring data). However, monitoring of landfill leachate in Ontario has not detected CBZ (Stafford 2008). Further investigation may be warranted to identify landfills as a potential source. An examination of CBZ (and other contaminants of emerging concern) in landfill leachate was conducted in the United States (Masoner *et al.* 2014). CBZ was detected in 75% of leachate samples, with a maximum measured concentration of 2.59 µg/L. Out of a total of 202 contaminants of emerging concern, 129 were detected in 19 landfills (12 municipal and 7 private) in the study. There is some manufacturing of CBZ in Canada (see Section 3.0). It is expected that manufacturing releases are very low (0.2 - 0.5% of product volume) (EAU-NSACB, 2014). However, releases from pharmaceutical formulation facilities have been identified as being 10 to 1,000 times higher in concentration when compared to releases of wastewater treatment effluents not influenced by these manufacturing facilities (Phillips *et al.* 2010).

5.0 ENVIRONMENTAL FATE AND BEHAVIOUR

5.1 Transformation Products

CBZ is highly metabolized in the human body, by the liver. Over 30 CBZ transformation products have been identified in humans, excreted via urine and feces (Table 2.1). Five major products have been identified in both aqueous (Bahlmann *et al.* 2014; Miao *et al.* 2005) and solid (Kinney *et al.* 2006; Miao *et al.* 2005) phases of wastewater treatment plant processes. Of the five, 10,11-dihydro-10,11-*trans*-dihydroxycarbamazepine (CBZ-DiOH) (CAS No. 58955-93-4) is one of the main transformation products found in human urine and plasma (Leclercq *et al.* 2009), and it can also form during wastewater treatment (López-Serna *et al.* 2012). CBZ-DiOH has been detected at higher concentrations in wastewater effluent, when compared to the parent compound CBZ (Leclercq *et al.* 2009; Miao *et al.* 2005) (Table 5.1).

Table 5.1. Mean concentration (with either ± standard deviation or range) of CBZ and its transformation product CBZ-DiOH (ng/L) as measured at various stages of wastewater treatment.

Sample Location	Sample and Units	CBZ (anticonvulsant)	CBZ-DiOH (transformation product)	Reference
Peterborough WWTP, ON, Canada[1]	Untreated wastewater (ng/L)	356.1 (±5.8)	1001.2 (±12.5)	Miao *et al.* 2005
Peterborough WWTP, ON, Canada[1]	Treated wastewater (ng/L)	251.0 (±6.3)	1081.2 (±13.0)	Miao *et al.* 2005
Peterborough WWTP, ON, Canada[1]	Untreated biosolids (ug/kg dry weight)	69.6 (±2.2)	7.5 (±0.7)	Miao *et al.* 2005
Peterborough WWTP, ON, Canada[1]	Treated biosolids (ug/kg dry weight)	258.1 (±4.7)	15.4 (±1.3)	Miao *et al.* 2005
France, WWTP-1[2]	Influent (ng/L)	235 (235-236)	1415 (1370-1460)	Leclercq *et al.* 2009
France, WWTP-1[2]	Effluent (ng/L)	258	1500	Leclercq *et al.* 2009
France, WWTP-2[3]	Influent (ng/L)	208 (193-224)	333 (323-344)	Leclercq *et al.* 2009
France, WWTP-2[3]	Trickling filter Effluent (ng/L)	146 (145-147)	896 (809-984)	Leclercq *et al.* 2009
France, WWTP-3[4]	Influent (ng/L)	416 (412-420)	325 (264-386)	Leclercq *et al.* 2009
France, WWTP-3[4]	Effluent (ng/L)	112 (86-139)	311 (311-311)	Leclercq *et al.* 2009

[1] Peterborough WWTP employed secondary (activated sludge) treatment with nitrification plus UV disinfection and an HRT of 12-18 hours.
[2] WWTP 1 employed biological treatment in activated sludge with a three-day HRT.
[3] WWTP 2 employed a trickling filter (two-day HRT) followed by a post-tertiary pond (20-day HRT), leading to a total 22-day HRT
[4] WWTP 3 employed a series of different waste stabilization ponds with depths between 1.4 and 3.1 m, with a total HRT of 78 days.

CBZ and metabolites in effluent from 24 municipal wastewater treatment plants in the State of Minnesota were measured by Writer *et al.* (2013). Concentrations (reported as the mean ± standard deviation) for CBZ and its major metabolites 10-OH-CBZ (10-hydroxy-carbamazepine) and CBZ-DiOH were 480 ±380 ng/L, 360 ± 400 ng/L, 120 ±100 ng/L. This indicates that in effluent, 10-OH-CBZ is generally the dominant metabolite, followed by CBZ-DiOH. Two potential factors that may be responsible for this higher 10-OH-CBZ concentration are 1) this transformation product is being transformed from oxcarbazepine (increasingly prescribed as an alternative to carbamazepine), or 2) microbial formation of 10-OH-CBZ is occurring during wastewater treatment. Unfortunately, influent concentrations were not measured. A similar finding of high 10-OH-CBZ concentrations (up to 1,170 ng.L) in wastewater effluent was reported by Leclercq *et al.* (2009), attributed to the rise in oxcarbazepine prescriptions. This was much higher than the highest reported 10-OH-CBZ concentration of 93 ng/L by Miao and Metcalfe (2003).

CBZ-DiOH has been reported as the only transformation product detected in surface waters, with concentrations exceeding those of the parent product (Kase *et al.* 2011; Miao and Metcalfe 2003) (Table 5.2). However, Writer *et al.* (2013) reported that 10-OH-CBZ is the major CBZ metabolite detected in Minnessota surface waters. Reporting on the occurrence and distribution of 58 parent pharmaceuticals and associated metabolites and transformation products in the Ebro River (Spain) and its tributaries included CBZ and three metabolites (2-OH-CBZ, 3-OH-CBZ, 10,11-epoxi-CBZ) and two transformation products formed during wastewater treatment (acridone and acridin) (López-Serna *et al.* 2012). The three metabolites can also be produced as transformation products during wastewater treatment, with only 10,11-epoxi-carbamazepine having pharmacological activity. The range in measured concentrations (ng/L) of these substances was much higher in the tributaries when compared to the Ebro River. Maximum concentrations (ng/L) reported in the tributaries were: CBZ (90.4), 2-OH-CBZ (61.7), 3-OH-CBZ (70), 10,11-epoxi-CBZ (1,667), acridone (not detected) and acridin (16.8). Maximum concentrations (ng/L) reported in the Ebro River were: CBZ (11.9), 2-OH-CBZ (5.38), 3-OH-CBZ (4.57), 10,11-epoxi-CBZ (214), acridone (17.5) and acridin (9.64).

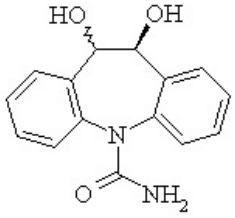
If the toxicity of the transformation products is found to be similar to or greater than CBZ, it may be of similar or even more concern when compared to the parent product CBZ (SMDS 2011). Much discussion exists in determining whether or not both parent compounds and transformation products are equally important for consideration in risk assessment, because these mixtures may show additive or synergistic toxicity. Some have suggested that there may be a need to consider the cumulative concentrations of CBZ and transformation products (e.g., 10-OH-CBZ, CBZ-DiOH and others) and not each chemical individually. For the purposes of deriving a CWQG, the same approach is followed as has been used for pesticides, where only the parent compound is taken into consideration.

Predicting environmental concentrations of CBZ and associated transformation products in aquatic receivers was conducted by Fenet *et al.* (2014), where consumption data and pharmacokinetics data was used to predict transformation product emissions from municipal wastewater treatment plants. Modelling was then used to predict environmental concentrations in a Mediterranean coastal zone, and compared with measured concentrations.

Table 5.2. Comparing concentrations of CBZ (ng/L) and its transformation product CBZ-DiOH (ng/L) as measured in Swiss surface waters and wastewater treatment plant effluents (Kase *et al.* 2011).

	Carbamazepine	**CBZ-DiOH**
CAS	298-46-4	58955-93-4
Group of substance	Anticonvulsant	Transformation product
Surface water **# detected / # measurements**	112/509	4/4
Surface water **Average concentration**	13	490
Surface water **90% percentile concentration**	43	1011
WWTP effluent **# detected / # measurements**	78/78	6/6
WWTP effluent **Average concentration**	482	1551
WWTP effluent **90% percentile concentration**	790	1882

Table 5.3. Physical and chemicals properties of CBZ-DiOH (SMDS 2011).

Property	**Description[1]**
Name	Trans-10,11-Dihydroxy-10,11-Dihydroxycarbamazepin; Carbamazepin-10,11-Dihydro-10,11-Dihydroxy
IUPAC name	(5S,6S)-5,6-dihydroxy-5,6-dihydrobenzo[b][1]benzazepine-11-carboxamide
CAS Number	58955-93-4
Structure	
Molecular formula	$C_{15}H_{14}N_2O_3$
SMILES code	c1ccc2c(c1)C(C(c3ccccc3N2C(=O)N)O)O
Molecular weight	270.28 $g \cdot mol^{-1}$
Water solubility	103.9 $mg \cdot L^{-1}$ (est)
Melting point	203.81°C (est); 208-210°C (not specified)
Boiling point	481.18°C (est)
Vapour pressure	4.05 E-10 Pa (est)
Octanol-water partition coefficient	log Kow= -0.21 (est)
Sediment-water partition coefficient	log Koc= -0.067 (est); log Koc= 1 (est)
Henry's law constant	1.054 E-09 $Pa \cdot m^3 \cdot mol^{-1}$ (est)
p*Ka*	13.72 (est)

[1] est = estimated via EPI Suite 4.0

In comparison to the parent compound carbamazepine, CBZ-DiOH is more polar due to being doubly hydroxylated (Löffler *et al.* 2005). CBZ-DiOH has an estimated melting point of 203.81 °C (SMDS 2010) (Table 5.3). It has a Henry's law constant reported as being 1.054 E-09 Pa m^3 mol^{-1} and a vapour pressure of 4.05 E-10. These values indicate that it has low potential for volatilization. The low K_{oc} (sediment-water partitioning coefficient) indicates that it will preferentially stay in solution, rather than partition to sediment or suspended solids. The low K_{ow} value also indicates that CBZ-DiOH is hydrophilic and will partition to water-based substances more so than organics. A log K_{ow} value of less than 2.5 indicates that a compound will have low potential for adsorption onto particulates (Miao *et al.* 2005).

5.2 Fate of Carbamazepine in Wastewater Treatment Facilities

CBZ has been detected in wastewater treatment plant influent and effluent in many countries including Canada, United States, Australia, France, Italy, Spain, Japan, and Finland, with highest concentrations being reported in Germany, Sweden, and Austria (Leclercq *et al.* 2009; Tabe *et al.* 2009). A thorough review of international literature published between 2002 and 2006 reporting on PPCPs (including CBZ) measured in influent and effluent is summarized in Glassmeyer *et al.* 2008. CBZ has been shown to be recalcitrant to conventional wastewater treatment processes (Bonvin 2013). The fate of CBZ during wastewater treatment is dependent on the physical and chemical properties of the compound, as well as treatment plant operating conditions.

The low K_d (solid liquid partitioning coefficient) values for CBZ (Table 2.1) indicate that sorption is not a significant removal mechanism for this substance, and sorption onto sludge will likely not be a significant removal pathway in municipal wastewater treatment. Generally, substances with a K_d coefficient of ≥500 are significantly sorbed onto sludge (Ternes *et al.* 2004). The low K_{ow} value (Table 2.1) also indicates that CBZ will not sorb to sludge and will remain predominantly in the aqueous phase. Compounds with high K_{ow} values (e.g. log K_{ow} >4) will tend to sorb to sludge whereas those with low K_{ow} values (e.g. log K_{ow} <2.5) will remain in the aqueous phase (Jones *et al.* 2005; Rogers 1996). The low value of Henry's law constant indicates that CBZ has low potential for volatilization. In wastewater treatment, CBZ will not likely be transferred from the water phase into the gas phase during aeration process (e.g., stripping). Substances with k_{O3} values greater than $5x10^4$ $M^{-1}s^{-1}$ indicate that ozonation will be efficient at transforming the compound. Based on the k_{O3} value (Table 2.1), CBZ can easily be oxidized by ozone (Huber *et al.* 2003). Effectiveness of ozonation has also been verified experimentally (Tabe *et al.* 2009). The nonaromatic double bonds in CBZ are responsible for the high reactivity with O_3 (Tabe *et al.* 2009). However, ozonation is a very expensive wastewater treatment technology. For example, in the case of Ontario, only one wastewater treatment facility in the province employs full-scale ozonation. In addition, toxic transformation products following ozonation have been reported. Removal of these transformation products may require the addition of a biofilter (V. Pileggi, Ontario Ministry of the Environment and Climate Change. Personal communication. 2014).

With respect to biological degradation, CBZ is not expected to undergo significant degradation (e.g. in aerobic reactors at wastewater treatment facilities). According to Ternes *et al.* (2005),

substances with a $k_{i,biol}$ value (specific biological degradation rate constant of pharmaceutical *i*) of less than 0.1 are not expected to undergo substantial removal via biological degradation (as is the case for CBZ) (Table 2.1). For substances with a $k_{i,biol}$ value of greater than 0.1 but less than 10, removal is strongly dependent on reactor configuration. More than 95 per cent removal via biological degradation is expected for substances with $k_{i,biol}$ values of greater than 10 (Ternes *et al.* 2005). Overall, the wastewater treatment process with the highest potential for removal of CBZ is ozonation, with biological degradation, sorption (filtration and activated granular carbon), and stripping offering the lowest potential for removal from wastewater. Minimal removal is also expected from chemical treatment such as coagulation, flocculation, and sedimentation (Tabe *et al.* 2009). Oxidation has been shown to be most effective at reducing CBZ concentrations in treated effluent, with reductions of more than 90 per cent (Lundstrom *et al.* 2010; Tabe *et al.* 2009). In general, high doses of ozone have been shown to produce toxic effluents when compared to lower doses of ozone (<5 mg/L) due to toxic transformation products (Petala *et al.* 2008).

The impact of hydraulic retention time (HRT), which is the average time that water soluble compounds in sewage are retained in a wastewater treatment facility, was investigated with respect to the removal of CBZ (Metcalfe *et al.* 2003b). It was concluded that within the typical HRTs in sewage treatment designs, CBZ is poorly removed. Note that "removed" here means transformation from the parent compound to any intermediate byproducts and not necessarily full mineralization. The percentage removal never exceeded 50 per cent, and concentrations of CBZ were often higher in effluent when compared to influent (see Section 6.1 for information on why CBZ can be detected in higher concentrations in effluent versus influent). The same study also investigated the impact of solids retention time (SRT), which is the average time activated sludge solids are retained in a wastewater treatment facility. In the case of mechanical plants (where the SRT varied from 1 to 53 days, depending on the type of STP), no trends were observed with respect to SRT and CBZ removal rates. In the case of seasonal discharge lagoons (where the HRT was greater than 150 days), the percent removal of CBZ varied from 5 to 25 per cent with one lagoon showing higher concentrations in effluent compared to influent (Metcalfe *et al.* 2003b).

A recent full-scale survey of four STPs conducted at select Ontario municipal wastewater treatment plants compared the efficiency of various treatment processes on the removal of PPCPs (including CBZ). For CBZ the median effluent concentration varied from 270-390 ng/L with no significant removals by any of the treatment facilities and treatment types (Pileggi and Tabe 2013). A pilot study comparing three different treatments (using the same influent source from one southern Ontario STP) for PPCP removal was assessed. CBZ concentrations were compared between influent or raw sewage (RS), primary effluent (PE), treatment with conventional activated sludge (CAS), treatment using a nitrification activated sludge (NAS) and biological nutrient removal (BNR) (V. Pileggi, Ontario Ministry of the Environment and Climate Change. Ppersonal communication. 2014). The boxplots in Figure 5.1 show overlap of the interquartile ranges, indicating that there was no difference in CBZ removal based on treatment. A chi-square test confirmed that there was no statistically significant difference in CBZ removal between CAS, NAS and BNR treatments ($p=0.877$). Median CBZ concentrations and standard deviation (ng/L) were: RS (238±69), PE (275±101), CAS (300±94), NAS (298±97) and BNR

(299±43). Simulated cold and warm (12 and 18 ^{o}C) seasons also showed no significant change in CBZ effluent concentrations (Pileggi, *et al.* 2013) consistent with full-scale facilities.

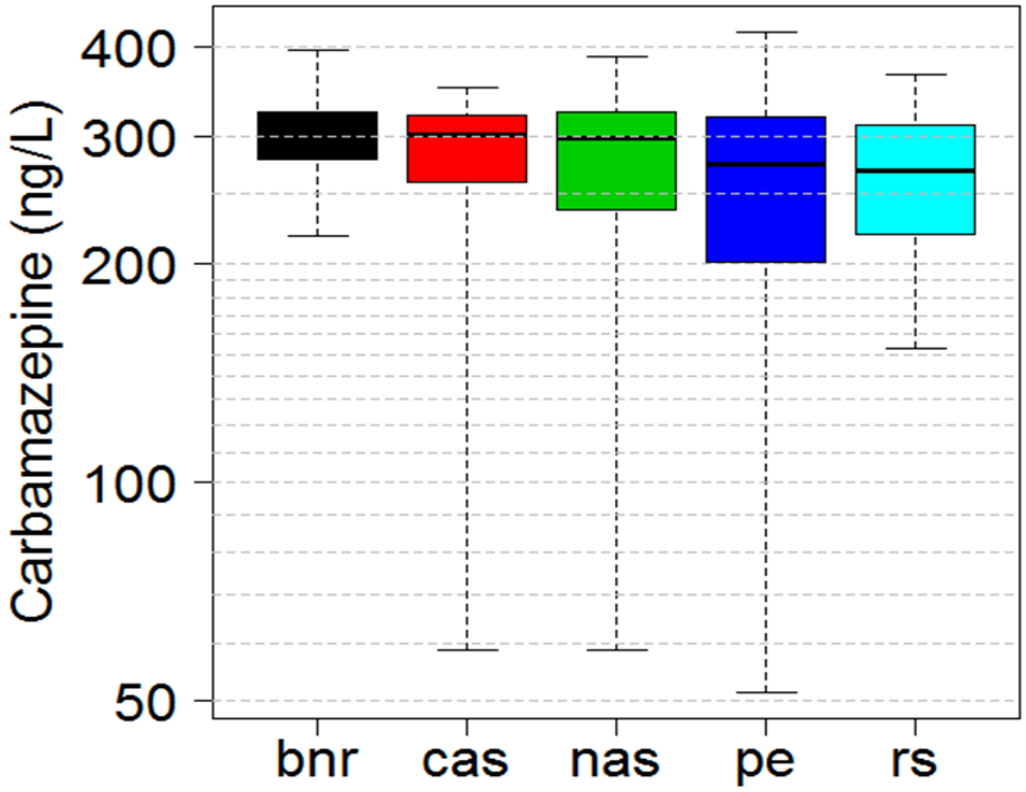


Figure 5.1. A comparison of CBZ concentrations in a pilot scale study where influent (raw sewage, rs) from a single source (a southern Ontario STP) was used. Three treatment technologies (CAS, NAS, BNR) were compared to evaluate CBZ removal, in addition to primary treatment (PE).

A study out of Australia also assessed a variety of treatment technologies for the removal of PPCPs, including CBZ (Ying *et al.* 2009). Plant A was treating sewage from a large municipality using secondary activated sludge and tertiary stage of six lagoons. The other three plants (B,C and D) were treating sewage from rural communities. Plant B used a biological process with two oxidation ditches and disinfection with chlorine. Plant C used a biological process with three bioreactors followed by disinfection with both UV and chorine. Plant D was composed of 10 lagoons in series (2 anaerobic, followed by 8 aerobic of which 3 were aerated). CBZ showed no significant changes in concentration at each of the respective STPs (measurements were taken at different stages of treatment at each respective facility).

Two studies assessed the effect CBZ may have on bacterial nitrite oxidation (Dokianakis *et al.* 2004) and methanogenesis (Fountoulakis *et al.* 2004) at sewage treatment facilities. CBZ (tested at 2, 6 and 10 mg/L) was found to cause only minor (if any) inhibition on the performance of nitrate oxidizing bacteria isolated from activated sludge. The concern was that inhibition of the conversion of nitrite to nitrate would result in accumulation of nitrite in STP effluent, a form of nitrogen more toxic than nitrate (Dokianakis *et al.* 2004). The impact of CBZ on the most sensitive microorganism group (methanogenes) was moderate (tested at 10, 50, 100, and 200

mg/L) (Fountoulakis *et al.* 2004). No significant impact on these two processes is expected, considering the lower concentration of CBZ detected at STPs versus those used in the studies (these concentrations are approximately 10,000 times the typical environmental concentrations).

5.3 Fate of Carbamazepine in Treatment Wetlands (Mesocosms)

Outdoor mescosms simulating multi-trophic, environmentally relevant, treatment wetlands were used to assess the dissipation of CBZ (and other pharmaceuticals) from a synthetic wastewater input (Cardinal *et al.* 2014). The synthetic wastewater with pharmaceuticals contained nominal TN and TP concentrations of 1.1 and 1.6 mg/L respectively (typically found in local lagoon effluent), in addition to six pharmaceuticals (CBZ added at nominal concentration of 7,600 ng/L). Following 28 days, no significant difference in removal of pharamaceuticals was observed in planted versus unplanted mesocosms. The average CBZ concentration in mesocosms was 910 ng/L, representing 88 per cent dissipation from the initial measured concentration. The observed half-life for CBZ was 9.1 days, where sorption (and to a lesser extent photodegradation) was a major removal process. Constructed wetlands may have benefit for wastewater polishing in Canadian prairie climates.

5.4 Fate of Carbamazepine in Water and Sediment

Since CBZ is highly resistant to conventional sewage treatment plant processes, it is one of the pharmaceuticals that is being detected in surface waters globally, with highest concentrations often reported near wastewater treatment effluent discharge points (Bonvin 2013). For this reason, CBZ is often used as a marker of sewage contamination (Kunkle and Radke 2012; Nakada *et al.* 2008; Zhang *et al.* 2008).

With respect to degradation processes in water, hydrolysis and microbial degradation do not appear to be significant for CBZ (Kunkle and Radke 2012; Lam *et al.* 2004). CBZ has also shown to be resistant to biodegradation in seawater (Zhang *et al.* 2008). The mean dissipation half life (DT50, time for concentration to be reduced by 50 per cent) for sea water is $DT50_{(seawater)}$ >100d (Benotti 2009, cited in EC 2013). Being resistant to biodegradation is necessary for CBZ to remain metabolically stable and hence pharmacologically active (in order to reach target site), so this is not surprising (Jos *et al.* 2003; Lissemore *et al.* 2006). CBZ is one of the pharmaceuticals most resistant to photodegradation in surface waters (Kunkle and Radke 2012). Photolysis data includes a DT50 ≈ 100 days (winter, 50ºN latitude) (Andreozzi 2003), $T_{1/2}$ = 3.5-87.5 days (Yamamoto 2009) and a photo-degradation half-life of 4.5 days (Lam and Mabury 2005). Photodegradation of CBZ has been shown to be reduced in the presence of humic acids (Andreozzi *et al.* 2002; Tabe *et al.* 2009), but is promoted in the presence of nitrate (Andreozzi *et al.* 2002). CBZ half-life reduced to 69.0, 24.5 and 11.2 h with increasing concentrations of nitrates (Andreozzi *et al.* 2002). According to the Persistence and Bioaccumulation Regulations (SOR/2000-107) under the *Canadian Environmental Protection Act (CEPA), 1999*, a substance is persistent if the half-life in water equals or exceeds 182 days. Therefore, based on these criteria, CBZ is not considered to be persistent. However, the potential for continuous presence exists due to its continual input into surface waters via municipal wastewater effluent.

An outdoor field microcosm was also used to study the persistance of CBZ in the aquatic environment, with the microcosm containing communities of fish, aquatic plants, zooplankton, phytoplankton, macrophytes, and bacteria (Lam *et al.* 2004). The mean dissipation half-life (DT50) of CBZ in the microcosm water was determined to be 82 days. This is similar to the finding of Loffler *et al.* (2005), who investigated the fate of CBZ in water/sediment systems, where field collected sediments were spiked with CBZ. The DT50 of CBZ for the water compartment only was 47 days (the DT50 for the sediment compartment was 328 days). The respective DT90 (time for concentration to be reduced by 90 per cent) values were >365 days (between one and two years) for the water compartment only and >>365 days (greater than two years) for water/sediment system (study duration was 100 days). The $T_{1/2}$ for river water was determined to range from 125 to 233 days (Yamamoto 2009 in EC 2013). Kunkle and Radke (2012) collected samples of river water (river Grundlach in Germany having a depth of 15 cm and a baseline flow of approximately 10 cm/s) for a distance of up to 12.5 km downstream from an STP outfall. Measured CBZ concentrations showed no change.

According to the Persistence and Bioaccumulation Regulations (SOR/2000-107) under *CEPA, 1999*, a substance is persistent if the half-life in sediment equals or exceeds 365 days. Therefore, based on these criteria, CBZ may be considered as being persistent in sediment.

With respect to sorption to sediment, Loffler *et al.* (2005) found CBZ to preferentially stay in solution, with some partitioning to soil/sediment. This is expected based on the log K_{oc} value of 2.227 to 3.123 (Table 2.1). CBZ is also expected to be present in a non-charged form under environmentally relevant pH conditions, with its high pK_a value of 13.94 to 15.37 (Table 2.1). Overall Loffler *et al.* (2005) concluded that CBZ is highly persistent and the potential for accumulation in sediment cannot be ruled out. Desorption of CBZ in spiked lake sediment was assessed in the presence of two benthic invertebrates (the midge *Chironomus dilutus* and the amphipod *Hyalella azteca*) and without organisms (Gilroy *et al.* 2012). Testing followed a standard 10-day exposure, as per the US Environmental Protection Agency. Bioturbation by the endobenthic invertebrate *C. dilutus* caused the greatest desorption (K_d desorption coefficient of 74) of CBZ from sediment, with lower desorption observed with *H. azteca* (K_d of 202). The midge *C. dilutus* is an endobenthic invertebrate inhabiting surificial sediment, and *H. azteca* is an epibenthic invertebrate which may or may not be in contact with sediment. Lowest desorption was observed with no organisms present (K_d = 263). This provided evidence that benthic invertebtrates can enhance the desorption of chemicals which have a *log* K_{ow} < 4, increasing potential for exposure and toxicity for other aquatic receptors. A survey of sediment in a marshland in Spain, impacted by STP effluent, reported 100 per cent detection of CBZ, where the maximum concentration was 1.7 ng/g (Vazquez-Roig *et al.* 2012). Williams and Kookona (2010) demonstrated that under steady state conditions, the likelihood of CBZ desorption into overlying water from sediment is decreased. This decrease was enhanced when tested sediments contained charcoal. However, work by Heberer (2002) indicates that polar compounds such as CBZ do not have a strong sorption capacity for natural organic matter (NOM) or sediment, and can pass through sediment via bank filtration and contaminate aquifer drinking water sources.

Kunkle and Radke (2012) assessed the fate of pharmaceuticals in a river (river Grundlach in Germany having a depth of 15 cm and a baseline flow of approximately 10 cm/s), including river

sediment pore water. Mini-piezometers were pushed into the river sediment (depth ranging from 5 to 35 cm), and pore water was sampled. CBZ was detected in pore water at all depths, with concentrations equal to concentrations measured in river (surface) water. Neither biotransformation nor dilution by groundwater were factors in the sampled sediment profiles of CBZ. Any variation of CBZ concentration with sediment depth was related to fluctuations of CBZ concentrations in river (surface) water. This study validated that CBZ is persistent in pore water.

Loffler *et al.* (2005) also investigated the fate of the transformation product CBZ-DiOH (10,11-dihydro-10,11-*trans*-dihydroxycarbamazepine) in the same water/spiked-sediment system. The DT50 value in water was much lower when compared to CBZ, and estimated at seven days. The DT50 for the water/sediment system was eight days. The DT90 for both water only and water/sediment was >365 days (between one and two years). The higher DT90 value was based on CBZ-DiOH concentrations remaining stable within the test system (35 per cent of initial concentration) from day 30 until the end of the experiment (Day 100). Overall, Loffler *et al.* (2005) concluded that CBZ-DiOH is persistent in the aquatic environment based on the low degradability of CBZ-DiOH in sewage treatment and its presence in surface waters. Due to the transformation product being more polar in nature (double hydroxylation) and its log K_{oc} value of -0.067 to 1 (Table 5.1), the affinity for CBZ-DiOH for sediments was greatly reduced when compared to CBZ. There is a low risk that CBZ-DiOH will accumulate in sediment (Loffler *et al.* 2005).

CBZ can take on continuous presence exposure characteristics when loading from sewage treatment facilities exceeds or equals water/sediment dissipation rates (half-lives). This is especially concerning for water bodies that are effluent dominated, where there is increased potential for accumulation of CBZ into aquatic life, and where exposures could occur over entire life-cycles (Ferrari *et al.* 2003; Ramirez *et al.* 2009). Chronic toxicity tests could underestimate the impacts of pharmaceuticals on aquatic organisms. They are relatively short when compared to the life span of tested organisms (e.g. seven-day *Ceriodaphnia dubia* exposure, ten-day *Danio rerio* early life stage exposure) (Ferrari *et al.* 2003). From the acute studies that are available for pharmaceuticals, many (90-100 per cent) produced effect concentrations greater than 1 mg/L (Webb 2001). Calculated acute to chronic toxicity ratios are not appropriate for the hazard assessment of pharmaceuticals (Webb 2001). In addition, chronic test methods are not designed to take into consideration toxicant transfer from parent to progeny (Ferrari *et al.* 2003). Some studies are now being conducted to investigate multi-generational exposure impacts (e.g., exposure of three successive generations of daphnids to CBZ) (Lamichhane *et al.* 2013). It must be noted that in the case of the Lamichane *et al.* 2013 study, it was suggested that there is no difference between the results obtained from a seven-day test versus the follow through to the F3 generation multi-generational test. The authors also saw effects at two orders of magnitude higher than environmentally relevant concentrations.

5.5 Fate of Carbamazepine Following Biosolids Application

Topp *et al.* (2008) investigated the release of PPCPs (including CBZ) from land amended with biosolids (e.g., treated sewage sludge) using either subsurface (10 cm) injection or surface

broadcast application followed by incorporation by cultivation (within 24 hours). Surface runoff was quantified following simulated precipitation up to 266 days post application. CBZ concentrations were continuously below the limit of quantification (4.9 ng/L) at the site that received subsurface injection (up to day 266 post-application). In contrast, the CBZ concentration measured one day post-broadcast application was 221 ± 88 ng/L. Mean CBZ concentration remained above the LOQ through to day 266, with detection up to nine months post-application (e.g., persistent in soil, even during winter season). This study also determined the kinetic parameters for the decline in CBZ concentration in simulated surface runoff following biosolids broadcast application. The days to reduce runoff concentration by 50 per cent of initial (dissipation time, DT50) is 18.95, the days to reduce runoff concentration by 90 per cent of initial (DT90) is 62.96 and the days to reduce the concentration to LOQ (DT_{LOQ}) is 104.0.

A survey for selected pharmaceuticals in seven surface water sites in the Grand River watershed in Southern Ontario – all identified as high risk for receiving agricultural runoff – was conducted by Lissemore *et al.* (2006). Three of the seven sites showed detection of CBZ (which was also identified as being one of the five most frequently detected analytes). All sampled sites were located upstream of major urban centres. Through examination of biosolids application records, Lissemore *et al.* (2006) identified the three sites with detected CBZ as areas in which biosolids had been applied adjacent and upstream some time during the four years previous to sampling. The range of detected CBZ was 0.16 to 24 ng/L, with a median of 1 ng/L (reporting limit = 0.06 ng/L). The other four sites where CBZ was not detected had no record of biosolids application.

5.6 Bioconcentration and Bioaccumulation

CBZ has a log K_{ow} value reported as ranging from 2.25 to 2.45 (Table 2.1) which is considered to be low, indicating that this compound will likely partition to water-based matrices more so than organics (CBZ has neutral lipophilicity; low K_{ow} substances preferentially are found in hydrophilic compartments such as blood serum). Based on the relationship between bioconcentration factor and log K_{ow}, CBZ is expected to bioconcentrate 10 to 100 times (Quinn *et al.* 2004). BCF values of <100 have been reported by European Union countries (Table 2.1) and based on this, secondary poisoning of predators is not considered relevant for CBZ (RIVM 2012). According to the Persistence and Bioaccumulation Regulations (SOR/2000-107) under *CEPA, 1999*, a substance is bioaccumulative if BAF or BCF is equal to or greater than 5,000, or if the log Kow is equal to or greater than five. Therefore, based on these criteria, CBZ is not considered to be bioaccumulative.

Zhou *et al.* (2008) applied the solid-phase microextraction (SPME) technique to measure both free (bioactive) and total (protein-bound) CBZ in fish muscle (dorsal-epaxial) tissue (where total is calculated using SPME-derived free concentration and the CBZ protein bindng value). The free concentration is what is bioactive, and the total concentration provides an assessment of the reservoir that has the potential to become bioactive (dependent on half-life of CBZ in vivo). Immature rainbow trout were exposed to a series of CBZ concentrations (0, 3.2, 32, 320 ng/L). The bioaccumulation factors (BAF, the slope of the curve between measured total tissue CBZ concentration and CBZ concentration in water) were 0.44 and 0.22 following 7 and 14 days, respectively. This indicates that CBZ metabolism is up-regulated over time. The rapid

accumulation of CBZ (over 24 hours) in rainbow trout plasma, and then a decrease over the balance of the 96-hour exposure, was also noted in a study by Huggett *et al.* (2004), indicating that CBZ metabolism in fish tissue may be occurring, even while the fish is still being exposed. Zhou *et al.* (2008) also made a comparison between CBZ binding to muscle (higher protein content) and plasma (lower protein content). CBZ displayed similar binding to muscle and plasma protein, likely due to its hydrophilic nature. Substances which are hydrophobic will result in increased binding to muscle protein.

Garcia *et al.* (2012) compared laboratory derived CBZ BCFs in the bluntnose minnow (*Pimephales notatus*) and the channel catfish (*Ictalurus punctatus*) with field measured CBZ BCFs in tilapia (*Oreochromis niloticus*). Tissue-specific accumulation was measured following a 42-day exposure to 298 ug CBZ/L with the minnow, and a 14-day exposure to 83 ug CBZ/L with the catfish. Wet-weight tissue specific BCFs for *P. notatus* were 1.9 (muscle) and 4.6 (liver), and for *I. puncatus* were 1.8 (muscle), 1.5 (liver), 7.1 (plasma), and 1.6 (brain) (the higher plasma BCF may be attributed to not reaching steady-state in the catfish). The tilapia *O. niloticus*, which resided in the wastewater treatment plant sand filter for years (therefore likely at steady-state), were exposed to an average CBZ effluent concentration of 274 ng/L. Tissue specific BAFs were 2.8 (muscle), 3.8 (liver) and 2.5 (plasma). These results indicate that laboratory BCF and field BAF values for CBZ in individual tissues are similar, with values of less than 10. As with Zhou *et al.* (2008), CBZ tissue concentrations are similar to plasma concentrations (when at steady-state).

The uptake (48 hours) and depuration (48 hours) of CBZ was assessed in the freshwater shrimp *Gammarus pulex* (a benthic detritivore) and the water boatman *Notonecta glauca* (a water column predator) (Meredith-Williams *et al.* 2012). Exposures were water-only. The uptake and depuration rates and resulting BCF values for CBZ in *G. pulex* are greater than in *N. glauca* (Table 5.4). The difference in uptake and depuration between the two species may be partially explained by the fact that these organisms occupy different locations within the water column. They may be exposed to different concentrations depending on how the compound settled out in solution, the conditions of the test (static, renewal, aeration etc). The ionization state of the exposure medium is likely not an issue since the solution pH may not be as relevant with CBZ having a pKa approaching 14.

Table 5.4. Modelled uptake and depuration rate constants and BCF data for the uptake of CBZ into two test species.

Parameters	Species	
	G. pulex	*N. glauca*
pH	8.51-8.9	7.85-7.95
DO ($mg\ L^{-1}$)	7.59	8.36
Temp (°C)	12.50	20.11
C_w (µg/L)	142.68 ± 2.38	100 ± 0.56
K_{in} ($L\ kg^{-1}d^{-1}$) mean ±SD (ML [95th percentiles])	5.199 ± 0.6551 (5.232 [3.990; 6.536])	0.292 ± 0.0479 (0.297 [0.202; 0.393])
K_{out} (d^{-1}) mean ±SD (ML [95th %iles])	0.7207 ± 0.1298 (0.7328 [0.482; 0.982])	1.181 ± 0.231 (1.198 [0.754; 1.666])
BCF ($L\ kg^{-1}$) [95th percentiles]	7.094 [5.472-8.935]	0.244 [0.172-0.330]

C_w = mean concentration in water over the study
K_{in} = uptake rate constant
K_{out} = depuration rate constant
BCF = bioconcentration factor

Based on BCF values, CBZ is not considered to be bioaccumulative (CEPA 1999; EU REACH 2006; US TSCA 1999). The lack of accumulation in fish, potentially due to metabolism, is supported by studies with mice (Amore *et al.* 1997). A study by Vernouillet *et al.* (2010) did identify a CBZ 24-hour BAF of 2.2 for algae (P. subcapitata), following exposure to an environmentally irrelevant concentration of 150 mg/L. This CBZ-infused algae was then fed to the crustacean *Thamnocephalus platyrus*, where accumulation was higher (12.6). However, the cnidarian *Hydra attenuata*, when fed the crustacean, only showed traces of CBZ in tissue. This indicated that "either the uptake of CBZ in the hydra was very weak, or there is a high detoxification activity in this cnidarian as revealed by increased heme oxidase activity, and cytochrome P450 3Af-like activity". When compared to other substances, the initial algae BAF is low. Vernouillet *et al.* (2010) states that the BAF in the micro-algae *Isochrysis galbana* following 2-hour exposure to nonylphenol was 6940. As well, 10-day exposures of bryophytes to oxolonic acid (250-450), flumequine (75-140), and oxytetracylcline (100-200) also produced much higher BaFs, in comparison to CBZ. BAF values greatly depend on the studied species, as well as the lipophilicity and ionization state of the chemical.
Wild fish (white sucker *Catostomus commersoni* and johnny darter *Etheostoma nigrum*) were collected in the Grand River watershed from three river reaches adjacent to two municipal sewage treatment plants (Zhou *et al.* 2008). SPME was used to determine the concentration of CBZ (and other PPCPs) in muscle tissue. CBZ was found to be below the limit of detection (LOD) in all cases (LOD = 50.4 pg/g).

A pilot study for the quantification of pharmaceuticals and personal care products (PPCPs) in fish tissue was undertaken by the United States Environmental Protection Agency (US EPA), whereby adult fish were sampled from five effluent dominated streams and one reference site (Ramirez *et al.* 2009). The sites (and fish species) sampled were Salt River in Phoenix, Arizona

(common carp *Cyprinus carpio*), Little Econlockhatchee River in Orlando, Florida (bowfin *Amia calva*), North Shore Channel in Chicago, Illinois (largemouth bass *Micropterus salmoides*), Taylor Run in Westchester, Pennsylvania (white sucker *Catostomus commersonii*), Trinity River in Dallas, Texas (smallmouth buffalo *Ictiobus bubalus*), and East Fork Gila River (reference site) in New Mexico (sonora sucker *Catostomus insignis*). Fish fillet and liver samples were analysed for PPCPs, including CBZ. CBZ (MDL of 0.54 ng/g) was not detected in either fillet or liver at the reference site. CBZ was only detected in fillet and liver tissue (in six out of six samples) at one site (Chicago, Illinois). The maximum (and mean) concentrations measured in fillet and liver were 3.1 (2.3) ng/g and 8 (6) ng/g, respectively. Concentrations of CBZ in water were not reported.

Fish tissue samples were collected along 13 rivers sites, downstream of STPs, along the Rhine, Danube and Elbe rivers (and tributaries) in Germany (Subedi *et al.*, 2012). CBZ was not detected in any of the samples (MDL = 0.19 ng/g).

In a study from Argentina, CBZ was one of the most frequently detected pharmaceuticals in the Suquia River basin (Cordoba), reaching sub µg/L concentrations (Vladés *et al.*, 2014). The bioconcentration of CBZ in the western mosquitofish (*Gambusia affinis*) was determined in a laboratory exposure. The estimated CBZ BCF was 0.7 and 0.9 L/kg when exposed to 10 and 100 µg/L CBZ, respectively.

A point of interest is the uptake of CBZ by terrestrial organisms and terrestrial plants following application of bisolids for amending soil as well as irrigation with reclaimed water. Kinney *et al.* (2012) examined CBZ uptake via biosolids amendment but did not find any CBZ in the corresponding worm tissues measured. However, research has documented uptake into plants via biosolids application (Holling *et al.* 2012) and irrigation with municipal effluent (Calderon-Preciado *et al.* 2011; Shenker *et al.* 2011).

6.0 CONCENTRATIONS IN CANADIAN WASTEWATER TREATMENT PLANTS AND SOURCE WATERS (SURFACE AND GROUND WATER)

CBZ has been described as a fairly persistent compound both in wastewater/sewage treatment processes and in surface water (Heberer 2002; Metcalfe *et al.* 2003a; Metcalfe *et al.* 2003b), making it a suitable indicator for tracking the presence of municipal sewage contamination in surface waters.

6.1 Wastewater Treatment Plant Influent and Effluent

A survey of both influent (raw sewage) and effluent (final treated sewage) from 18 sewage treatment facilities (with varying operating conditions and wastewater inputs) across 14 Canadian municipalities was conducted by Metcalfe *et al.* (2003b) in 1998 through 1999. CBZ was detected in all samples. The maximum (and median) CBZ concentration measured in influent was 1,900 (700) ng/L (detection limit of 500 ng/L) and in effluent was 2,300 (700) ng/L (detection limit of 100 ng/L) indicating that concentrations in influent and effluent are essentially

equal (the parent CBZ compound is highly resistant to degradation during sewage treatment processes) and sometimes higher in effluent. Glucuronide conjugate formation (glucuronidation) is an important detoxification pathway for many substances in humans (Figure 6.1). It involves the addition of glucuronic acid to a substance (Bonvin 2013). CBZ *N*-glucuronide and glucuronides of hydroxylated transformation products have been found to be significant urinary metabolites, which end up in sewage wastewater (Staines *et al.* 2004). Even when less than 2 per cent of CBZ is excreted unmetabolized in human urine (Table 2.1), the cleavage of the glucoronide conjugates increases the effluent concentration of CBZ parent product. Cleavage of CBZ glucuronide conjugates by bacterial degradation back to parent compound during wastewater treatment may be why CBZ concentrations in effluent are often higher than in influent (Miao and Metcalfe 2003). This may also be a relevant point for the other hydroxlated metabolites which in turn may also undergo dehydration restoring the parent form. An alternative theory is that many of the negative removals (e.g. CBZ concentrations greater in final effluents than in influents) may be due to inappropriate sampling strategies in the wastewater treatment plant (Ort *et al.* 2010).

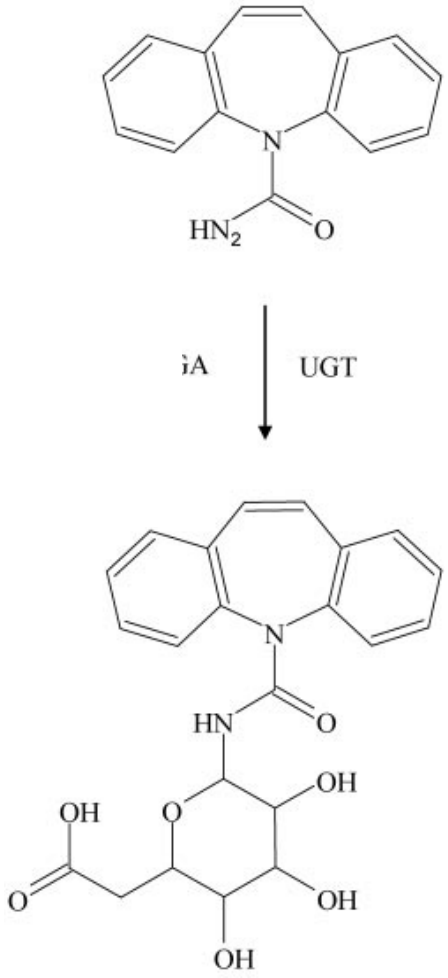


Figure 6.1. Formation of CBZ glucuronide. UGT (uridine diphosphate glucuronosyltransferase) isoform is responsible for the *N*-glucuronidation of CBZ, where CBZ is specifically glucuronidated by human UGT2B7 (Staines *et al.* 2004).

In 2010 and 2011, Environment Canada engaged in a survey of PPCPs in wastewater treatment systems. The goal of the survey was to understand the occurrence and fate of PPCPs in systems under conditions and temperatures typical of Canadian conditions, with samples being collected in both summer and winter (Smyth 2011). Four types of facilities were sampled, including a facultative lagoon, an advanced primary treatment system, a secondary activated sludge system and an advanced tertiary system. CBZ was detected in all influent (range 201 to 1,340 ng/L) and effluent (range 273 to 1,230 ng/L) samples from all four systems (reporting limit of 1.48 to 7.04 ng/L). Summer CBZ removal efficiencies were negative (<0% in primary treatment), zero (advanced treatment), and poor (0-10% in lagoon and secondary treatment). Winter removal efficiency was found to be negative (<0%) for all systems.

Grab samples of effluent from two municipal STPs in Alberta (Bonnybrook and Fish Creek) were collected periodically from October 2002 to July 2004 (Chen *et al.* 2006). Both STPs employed tertiary treatment, nutrient removal and UV disinfection. Measured CBZ concentrations were as follows: Bonnybrook sampled in 2002 (505 ng/L), Bonnybrook sampled in 2003 (925±31 ng/L), and Fish Creek sampled in 2003 (702±12 ng/L). Data collected at municipal wastewater facilities in the province of Alberta in fall 2007 showed no detections of CBZ in wastewater influent from smaller municipalities (n=4, DL = 10 ng/L). In contrast, CBZ was detected in 60 per cent (18/30) of treated municipal wastewater samples from 2004 to 2007 (range: < 10 to 2,200 ng/L; median = 120 ng/L) (AESRD 2014).

Additional monitoring out of Alberta involved the use of passive samplers (POCIS, or Polar Organic Chemical Integrative Samplers) for detection of pharmaceuticals, including CBZ, in treated wastewater from three STPs (MacLeod and Wong 2010; MacLeod *et al.* 2007). Passive sampling allows for a better representation of the concentration over time (time weighted average concentration) when compared to grab sampling. POCIS samplers were installed in a secondary equivalent lagoon (Lac La Biche) servicing a small rural community (4,000) over six sampling periods, each being 35 to 51 days in duration, from July 2007 to April 2008. Time weighted average (TWA) CBZ concentration ranged from 32 to 77 ng/L (MacLeod and Wong 2010), indicating that CBZ is present in smaller municipalities. No significant temporal changes in CBZ concentrations were detected. POCIS samplers were also deployed in two urban tertiary mechanical plants employing UV disinfection (Edmonton Gold Bar servicing 750,000 and Edmonton Capital Region servicing 250,000), from December 2007 to March 2008. TWA CBZ concentrations in Gold Bar effluent ranged from 150 to 250 ng/L, and at Capital Region effluent ranged from 190 to 360 ng/L.

Effluent from both the City of Regina (CAS) and the City of Saskatoon (CAS-BNR) wastewater treatment plants was collected in 2014 by the Water Security Agency of Saskatchewan, Environmental Protection Services Section. The highest reported CBZ concentration was 264 ng/L at the Saskatoon plant (O. S. Thirunavukkarasu, Water Security Agency. Personal communication. March 2015).

A one-time sampling event was conducted in 2007 at three wastewater treatment facilities in Manitoba (WQMS, 2012). Two sampled lagoons had CBZ effluent concentrations of 34.9 ng/L (Dauphin Lake) and 41.8 ng/L (Manning Canal Drainage System). A mechanical plant discharging into the Red River had an effluent CBZ concentration of 13.5 ng/L. A study by

Carlson *et al.* (2013) assessed CBZ (in addition to other organic micropollutants) in two lagoons (Morden and Winkler, both discharging to Dead Horse Creek) on both the lagoon and at the outfall. Both grab and POCIS samples were collected in the lagoons in June 2010, and grab only was used to collect samples at the outfall in June and July 2010. The grab samples were extracted by SPE. The table below provides the results (Table 6.1).

Table 6.1. Mean concentrations (min and max, ng/L) of CBZ measured in Morden and Winkler lagoons and outfalls into Dead Horse Creek by grab/SPE and POCIS in 2010.

Morden				Winkler			
Lagoon		Outfall		Lagoon		Outfall	
June 28 (SPE)	June 2-22 (POCIS)	June 28 (SPE)	July 6 (SPE)	June 28 (SPE)	June 2-22 (POCIS)	June 28 (SPE)	July 6 (SPE)
68.3 (56.5, 75.8)	172.2 (153.1, 191.2)	Not detected	88.5 (85.2, 91.2)	Not detected	92.5 (80.8, 104.2)	Not detected	119 (105, 128)

A survey of PPCPs in Ontario sewage treatment plant effluents discharging to the Lower Great Lakes was conducted in 2002 by Metcalfe *et al.* (2003a). CBZ was detected at all four facilities. The mean (standard deviation) measured at each facility was: Peterborough STP - 126 (3) ng/L, Burlington STP – 64 (5) ng/L, Little River STP – 112 (5) ng/L, and West Windsor STP – 7 (1) ng/L. The Little River STP was again surveyed for PPCPs by Hua *et al.* (2006) in 2002 and 2003 to assess the influence of seasonal changes on PPCP effluent concentrations. CBZ was detected in all effluent samples, with concentrations ranging from 184 to 368 ng/L, and was relatively consistent when compared among the sampling seasons. CBZ concentrations measured in Little River STP effluent were also reported by the Water Research Foundation (Tabe *et al.* 2009), with detection in all 8 samples. Effluent CBZ concentrations ranged from 361 to 735 ng/L. No information was retrieved relating number of patients/hospitals/psychiatric institutions in the areas where CBZ was detected in high concentrations in effluent (e.g. Little River STP).

Monitoring of PPCPs at six wastewater treatment plants in Québec from 2003 to 2006 resulted in a maximum detected CBZ influent concentration of 890 ng/L, with no detection in treated wastewater effluent (< detection limit of 5 ng/L) (MDDELC 2015). A seasonal comparison of wastewater influent showed no detection of CBZ in winter (< detection limit of 25 ng/L), and 14 per cent detection in summer (maximum of 890 ng/L). Overall, influent CBZ detection in this study was lower than reported in other jurisdictions (>33 to 100%). A second sampling campaign took place in 2008 to 2009, where maximum CBZ detected in wastewater influent and effluent was 1,400 and 520 ng/L, respectively.

Eight sewage treatment plants located in Atlantic Canada were sampled for pharmaceuticals in effluent (Brun *et al.* 2006). CBZ was detected in all effluents (median concentration of 79 ng/L), with spring concentrations ranging from non-detect (nd) to 88 ng/L, and summer concentrations ranging from 66 to 240 ng/L (detection limit of 20 ng/L). The spring (sp) and summer (su) CBZ concentrations (sp, su) (ng/L) for the eight STPs was: Halifax, Nova Scotia (45, 130), Fredericton, New Brunswick (97, 83), Charlottetown, PEI (64, 240), Grand Falls-Windsor, Newfoundland and Labrador (nd, 75), Sussex, New Brunswick (41, 98), Springhill, Nova Scotia (88, 180), Summerside, Prince Edward Island (24, 66), and Gander, Newfoundland and Labrador (nd, 170) (Brun *et al.* 2006).

6.1.1 Constructed Wetlands

Many small communities (populations of ≤10,000) in the Province of Manitoba are serviced by lagoons. In order to be able to meet stricter federal and provincial effluent release guidelines, the use of constructed wetlands as an additional treatment step is being investigated (Anderson *et al.* 2013; Cardinal *et al.* 2014). The community of Grand Marais in Manitoba treats wastewater using an intermittent secondary-equivalent lagoon, from which wastewater flows into a treatment wetland before discharge into Lake Winnipeg (Anderson *et al.* 2013). Both grab (SPE extracted) and POCIS samples were collected before (May 22 and June 15, 2012) and after (July 16 and July 23, 2012) lagoon release, for a total of seven sampling locations: 1) in the secondary treatment lagoon, 2) at the outfall of the lagoon, 3) mid-channel to the treatment wetland, 4) at the point of inflow from channel into treatment wetland, 5) east wetland, 6) west wetland, and 7) outlet of the wetland. The highest CBZ concentrations were measured at the lagoon outfall (500 ng/L by POCIS) and in the lagoon (380 ng/L by SPE). All other CBZ measurements were <100 ng/L. A significant reduction in CBZ concentration was detected between entry and discharge of treatment wetland, suggesting that this additional treatment step may reduce CBZ concentrations in final effluent.

6.2 Wastewater Treatment Plant Sewage Biosolids

The Environment Canada survey of PPCPs in wastewater systems also included quantification of PPCPs in biosolids (Smyth 2011). CBZ was detected in all samples from all four systems, with measured concentrations ranging from 37.1 ng/g to 431 ng/g (the reporting limit ranged from a minimum of 2.64 ng/g to a maximum of 3.05 ng/g). T-test comparisons of summer and cold CBZ biosolids concentrations by WWTP and season showed that CBZ concentrations were highest in winter biosolids samples collected from the primary, secondary and advanced facilities (α=0.05).

A comprehensive assessment of emerging substances of concern (which included CBZ) in Canadian biosolids was reported by Hydromantis *et al.* (2010) and submitted to the Biosolids Task Group (BTG) of CCME. CBZ was found at detectable levels in all 31 samples of treated sludges and biosolids, with a median concentration of 66.6 ng/g total solids (TS) dw (sample detection limits were determined for carbamazepine in each matrix, and as a result no single "representative" detection limit is provided). Aerobic treatment of sludges (composting) was found to be effective at removing CBZ (in addition to azithromycin, ciprofloxacin, miconazole, triclosan, triclocarban, diphenhydramine, gemfibrozil, thiabendazole). None of the other treatment processes were found to be effective. The different treatment processes examined, however, were not replicated sufficiently to draw statistical inferences. Anaerobic digestion either resulted in no change or increased CBZ concentrations. Unlike other substances that were bound to the solid phase, CBZ could be lost to other process side-streams such as dewatering filtrate, leachate or digester supernatant.

6.3 Septic Systems

A survey of effluent from four septic systems in southern Ontario provincial parks was conducted in spring (June) and fall (October) 2013 (S. Kleywegt, Ontario Ministry of Environment and Climate Change. Personal communication. 2013). CBZ was detected at all sites, with spring and fall CBZ concentrations ranging from 10 to 317 ng/L and 39 to 242 ng/L, respectively.

6.4 Surface Water

A national survey of disinfection by-products and selected emerging contaminants (including CBZ) in raw drinking water sources was conducted by Health Canada in the summer and winter of 2009 and 2010. Sites were selected to be representative of all provinces and territories as well as to give an equilibrated selection of water systems serving large, medium and small populations (river, lake, groundwater well). The analysis was done by SPE-LCMS and an isotopic internal standard was added to the samples at time of sample collection, to account for any loss of analyte during shipment or processing. The CBZ data is shown in Table 6.2 (A.-M. Tugulea, Health Canada. Personal communication. May 2014).

Table 6.2. CBZ concentrations (ng/L) detected in the national survey of disinfection by-products and selected emerging contaminants in raw drinking water sources, Health Canada, 2009 and 2010 (Tugulea, 2014).

Location	Summer 2009[1]									Winter 2009[1]								
	River			Lake			Well			River			Lake			Well		
	Min	Max	Det. Freq	Min	Max	Det. Freq	Min	Max	Det. Freq	Min	Max	Det. Freq	Min	Max	Det. Freq	Min	Max	Det. Freq
Québec	6	10	5/5	na	6	1/1	na	<mdl	0/1	3	14	5/5	na	<mdl	0/1	na	<mdl	0/1
Ontario	<mdl	10	3/7	na	<mdl	0/3	nm	nm	nm	<mdl	10	3/7	na	<mdl	0/3	nm	nm	nm
Saskatchewan	15	16	2/2	nm	nm	nm	na	<mdl	0/2	16	34	2/2	nm	nm	nm	na	<mdl	0/2
British Columbia	na	<mdl	0/2	na	<mdl	0/3	na	<mdl	0/2	na	<mdl	0/2	na	<mdl	0/3	na	<mdl	0/2
Newfound-Land and Labrador	na	<mdl	0/1	8	28	2/2	na	<mdl	0/1	na	<mdl	0/1	na	<mdl	0/2	na	<mdl	0/1
Prince Edward Island	nm	nm	nm	nm	nm	nm	<mdl	9	1/3	nm	nm	nm	nm	nm	nm	na	<mdl	0/3
Location	**Summer 2010[1]**									**Winter 2010[1]**								
	River			Lake			Well			River			Lake			Well		
	Min	Max	Det. Freq	Min	Max	Det. Freq	Min	Max	Det. Freq	Min	Max	Det. Freq	Min	Max	Det. Freq	Min	Max	Det. Freq
British Columbia	na	10	1/1	nm	nm	nm	nm	nm	nm	na	<mdl	0/1	nm	nm	nm	nm	nm	nm
Yukon	nm	nm	nm	na	<mdl	0/1	na	<mdl	0/2	nm	nm	nm	na	<mdl	0/1	na	<mdl	0/2
Northwest Territory	na	<mdl	0/1	na	<mdl	0/2	nm	nm	nm	na	7	1/1	<mdl	131	1/2	nm	nm	nm
Nunavut	nm	nm	nm	<mdl	312	1/3	nm	nm	nm	nm	nm	nm	<mdl	49	1/3	nm	nm	nm
Manitoba	15	28	2/2	<mdl	12	1/2	nm	nm	nm	10	11	2/2	na	<mdl	0/2	nm	nm	nm
New Brunswick	na	10	1/1	na	6	1/1	3	18	3/3	na	<mdl	0/1	na	<mdl	0/1	na	<mdl	0/3
Nova Scotia	nm	nm	nm	na	<mdl	0/2	<mdl	5	1/2	nm	nm	nm	na	<mdl	0/2	na	<mdl	0/2
Alberta	<mdl	17	2/3	na	11	1/1	na	<mdl	0/2	na	<mdl	0/3	na	<mdl	0/1	na	<mdl	0/2
First Nations Territory in southern Ontario	<mdl	49	1/1	nm	nm	nm	nm	nm	nm	<mdl	49	1/1	nm	nm	nm	nm	nm	nm

Det. Freq = detection frequency
nm = not measured
na = not applicable
mdl = method detection limit
[1] CBZ method detection limit = 1 ng/L

A First Nations food, nutrition and environment study was funded by Health Canada, which included testing for the presence of pharmaceuticals in surface water (specifically fish harvesting areas and areas used as source water for drinking water). Data has been released for First Nations communities located in British Columbia (Chan *et al.* 2011), Manitoba (Chan *et al.* 2012) and Ontario (Chan *et al.* 2014). The results for CBZ are presented in Table 6.3.

Table 6.3. Level of CBZ (ng/L) in surface water, by total and by ecozone, for First Nations communities in British Columbia, Manitoba, and Ontario. CBZ detection limit is 0.5 ng/L.

Province and Ecozone	Maximum (ng/L)	Detection Frequency
British Columbia		
-All Ecozones Combined	No detection	0/62
Manitoba		
-All Ecozones Combined	4.74	3/36
-Prairies/Plains:	<0.5	0/3
-Prairies/Subarctic	<0.5	0/3
-Boreal Plains/Plains	<0.5	0/3
-Boreal Plains/Subarctic	4.74	3/3
-Boreal Shield/Subarctic	<0.5	0/15
-Taiga Shield/Subarctic	<0.5	0/6
-Hudson Plains/Subarctic	<0.5	0/3
Ontario		
-All Ecozones Combined	39.6	26/95
-Boreal Shield/Subarctic	<0.5	0/24
-Boreal Shield/Northeast	39.6	2/16
-Hudson Plains/Subarctic	8.08	5/28
-Mixedwood Plains/Northeast	32.9	19/27

The Ontario Ministry of the Environment conducted a survey of emerging contaminants of concern, which included the pharmaceutical CBZ, in select source waters in the vicinity of 17 drinking water treatment plants (Kleywegt *et al.* 2011). CBZ was one of the most frequently detected pharmaceuticals detected in river and lake source waters (10 of 17 source waters) (Table 6.4).

Table 6.4. Number of detections and concentrations of CBZ (ng/L) in untreated source waters (n=125) from river and lake sources near drinking water treatment plant intakes in Ontario (Kleywegt *et al.* 2011).

Source	Number of Detections	Detection Percentage (%)	Detection Limit	Median	95th Percentile	Maximum
Untreated Source Water	63	50	1	3	152	749

Surface water downstream of the Little River STP in Windsor, Ontario (Tabe *et al.* 2009; see Section 6.1 for effluent concentrations) was sampled and analyzed for CBZ. Little River STP discharges effluent into Little River which flows into the Detroit River. At the point of confluence of the two rivers CBZ concentrations ranged from 14 to 508 ng/L (method and instrument detection limits of 4.3 and 0.004 ng/L, respectively). Concentrations in raw Detroit River source water near the intakes of the drinking water treatment plants (Windsor Water Treatment Plant and Detroit Water Works Park Water Treatment Plant) showed CBZ concentrations ranging from 0.1 to 2 ng/L. The distance from the Little River STP effluent

outfall to both the Windsor Water Treatment Plant and the Detroit Water Works is approximately 8 km (S. Tabe 2013).

A survey of untreated source water near drinking water treatment facilities was also conducted by Kormos (2007) in a southern Ontario watershed. Two river source water samples near two associated drinking water treatment plants (one downstream from the second) were sampled. CBZ was detected in all raw water. CBZ raw water concentrations at Facility A ranged from 2.8 ng/L to 27.6 ng/L, whereas mean concentrations at Facility B (downstream of Facility A) were much higher ranging from 7.8 ng/L to 988.3 ng/L. Raw water CBZ concentrations were highest in summer and early fall months. This may be associated with low receiver water levels due to the summer dry season.

A survey of surface water in the lower Great Lakes area (Canada) conducted in 2000 (initial survey) and 2002 (adjacent to discharges of effluents from sewage treatment plants) was conducted by Metcalfe *et al.* (2003a), in which similar CBZ (detection limit of 1 ng/L) concentrations were reported as in Kleywegt *et al*. (2011) and Kormos (2007). Select regions of the Detroit River, Hamilton Harbour, and other sites in Eastern Lake Ontario were sampled in 2000, with CBZ detection of 73, 64 and 7 per cent, respectively. The 2002 survey sampled surface water immediately adjacent to discharges from the Peterborough STP (Otonabee River), Burlington STP (Hamilton Harbour), Little River STP in Windsor (Little River) and Windsor STP (Detroit River). Another survey of surface water on the Canadian side of the Detroit River, close to the Little River STP, was conducted by Hua *et al.* (2006) in 2002 and 2003. All results are presented in Table 6.5.

Table 6.5. CBZ concentrations (ng/L) measured in Ontario surface waters from various surveys.

Location	Survey Year	Detection Limit	Mean	Median	Maximum	Reference
Detroit River	2000	1	na	185	650	Metcalfe *et al.* 2003a
Hamilton Harbour	2000	1	na	120	310	Metcalfe *et al.* 2003a
Hamilton Harbour	2002	1	23	na	na	Metcalfe *et al.* 2003a
Various sites in Eastern Lake Ontario	2000	1	na	20	20	Metcalfe *et al.* 2003a
Otonabee River	2002	1	2	na	na	Metcalfe *et al.* 2003a
Little River	2002	1	80	na	na	Metcalfe *et al.* 2003a
Little River	2002/03	0.05	na	na	40	Hua *et al.* 2006
Detroit River	2002	1	4	na	na	Metcalfe *et al.* 2003a

na = not available

Surface water CBZ data was collected by the Water Quality Management Section of Manitoba Conservation and Water Stewardship in 2006 and 2007 (Table 6.6). An additional survey was conducted by Carlson *et al.* (2013), with sampling of Dead Horse Creek, a tributary of the Red River which empties into Lake Winnipeg. Five sites were sampled. Grab samples were collected from June to July 2010 (followed by SPE), and POCIS samples were collected from June to

November 2010 and from June to July 2011. The range (maximum to minimum) measured CBZ concentrations (using both methods) is presented in Table 6.6. Monitoring of surface water in the North Saskatchewan River (December 2002) downstream of the effluent outfall from both the Gold Bar and Capital Region STPs showed detection of CBZ (35 – 171 ng/L) (Sosiak and Hebben 2005). From August to September 2006 and September to October 2006, POCIS samplers were deployed in the North Saskatchewan River downstream of the Gold Bar STP, but upstream of the Capital Region STP (MacLeod *et al.* 2007). The time weighted average (TWA) CBZ concentration was 3.6 – 92 ng/L. Surface water data collected from Long Term River Network sites in Alberta from 2004 to 2012 showed detection of CBZ in 6 out of 250 samples (2.4 per cent detection frequency) (AESRD 2012) (Table 6.6). Overall, detections in Alberta surface water only occurred in rivers downstream of municipal wastewater effluent discharges.

Brun *et al.* (2006) investigated the occurrence of pharmaceuticals in wastewater treatment plant effluent and associated surface water receivers in four Atlantic provinces (Table 6.6; effluent data Section 6.1). The persistence of CBZ was confirmed when detected in a small stream 17 km downstream of a sewage treatment plant, suggesting small receiving water bodies may be more at risk than larger surface water bodies (Brun *et al.* 2006).

Table 6.6. CBZ concentrations (ng/L) measured in surface waters in Alberta, Sakatchewan, Manitoba, and Atlantic Canada.

Water Source	City, Province	Name of Water Body	LOQ	Max	Median	Range	Detection Frequency	Reference
River	Alberta	Battle River d/s of Ponoka	10 (MDL)	359			1/250	AESRD 2012
		Bow River d/s of Calgary	10 (MDL)	28		16-28	4/250	AESRD 2012
		North Saskatchewan River d/s of Edmonton	10 (MDL)	11.3			1/250	AESRD 2012
River	Saskatchewan	Wascana Creek, upstream of wastewater treatment plant		nd			No. of samples not reported	EAU-NSACB. 2014
		Wascana Creek, downstream of wastewater treatment plant		321			No. of samples not reported	EAU-NSACB. 2014
		Swift Current, upstream of wastewater treatment plant		nd			No. of samples not reported	EAU-NSACB. 2014
		Swift Current, downstream of wastewater treatment plant		nd			No. of samples not reported	EAU-NSACB. 2014
		South Saskatchewan River, upstream of wastewater treatment plant		nd			No. of samples not reported	EAU-NSACB. 2014

Water Source	City, Province	Name of Water Body	LOQ	Max	Median	Range	Detection Frequency	Reference
		South Saskatchewan River, downstream of wastewater treatment plant		nd			No. of samples not reported	EAU-NSACB. 2014
River	Manitoba	Red River at south gate of floodway		17		12.1-17	2/2	WQMS 2012
		Red River at Selkirk Bridge		583		6.75-583	6/6	WQMS 2012
		Vermillion River		8.34			1/1	WQMS 2012
		Rat River watershed		35.3			1/1	WQMS 2012
		Dead Horse Creek, upstream of Morden sewage outfall	3.0[a] 1.2[b] 1.0[c]	1.5 (grab)[d]	Not detected (POCIS)[e]	<LOQ-1.5 (grab)[d]		Carlson *et al.* 2013
		Dead Horse Creek, downstream of Morden sewage outfall	3.0[a] 1.2[b] 1.0[c]	85.1 (grab)[d], 18.9 (POCIS)[e]		<LOQ-85.1 (grab)[d], <LOQ-18.9 (POCIS)[e]		Carlson *et al.* 2013
		Dead Horse Creek, upstream of Winkler sewage outfall	3.0[a] 1.2[b] 1.0[c]	76.2 (grab)[d], 18.2 (POCIS)[e]		<LOQ-76.2 (grab)[d], <LOQ-18.2 (POCIS)[e]		Carlson *et al.* 2013
		Dead Horse Creek, downstream of Winkler sewage outfall	3.0[a] 1.2[b] 1.0[c]	133 (grab)[d], 47.7 (POCIS)		<LOQ-133 (grab)[d], <LOQ-47.7 (POCIS)[e]		Carlson *et al.* 2013
		Dead Horse Creek, near the confluence of Dead Horse Creek and Plum River	3.0[a] 1.2[b] 1.0[c]	67.3 (grab)[d], 24.0 (POCIS)[e]		<LOQ-67.4 (grab)[d], <LOQ-24.0 (POCIS)[e]		Carlson *et al.* 2013
Marine waters	Halifax, NS	Bedford Bay/Basin – spring sampling	20	nd			0/1	Brun *et al.* 2006
		Bedford Bay/Basin – summer sampling	20	nd			0/1	Brun *et al.* 2006
River	Fredericton, NB	Saint John River – spring sampling	20	nd			0/1	Brun *et al.* 2006
		Saint John River – summer sampling	20	nd			0/1	Brun *et al.* 2006
River	Charlotte town, PE	Hillsborough River – spring sampling	20	nd			0/1	Brun *et al.* 2006
		Hillsborough River – summer sampling	20	nd			0/1	Brun *et al.* 2006

Water Source	City, Province	Name of Water Body	LOQ	Max	Median	Range	Detection Frequency	Reference
River	Grand Falls–Windsor, NL	Exploits River – spring sampling	20	nd			0/1	Brun *et al.* 2006
		Exploits River – summer sampling	20	nd			0/1	Brun *et al.* 2006
River	Sussex, NB	Kenebecasis River – spring sampling	20	nd			0/1	Brun *et al.* 2006
		Kenebecasis River – summer sampling	20	nd			0/1	Brun *et al.* 2006
Small streams (few metres wide)	Spring hill, NS	na – spring sampling	20	62			1/1	Brun *et al.* 2006
		na – summer sampling	20	170			1/1	Brun *et al.* 2006
	Summerside, PE	na – spring sampling	20	nd			0/1	Brun *et al.* 2006
		na – summer sampling	20	40			1/1	Brun *et al.* 2006
	Gander, NL	na – spring sampling	20	nd			0/1	Brun *et al.* 2006
		na – summer sampling	20	nd			0/1	Brun *et al.* 2006

LOQ = limit of quantification
MDL = method detection limit
nd = not detected
na = not available[a] = Limit of Quantification (LOQ) for POCIS and SPE grab samples by Q-Trap analysis
[b] = LOQ for POCIS sampler by Agilent MS analysis
[c] = LOQ for SPE grab sample by Agilent MS analysis
[d] = grab samples followed by SPE collected in June to July 2010
[e] = POCIS samples collected June to November 2010 and June to July 2011

Lajeunesse and Gagnon (2007) sampled for CBZ in the St Lawrence River both upstream and downstream of the Montréal wastewater treatment plant (employing primary treatment technology), with detection at 8 km downstream (Table 6.7). It appears that there is only a 50 per cent dilution at 8 km downstream. The plume from the Montréal wastewater treatment plant is very defined and persists for several kilometres downstream of the discharge.

Table 6.7. CBZ concentrations (ng/L) detected in the St Lawrence River in samples collected both upstream and downstream of the Montréal wastewater treatment plant (Lajeunesse and Gagnon 2007).

1.0 km upstream	0.5 km downstream	2.5 km downstream	4.5 km downstream	8 km downstream
0.8	7.4	5	4	3.5

Monitoring of PPCPs in raw water at eight drinking water intakes in Québec from 2003 to 2006 showed no detection of CBZ (< detection limit of 5 ng/L) (MDDELC 2015).

6.5 Groundwater

CBZ has been shown to poorly sorb to sediments, soils and/or colloids (natural organic matter) and can pass through sediment during bank filtration into groundwater aquifers (Tabe *et al.* 2009). The reported data below is for untreated (raw) groundwater.

The Ontario Ministry of the Environment survey of emerging contaminants of concern in southern Ontario drinking water supplies also included sampling of groundwater (Kleywegt *et al.* 2011). Results were obtained from only five samples taken from two drinking water systems. CBZ was not detected in any of the samples.

In contrast, the survey of pharmaceuticals in southern Ontario drinking water supplies conducted by Kormos (2007) provided evidence that CBZ is found in groundwater drinking water supplies. Eight wells were sampled in total, two deep groundwater reference wells plus six wells under the direct influence of surface water (GUDI wells). Two wells had detectable levels of CBZ, one of which was a rural well and the second an urban well. Higher concentrations were detected in the urban well, with both wells having CBZ concentrations lower than raw surface water concentrations (Table 6.5).

Table 6.8. CBZ concentrations (ng/L) detected in raw water collected from two southern Ontario groundwater wells (Kormos 2007).

Sampling Date	Urban Well	Rural Well
September 2005 (Rep 1)	11.5	4.2
September 2005 (Rep 2)	12.2	4.1
October 2005 (Rep 1)	9.8	3.2
October 2005 (Rep 2)	11.0	4.1

Stafford (2008) also surveyed three groundwater sources in southern Ontario. Two were down gradient from landfills, with one landfill being active (Waterloo landfill, Waterloo, Ontario) and the second one closed and no longer accepting waste (Cambridge landfill, Cambridge, Ontario – accepted primarily domestic waste with some commercial and institutional). All groundwater samples down gradient from the landfills had no detection of CBZ (instrument and method MDLs of 3 and 0.2 ng/L, respectively) suggesting that degradation and attenuation of these pharmaceuticals may be occurring (e.g. a thick unsaturated zone, strongly reducing conditions, high sorptive capacity of the waste). The third source was receiving septic system discharge (Long Point Provincial Park). In the groundwater sample receiving septic discharge, CBZ was one of two pharmaceuticals (the second being ibuprofen) that exhibited the highest concentration (maximum of 2,050 ng/L) with detection in the septic plume both horizontally and vertically (as far as 30 m from the septic bed infiltration zone).

Sampling of 35 wells in Alberta between 2010 and 2012 showed no detection of CBZ (detection limit of 10 ng/L) (AESRD 2014).

6.6 Sediment

Sediments sampled (n=113) between 2005 and 2008 from Alberta's Long-term River Network sites and five sites immediately downstream of STP effluent discharges showed no detection of

CBZ (detection limit of 10 ppm) (AESRD 2014). No other sediment concentration estimates for CBZ were found for the Canadian environment (Dove 2013).

A USGS survey of PPCPs conducted in Pennsylvania waterways from 2006 to 2009 showed no detection of CBZ in sediments downstream of municipal sewage treatment facilities (Reif *et al.* 2012). Laboratory based examination of the fate of CBZ in spiked sediments showed that after 100 days, 40 per cent of CBZ was detected in the sediment (Loffler *et al.* 2005).

7.0 ENVIRONMENTAL TOXICITY

This section presents a review of the scientific literature on the toxicity of CBZ to aquatic biota. The focus of the review is on the short- and long-term effects of CBZ on the survival, growth, reproduction and other endpoints of aquatic organisms. Effects data identified in the open literature were evaluated using the CCME (2007) data acceptability criteria for water quality guideline derivation.

7.1 Freshwater Aquatic Toxicity

Based on CBZ concentrations detected in Canadian surface waters (Section 6.3) and the toxicity data presented below, acute toxicity is not expected. Because of the low concentration, but persistent occurrence of carbmazepine in the aquatic environment, chronic toxicity is more likely to occur than acute toxicity (Ferrari *et al.* 2004). Ferrari *et al.* (2003) calculated the acute to chronic ratio for *C. dubia* (EC50/chronic NOEC) resulting in a very high value of 3,108 (although it was noted that the exposures were conducted in darkness). There is also potential for unknown mixture effects involving CBZ (and its transformation products) with other chemicals (and their associated transformation products) in the aquatic environment (Jos *et al.* 2003). Of particular concern is the co-exposure to pharmaceuticals with similar modes of action (e.g. sedation, anxiety reduction) that alone may not be toxic, but when combined, may increase toxic potential (Brandao *et al.* 2013).

Standard chronic toxicity tests may not utilize endpoints adequate for assessing specific effects associated with low-level exposure to pharmaceuticals such as CBZ. Standard tests assess impacts on mortality, growth and reproduction. Pharmaceuticals can elicit low dose effects due to being designed for biological activity. Perhaps more sensitive and specific endpoints would be more useful, such as developmental abnormalities, sex ratios or metabolic perturbations (e.g., biomarkers), where testing may cover multiple generations, but the ecological relevance of these would need to be established and validated (Ferrari *et al.* 2003). One example of a pharmaceutical elicting changes in behavior at environmentally relevant concentrations is the anti-anxiety benzodiazepine drug (oxazepam) (Brodin *et al.* 2013). Europan perch (*Perca fluviatilis*) exposed for seven days to 1.8 µg/L for seven days (river water concentration was 0.58 µg/L) displayed changes including increased activity, reduced sociality and higher feeding rate, all of which can lead to ecosystem-level consequences. In contrast, behavioural changes in *Hydra attenuata* were observed only at environmentally irrelevant CBZ concentrations (3,760 µg/L) (Quinn *et al.* 2008).

7.2 Toxicity to Invertebrates

7.2.1 Short-term Effects

Studies that were found to be acceptable (see CCME 2007 for toxicological data quality characterization) and given consideration for derivation of the short-term benchmark concentration are listed in Table 7.1. All reviewed studies are listed in the appendix.

In some of the exposures, solvent was used in the preparation of a CBZ stock solution. According to the European Commission (2011) Technical Guidance for Deriving Environmental Quality Standards, concentrations of a solvent should not exceed 100 mg/L (or 100 µL/L or 0.01 per cent). In the above listed studies, solvent concentrations used were within acceptable limits and the survival of organisms in solvent control solutions was acceptable.

The most sensitive organism from the acceptable short-term studies was *Hydra attenuata*, with a 96-hour toxicity threshold for morphological changes of 2,240 µg/L (Quinn *et al.* 2008). A 96-hour EC50 based on morphology of 15,520 µg/L was also derived for *H. attenuata* (Quinn *et al.* 2008).

The 96-hour EC50 (feeding behavior) result is similar to that obtained in other studies (Blaise *et al.* 2007; Hernando *et al.* 2006). De Lange *et al.* (2006) studied the effect of exposure to low concentrations (maximum of 1,000 µg/L) of CBZ on the behavioural responses of *Gammarus pulex*. No significant difference was observed across concentrations, but a general trend was observed following 1.5 hours of observation. At low concentrations (0.001 and 0.01 ug/L) activity was reduced, with an increased activity or flight response starting at higher concentrations (≥ 0.1 µg/L) although the differences were not found to be statistically different from control. The sensitivity of *G. pulex* could not be established from the short 1.5 hour exposure using the endpoints of decreased activity / locomotion, ventilation and feeding, where LOEC was >1,000 µg/L (De Lange *et al.* 2006).

Short-term studies that were reviewed, but not used for short-term benchmark derivation (with accompanying rationale) are listed in the appendix. The following describes a selection of the studies that reported on some non-traditional endpoints that were not used for short-term benchmark derivation.

Table 7.1. Toxicity data points for invertebrates considered for the derivation of a carbamazepine short-term benchmark concentration.

Species	Common Name	Life Stage	Endpoint	Effect Conc. (µg/L)	Study Ranking	Reference
Ceriodaphnia dubia	water flea	<24 hours old	48 hour LC50	7,070	2	Lamichhane *et al.* 2013
Ceriodaphnia dubia	water flea	<24 hours old	48 hour EC50 (inhibition of mobility)	77,700	2	Ferrari *et al.* 2003; Ferrari *et al.* 2004
Daphnia magna	water flea	<24 hours old	48 hour EC50 (inhibition of mobility)	>13,800	2	Ferrari *et al.* 2003; Ferrari *et al.* 2004
Daphnia magna	water flea	<24 hours old	48 hour EC50 (inhibition of mobility)	97, 816	2	Jos *et al.* 2003
Daphnia magna	water flea	<24 hours old	48 hour EC50 (inhibition of mobility)	>100,000	2	Cleuvers 2003
Daphnia magna	water flea	<24 hours old	48 hour EC50 (inhibition of mobility)	111,000	2	Han *et al.* 2006
Daphnia magna	water flea	<24 hours old	48 hour EC50 (inhibition of mobility)	>100,000	2	Kim *et al.* 2007
Daphnia magna	water flea	<24 hours old	48 hour EC50 (inhibition of mobility)	67,500	2	Pfluger *et al.* 2000
Daphnia magna	water flea	<24 hours old	48 hour EC50 (inhibition of mobility)	>10,000	2	Harada *et al.* 2008
Daphnia magna	water flea	<24 hours old	48 hour EC50 (inhibition of mobility)	76,300	2	Kim *et al.* 2007
Hydra attenuata	hydra		96 hour EC50 (decreased feeding behavior)	3,760	2	Quinn *et al.* 2008
Hydra attenuata	hydra		96 hour LC50	29,400	2	Quinn *et al.* 2008
Hydra attenuata	hydra		96 hour EC50 (morphological changes)	15,520	2	Quinn *et al.* 2008
Hydra attenuata	hydra		96 hour toxicity threshold (morphological changes)	2,240	2	Quinn *et al.* 2008

Quinn *et al.* (2004) used molecular markers to assess effects of CBZ on *Hydra attenuata* (these endopoints are not considered for guideline derivation unless they are directly linked to survival, growth or reproduction – see CCME 2007). The inducible threshold concentration for oxidative metabolism (heme oxidase and lipid peroxidation) was found at an environmentally-relevant concentration (7.09 µg/L) following 48 hours of exposure. Biotransformation capacity (Phase I - measured by cytochrome P450-mediated activity and Phase II - activity of glutathione S-transferase and sulfotransferase) was also induced at a threshold concentration of 47.25 µg/L after 48 hours.

Gagné *et al.* (2006) compared immune response in *Elliptio complananta* by exposing hemolymph *in vitro* for 24 hours to increasing concentrations of CBZ (as well as other individual PPCPs in separate tests) and comparing immunotoxic effects observed in whole organisms exposed to treated wastewater for 60 days. With the *in vitro* exposure, CBZ was found to be among the most potent of the tested PPCPs in altering immune function. The endpoint threshold concentrations measured *in vitro* for the various PPCPs were 10-100 times higher than observed in whole organisms exposed to effluents (where exact composition of effluent was unknown).

In general, acutely-toxic effects at environmentally-relevant concentrations[1] are not expected. The lowest reported short-term effect concentration is three orders of magnitude greater than the highest measured CBZ concentration in Canadian surface waters (Section 6.3), therefore risk of acute toxicity to invertebrates appears to be negligible.

7.2.2 Long-term Effects

Long-term exposures with six organisms from six studies were given consideration for derivation of the long-term water quality guideline and are listed in Table 7.2. All reviewed studies are listed in the appendix.

Table 7.2. Toxicity data points for invertebrates considered for the derivation of a carbamazepine long-term water quality guideline.

Species	Common Name	Life Stage	Endpoint	Effect Conc. (µg/L)	Study Ranking	Reference
Brachionus calyciflorus	Rotifer	Newly hatched	48 hour NOEC (reproduction)	377	2	Ferrari *et al.* 2003; Ferrari *et al.* 2004
Brachionus calyciflorus	Rotifer	Newly hatched	48 hour LOEC (reproduction)	754	2	Ferrari *et al.* 2003; Ferrari *et al.* 2004
Ceriodaphnia dubia	Water flea	<24 hours old	7 day NOEC (reproduction)	25	2	Ferrari *et al.* 2003; Ferrari *et al.* 2004
Ceriodaphnia dubia	Water flea	<24 hours old	7 day LOEC (reproduction)	100	2	Ferrari *et al.* 2003; Ferrari *et al.* 2004
Ceriodaphnia dubia	Water flea	<24 hours old	7 day LOEC (reproduction)	100	2	Lamichhane *et al.* 2013
Ceriodaphnia dubia	Water flea	<24 hours old	7 day LOEC (fecundity, 3 broods, F0, F1, F2)	264.6	2	Lamichhane *et al.* 2013
Ceriodaphnia dubia	Water flea	<24 hours old	7 day NOEC (fecundity, 3 broods, F0, F1, F2)	196.7	2	Lamichhane *et al.* 2013
Ceriodaphnia dubia	Water flea	<24 hours old	14 day LOEC (fecundity, 1-7 broods, F0, F1)	196.7	2	Lamichhane *et al.* 2013
Ceriodaphnia dubia	Water flea	<24 hours old	14 day NOEC (fecundity, 1-7 broods, F0, F1)	104	2	Lamichhane *et al.* 2013

[1] Highest measured CBZ concentration in Canadian surface waters is 0.988 ug/L (Kormos 2007). Highest measured CBZ concentration in Canadian STP effluent is 2.3 ug/L (Metcalfe *et al.*. 2003b).

Species	Common Name	Life Stage	Endpoint	Effect Conc. (µg/L)	Study Ranking	Reference
Ceriodaphnia dubia	Water flea	<24 hours old	14 day LOEC (fecundity, 1-7 broods, F2)	264.6	2	Lamichhane *et al.* 2013
Ceriodaphnia dubia	Water flea	<24 hours old	14 day NOEC (fecundity, 1-7 broods, F2)	196.7	2	Lamichhane *et al.* 2013
Ceriodaphnia dubia	Water flea	<24 hours old	14 day LOEC (fecundity, age at first reproduction, F0, F1)	≥264.6	2	Lamichhane *et al.* 2013
Ceriodaphnia dubia	Water flea	<24 hours old	14 day NOEC (fecundity, age at first reproduction, F0, F1)	264.6	2	Lamichhane *et al.* 2013
Ceriodaphnia dubia	Water flea	<24 hours old	14 day LOEC (fecundity, age at first reproduction, F2)	264.6	2	Lamichhane *et al.* 2013
Ceriodaphnia dubia	Water flea	<24 hours old	14 day NOEC (fecundity, age at first reproduction, F2)	196.7	2	Lamichhane *et al.* 2013
Ceriodaphnia dubia	Water flea	<24 hours old	14 day LOEC (growth, neonate body length (mm), F0, F1, F2)	≥264.6	2	Lamichhane *et al.* 2013
Ceriodaphnia dubia	Water flea	<24 hours old	14 day NOEC (growth, neonate body length (mm), F0, F1, F2)	264.6	2	Lamichhane *et al.* 2013
Ceriodaphnia dubia	Water flea	<24 hours old	14 day LOEC (growth, adult body length (mm), F0, F1)	≥264.6	2	Lamichhane *et al.* 2013
Ceriodaphnia dubia	Water flea	<24 hours old	14 day NOEC (growth, adult body length (mm), F0, F1)	264.6	2	Lamichhane *et al.* 2013
Ceriodaphnia dubia	Water flea	<24 hours old	14 day LOEC (growth, adult body length (mm), F2)	264.6	2	Lamichhane *et al.* 2013
Ceriodaphnia dubia	Water flea	<24 hours old	14 day NOEC (growth, adult body length (mm), F2)	196.7	2	Lamichhane *et al.* 2013
Ceriodaphnia dubia	Water flea	<24 hours old	14 day LOEC (growth, neonate dry weight (ug), F0)	≥264.6	2	Lamichhane *et al.* 2013
Ceriodaphnia dubia	Water flea	<24 hours old	14 day NOEC (growth, neonate dry weight (ug), F0)	264.6	2	Lamichhane *et al.* 2013

Species	Common Name	Life Stage	Endpoint	Effect Conc. (µg/L)	Study Ranking	Reference
Ceriodaphnia dubia	Water flea	<24 hours old	14 day LOEC (growth, neonate dry weight (ug), F1)	196.7	2	Lamichhane *et al.* 2013
Ceriodaphnia dubia	Water flea	<24 hours old	14 day NOEC (growth, neonate dry weight (ug), F1)	104	2	Lamichhane *et al.* 2013
Ceriodaphnia dubia	Water flea	<24 hours old	14 day LOEC (growth, neonate dry weight (ug), F2)	264.6	2	Lamichhane *et al.* 2013
Ceriodaphnia dubia	Water flea	<24 hours old	14 day NOEC (growth, neonate dry weight (ug), F2)	196.7	2	Lamichhane *et al.* 2013
Daphnia magna	Water flea	<24 hours old	21 day NOEC (mortality)	4,000	1	Liebig 2005
Daphnia magna	Water flea	<24 hours old	21 day NOEC (reproduction)	400	1	Liebig 2005
Daphnia magna	Water flea	<24 hours old	21 day NOEC (growth)	400	1	Liebig 2005
Daphnia magna	Water flea	<24 hours old	21 day LOEC (mortality)	12,600	1	Liebig 2005
Daphnia magna	Water flea	<24 hours old	21 day LOEC (reproduction)	1,260	1	Liebig 2005
Daphnia magna	Water flea	<24 hours old	21 day LOEC (growth)	1,260	1	Liebig 2005
Daphnia pulex	Water flea	<24 hours old	5 day (juvenile period) LOEC for body length / somatic growth rate (delayed maturation)	200	2	Lurling *et al.* 2006
Daphnia pulex	Water flea	<24 hours old	14 day (entire experimental period) LOEC for body length / somatic growth rate (delayed maturation)	>200	2	Lurling *et al.* 2006
Daphnia pulex	Water flea	<24 hours old	14 day (entire experimental period) LOEC size at first reproduction (mm)	>200	2	Lurling *et al.* 2006
Daphnia pulex	Water flea	<24 hours old	14 day (entire experimental period) LOEC survival (survival of 67%, compared to control & solvent	100	2	Lurling *et al.* 2006

Species	Common Name	Life Stage	Endpoint	Effect Conc. (µg/L)	Study Ranking	Reference
			control, both at 83%)			
Daphnia pulex	Water flea	<24 hours old	14 day (entire experimental period) LOEC spine length	>200	2	Lurling *et al.* 2006
Chironomus dilutus	midge	12 days old	10 day LC25 (survival)	23,700	1	Dussault *et al.* 2008
Chironomus dilutus	midge	12 days old	10 day LC10 (survival)	9,500	1	Dussault *et al.* 2008
Chironomus dilutus	midge	12 days old	10 dayEC25 (growth)	4,600	1	Dussault *et al.* 2008
Chironomus dilutus	midge	12 days old	10 day EC10 (growth)	2,600	1	Dussault *et al.* 2008
Hyalella azteca	amphipod	7-14 days old	10 day LC25 (survival)	2,300	1	Dussault *et al.* 2008
Hyalella azteca	amphipod	7-14 days old	10 day LC10 (survival)	600	1	Dussault *et al.* 2008
Hyalella azteca	amphipod	7-14 days old	10 day EC25 (survival)	6,100	1	Dussault *et al.* 2008
Hyalella azteca	amphipod	7-14 days old	10 day EC10 (survival)	2,400	1	Dussault *et al.* 2008

All other studies were classified as unacceptable, with brief rationale for classification given below.

The order of sensitivity to CBZ following chronic exposure (most to least sensitive) was *C. dubia = D. pulex > B. calyciflorus > D. magna > H. azteca > C. dilutus*. The endpoint measured with *C. dubia* was reproduction, with a seven-day (nominal) NOEC and (nominal) LOEC of 25 µg/L and 100 µg/L, respectively (Ferrari *et al.* 2003; Ferrari *et al.* 2004). The 14-day survival (nominal) NOEC and (nominal) LOEC for *D. pulex* was 10 and 100 µg/L, respectively (Lurling *et al.* 2006). The 48-hour reproduction (nominal) NOEC and (nominal) LOEC for *B. calyciflorus* was 377 and 754 µg/L, respectively. The 21-day NOEC and LOEC for *Daphnia magna* for both growth and reproduction was 400 and 1,260 µg/L, respectively (Liebig 2005). The study by Liebig 2005 was not obtained and therefore not reviewed. However, this study was reported in RIVM 2014, and had been scored a Klimisch ranking of 1 (i.e., best quality study) following review by various jurisdictions (European Commission, Switzerland, Germany, France) (RIVM 2014). The ten-day LC10 for *Hyalella azteca* was 600 µg/L (Dussault *et al.* 2008). The most tolerant species, *C. dilutus*, showed a ten-day EC10 for growth of 2,600 µg/L (Dussault *et al.* 2008). The reported concentrations from Dussault *et al.* (2008) were based on measured concentrations.

The impact from exposure to CBZ on growth and life-history characteristics was investigated using *Daphnia pulex* (Lurling *et al.* 2006). 14-day exposures were conducted in darkness. Females exposed to 1 µg/L CBZ matured slightly earlier and produced more offspring when compared to controls and higher treatments (up to 200 µg/L CBZ). At the highest test concentration (200 µg/L), reduced survival, delayed time to first reproduction, and reduced rate of population growth was noted.

Long-term studies that were reviewed but not used for long-term guideline derivation (with accompanying rationale) are listed in the appendix. The following describes a selection of the studies that were not used.

Although results from water-only exposures are appropriate for consideration in water quality guideline derivation, the following provides an overview of results obtained using sediment-exposures. The 28-day life-cycle spiked sediment assays conducted with *Lumbriculus variegatus* and *Chironomus riparius* by Oetken *et al.* (2005) reported significant effects on emergence for the chrionomid, but no effects for the oligochaete. The 28-day LOEC sediment CBZ concentration was 234 ug/kg dw [ppb] (332 µg/L [ppb] overlying water concentration). Overlying water concentrations were reported as high as 2,850 µg/L [ppb] when the sediment CBZ concentration was 2,900 ug/kg dw [ppb]. Dussault *et al.* (2008) stated that it was difficult to evaluate whether the observed impacts to *Chironomus* were caused by sediment-bound CBZ, overlying water CBZ, or both in the Oetken *et al.* (2005) study. Spiked sediment toxicity tests (ten-day) conducted by Gilroy *et al.* (2012) were conducted using Imhoff settling cones, which provide a high water to sediment ratio (39:1), allowing for an improved assessment of exposure to sediment-bound contaminants. Even at the highest test concentration of 56 mg/kg dry [ppm] weight for the exposure with *C. dilutus*, and 35 mg/kg dry [ppm] weight for the exposure with *H. azteca*, no toxic effects were observed (Gilroy *et al.* 2012). Highest measured overlying water concentrations in these sediment exposures were 410 µg/L with *C. dilutus*, and 120 µg/L with *H. azteca* (Gilroy *et al.* 2012)

Effect concentrations (using traditional endpoints) for long-term invertebrate exposures to CBZ are an order of magnitude greater than the highest reported concentration of CBZ in Canadian surface waters[2]. The risk to invertebrate receptors appears to be low.

7.3 Toxicity to Fish

7.3.1 Short-term Effects

Seven studies with four fish species were given consideration for derivation of the short-term benchmark concentration. These are listed in Table 7.3. All reviewed studies are listed in the appendix.

[2] Highest measured CBZ concentration in Canadian surface waters is 0.988 ug/L (Kormos 2007). Highest measured CBZ concentration in Canadian STP effluent is 2.3 ug/L (Metcalfe *et al.*. 2003b).

Table 7.3. Toxicity data points for fish considered for the derivation of a carbamazepine short-term benchmark concentration.

Species	Common Name	Life Stage	Endpoint	Effect Conc. (µg/L)	Study Ranking	Reference
Cyprinus carpio	Common carp	7.5 cm; 15.0 g	24 hour LC50	59,700	2	Malarvizhi *et al.*, 2012
Danio rerio	Zebrafish	2 hour old embryos	72 hour EC50 (growth retardation)	86,500	2	van den Brandhof and Montforts 2010
Danio rerio	Zebrafish	16 weeks old (3.0 cm, 132 mg)	96h LC50	35,400	1	Liebig 2005
Danio rerio	Zebrafish	Fertilized embryos (2-2.5 hours post fertilization)	72 hours post hatch EC50 (teratogenicity)	52,452	2	Weigt *et al.* 2011
Oryzias latipes	Japaese medaka	Length 2.0 ± 1.0 cm	48 hour LC50	35,400	2	Kim *et al.* 2007
Oryzias latipes	Japaese medaka	Length 2.0 ± 1.0 cm	96 hour LC50	45,870	2	Kim *et al.* 2009
Oncorhynchus mykiss	Rainbow trout	Juveniles (64.3 g)	96 hour LC50	19,900	2	Li *et al.* 2011

Two-hour-old Zebrafish embryos were exposed to CBZ for 72 hours, as per draft (at time of testing) OECD standard guideline for fish embryo testing (van den Brandhof and Montforts 2010). Additional testing (which was not reported in the manuscript) assessing an exposure duration of 96 hours, as per final OECD standard guideline, was evaluated in comparison to the 72 hour exposure, and it was determined that sensitivity of the test was not enhanced. Rather, the increased exposure time of 96 hours (versus 72 hours) allowed for additional teratogenic endpoints to be incorporated. Overall, the 72 hour exposure was found to be as sensitive as regular acute studies with fish. The 72 hour EC50 for growth reduction was 86,500 µg/L (with a NOEC of 30,600 µg/L). The study by Weigt *et al.* (2011) was to develop a three-day Zebrafish embryo teratogenicity assay. The three-day teratogenicity EC50 derived in this study (52,452 µg/L) was lower than that of van den Brandhof and Montforts (2010). Considering that the Weigt *et al.* (2011) study was related to development of a new assay, with minimal water quality reporting, the van den Brandhof and Montforts (2010) study was selected as higher ranking. The Zebrafish study by Liebig 2005 was not obtained and therefore not reviewed. However, this study was reported in RIVM 2014, and had been scored a Klimisch ranking of 1 following review by various jurisdictions (European Commission, Switzerland, Germany, France) (RIVM 2014). The order of sensitivity to CBZ following acute exposure (most to least sensitive) was *O. mykiss* > *O. latipes* = *D. rerio* > *C. carpio.*

Short-term studies that were reviewed but not used for short-term benchmark derivation (with accompanying rationale) are listed in the appendix. The following describes a selection of the studies that were not used.

Malarvizhi *et al.* (2012) exposed adult Common carp (Cyprinus carpio) for 24 hours to a single concentration of CBZ (a pre-determined 24-hour LC50 of 59,700 µg/L). An assessment of the resulting enzymological changes in the gill, liver and muscle tissue was made. CBZ was shown to induce alterations of the enzymes glutamate oxaloacetate transaminase (GOT), glutamate

pyruvate transaminase (GPT) and lactate dehydrogenase (LDH). GOT activity decreased in all organs - gill, liver and muscle, whereas GPT and LDH activity was increased in liver and muscle and decreased in gill. It was suggested by Malarvizhi *et al.* (2012) that these three enzymes may be used as biomarkers to monitor the toxic levels of pharmaceuticals in aquatic organisms.

Li *et al.* (2010b) demonstrated that following a two-hour exposure to CBZ, spermatozoa from the Common carp displayed induction of oxidative stress (increased LPO and CP – see glossary), as well as a reduction/inhibition of antioxidant enzyme activity. Spermatazoa motility and velocity were also significantly reduced compared to control, but viability was not affected. The lowest exposure concentration (200 µg/L) was well above what Li *et al.* (2010b) reported as environmentally-relevant concentrations (0.1 to 1.3 µg/L). What is unknown is the effect of CBZ on the quality of spermatozoa in wild fish exposed to low concentrations over a life-time and resulting impacts to reproduction. This study was not used because it assessed sperm quality parameters and markers of oxidative stress in vitro.

The cumulative daily embryo production in Zebrafish exposed to environmentally relevant concentrations of CBZ (0.5 and 10 µg/L) for six weeks was shown to significantly decrease compared to controls (discussed further in Section 7.3.2). No significant difference was found between the low (0.5 µg/L) and high (10 µg/L) doses (Galus *et al.* 2013a). Interestingly, this parental Zebrafish exposure to CBZ did not adversely impact offspring survival, hatching success and rate of developmental abnormalities. In contrast, direct exposure of fertilized Zebrafish embryos to low and high dose CBZ did show adverse impacts with respect to survival, but no impacts on developmental abnormalities (Galus *et al.* 2013a). The 96-hour (post-hatch) LOEC for increased mortality compared to control was found to be statistically significant at 0.5 µg/L CBZ. This study used a DMSO solvent concentration of 0.004 per cent (the solvent did not elevate mortality above background). Surprisingly, a statistically significant decrease in mortality compared to control was observed at the highest test concentration of 10 µg/L. This is suggestive of a non-monotonic concentration-response, however is not conclusive since only two CBZ concentrations (low and high, plus a control) were tested. A recent paper published by the United States Environmental Protection Agency (US EPA 2013) summarizing the state-of-the-science on non-monotonic dose responses identified several chemicals that have displayed this response to aquatic organisms. Carbamazepine was not included in the US EPA list. Further investigation into CBZ and non-monotonic dose responses would be required.

The study by Nassef *et al.* (2010) (using ethanol solvent and triolein as an injection vehicle) nanoinjected CBZ into Japanese medaka embryos 8-hours post-fertilization (1, 5 or 12 ng per egg in triolein). This was done in order to mimic maternal transfer to fish embryos, followed by assessment of impacts to fish development and survival. CBZ injected embryos showed a significant decrease in number of survivors, a significant increase in the number of hemorrhaged embryos, significantly delayed embryonic development, and a significant decrease in number of hatched embryos. The study by Galus *et al.* (2013a) (discussed earlier) showed that Zebrafish embryos collected from CBZ exposed adults displayed no adverse impact on survival, hatching success, or rate of developmental abnormalities. Direct comparison between these two studies is not possible (based on method of CBZ exposure of injection versus maternal transfer). In the nanoinjection study, CBZ may be a completely different compound when compared to waterborne CBZ. In addition the exposure routes are completely different. With the nanoinjection study, there may also be artifacts as a result of the injection procedure. What is interesting is that the log K_{ow} for CBZ of 2.25 to 2.45 (Table 2.1) is considered to be low,

implying that uptake into lipid (maternal transfer into embryos) would be low. This may explain why no effects were observed in Galus *et al.* (2013a).

Laville *et al.* (2004) tested the toxicity of CBZ using two fish hepatocyte models, PLHC-1 (derived from the hepatocellular carcinoma of the Topminnow *Poeciliopsis lucida*) and PRTH (Rainbow trout hepatocytes). Out of the nine pharmaceuticals tested, CBZ was least cytotoxic when tested with the PLHC-1 fish cell line (3h EC50 of 153,812 µg/L). Increased cytoxicity (cell viability) was observed with the PRTH fish cell line (75,134 µg/L). Interaction with the cytochrome P450 1A (CYP1A) enzyme was assessed using the EROD assay, with no induction of EROD in the PLHC-1 fish cell line. EROD activity was inhibited by CBZ in the PRTH fish cell line (15 min EC50 of 75,134 µg/L).

A variety of endpoints were tested using the RTG-2 cell line tested by Jos *et al.* (2003). This cell line was not very sensitive to CBZ, with the most sensitive endpoint being the neutral red assay (reduced uptake of vital dye by lysosomes) with 24-, 48- and 72-hour EC50 values of 117,190, 111,661 and 116,954 µg/L, respectively.

The effects of CBZ on the fish cerebral antioxidant system were tested *in vitro*, with Rainbow trout brain homogenates exposed to a series of CBZ concentrations for 0, 60 and 120 minutes (Li *et al.* 2010a). The nominal exposure concentrations included an environmentally relevant concentration (1.0 µg/L), 1 per cent of the 96-hour EC50 (200 µg/L) and 10 per cent of the 96-hour EC50 (2,000 µg/L). Adaptive stress responses were induced at the environmentally relevant concentration of 1.0 µg/L, with brain homogenates showing increased antioxidant responses compared to previous exposures conducted in vivo. Oxidative stress responses were also apparent at the higher CBZ concentrations.

As with invertebrates, acute toxic effects to fish at environmentally relevant concentrations[3] are not expected.

7.3.2 Long-term Effects

Four studies presented data for three fish species that were considered for long-term guideline derivation, and are listed in Table 7.4. All reviewed studies are listed in the appendix.

[3] Highest measured CBZ concentration in Canadian surface waters is 0.988 ug/L (Kormos 2007). Highest measured CBZ concentration in Canadian STP effluent is 2.3 ug/L (Metcalfe *et al.*. 2003b).

Table 7.4. Toxicity data points for fish considered for the derivation of a carbamazepine long-term water quality guideline.

Species	Common Name	Life Stage	Endpoint	Effect Conc. (µg/L)	Study Ranking	Reference
Danio rerio	Zebrafish	Embryos, 3 hours post fertilization	10 day NOEC (fish embryo and larvae mortality)	25,000	2	Ferrari *et al.* 2003; Ferrari *et al.* 2004
Danio rerio	Zebrafish	Embryos, 3 hours post fertilization	10 day LOEC (fish embryo and larvae mortality)	50,000	2	Ferrari *et al.* 2003; Ferrari *et al.* 2004
Oncorhynchus mykiss	Rainbow trout	10-12 months old (264.33 ± 39.97g) , female and male	42 day LOEC (lower condition factor)	2,000	2	Li *et al.* 2009
Oncorhynchus mykiss	Rainbow trout	10-12 months old (264.33 ± 39.97g) , female and male	42 day NOEC (lower condition factor)	200	2	Li *et al.* 2009
Pimephales promelas	Fathead minnow	eggs <48 hours old	28 day NOEC (survival)	862	2	Overturf *et al.* 2012
Pimephales promelas	Fathead minnow	eggs <48 hours old	28 day LOEC (survival)	>862	2	Overturf *et al.* 2012
Pimephales promelas	Fathead minnow	eggs <48 hours old	28 day NOEC (growth)	862	2	Overturf *et al.* 2012
Pimephales promelas	Fathead minnow	eggs <48 hours old	28 day LOEC (survival)	>862	2	Overturf *et al.* 2012

Ferrari *et al.* (2003, 2004) assessed the impact of CBZ on the hatching rate and embryonic mortality of Zebrafish *(Danio rerio)* embryos in a ten-day exposure, following a standard ISO (International Organization for Standardization) test procedure 12890 (1999). The toxicity observed was low, with a ten-day NOEC for fish embryo and larvae mortality of 25,000µg/L. The low sensitivity of the embryos may be due to the age of the Zebrafish eggs, as exposures were initiated with eggs three hours post-fertilization and where chorion hardening may have occurred. Instead, eggs at the 0.5-hour stage post-fertilization would be optimal (Ferrari *et al.* 2003). Another suggestion for the low sensitivity is that the endpoints measured are not sensitive enough to detect the specific effects associated with CBZ. More specific and sensitive endpoints could include developmental abnormalities, sex ratios or biomarkers (metabolic perturbations), as well as testing over multiple generations (Ferrari *et al.* 2003).

Li *et al.* (2009) investigated the chronic effects of CBZ on the physiological condition status and muscle-based biomarkers following a 42-day exposure with Rainbow trout *(Oncorhynchus mykiss)*. Condition factor (CF) was significantly lower in the group exposed to the highest test concentration (LOEC of 2,000 µg/L), with no change observed in hepatosomatic index (HSI) (LOEC >2,000 µg/L). The most sensitive endpoints were the muscle-based biomarkers with NOEC and LOEC values of 1 and 200 µg/L, respectively. The endpoints related to gross

morphometric indices (CF and HSI) are more ecologically relevant, in comparison to biomarkers of antioxidant response and energy metabolic parameters.

Overturf *et al.* (2012) conducted an early life stage exposure using fathead minnow (*Pimephales promelas*) eggs (<48 hours old) to a CBZ concentration series. A NOEC of 862 µg/L (survival and growth) was reported in this study. This is two magnitudes higher than the highest reported CBZ effluent concentration of 2.3 µg/L reported by Metcalfe *et al.* (2003b).

Long-term studies that were reviewed but not used for long-term guideline derivation (with accompanying rationale) are listed in the appendix. The following describes a selection of the studies that were not used.

Fish exposed to environmental toxicants have been shown to respond by altering enzyme activity (either through induction or inhibition of enzyme levels) which can lead to tissue damage (Malarvizhi *et al.* 2012). These types of endpoints are not considered suitable for guideline derivation unless a strong ecological relevance can be established. One study which was reviewed but not used for guideline derivation included both an acute (24-hour) and chronic (35-day) exposure of adult Common carp (*Cyprinus carpio*) to CBZ (Malarvizhi *et al.* 2012). The acute exposure exposed fish to a pre-determined 24-hour LC50 CBZ concentration of 59,700 µg/L, whereas the chronic exposure exposed fish to 1/10 of the 24-hour LC50 (5,970 µg/L). Following both the acute and chronic exposures, fish tissues (gill, liver and muscle) were collected and processed for enzymological levels. The CBZ exposure concentrations used in this study are not considered to be environmentally relevant.

Triebskorn *et al.* (2007) argued that standard toxicity tests are not testing endpoints sensitive enough to detect impacts of pharmaceuticals, as these do not take into account the specific mode of action of pharmaceuticals. The 28-day exposure conducted by Triebskorn *et al.* (2007), with the Common carp *(Cyprinus carpio)*, investigated cellular impacts through histological and cytological investigation of liver, kidney, and gills (exposure concentrations were 1, 5, 20, 50, and 100 µg/L with deviations of measured from nominal concentrations ranging from 0.54 per cent to 10.6 per cent). In the case of the liver, a slight reaction with no clear dose-response relationship was observed (increased amount of macrophages, membrane material), with a LOEC (liver) of >100 µg/L. In the case of the gills, a moderate reaction was observed (epithelial lifting, hyperplasia and hypertrophy of mucus cells). The LOEC (gills) was 20 µg/L, but no clear dose-response was observed. The strongest effect was observed in the kidney, with a LOEC of 1 µg/L. Strongest reactions of CBZ were in proximal tubule 2 (mainly involved in the reabsorption of inorganic molecules and bivalent ions) and in the distal portions of the tubules (where univalent ions are reabsorbed). CBZ is known to be a sodium channel blocker, and therefore the observed effect could be related to its influence on salt and water balance in the kidney (influence on Na^+ homeostasis and resulting cellular effects in the kidney) (Triebskorn *et al.* 2007). No discussion on the ecological relevance of these observations was provided.

The six-week exposure with Zebrafish *(Danio rerio)* by Galus *et al.* (2013a) showed that adult females exposed to CBZ concentrations of 0.5 (low) and 10 µg/L (high) showed a statistically significant decrease in cumulative daily embryo production compared to control (DMSO solvent was used, where the final concentration did not exceed 0.004 per cent and was shown to not impact any measured endpoint). Exposure to low concentrations also increased the occurrence of atreitic oocytes in females. Males displayed no observable effects on testis morphology, nor was

there any occurrence of apoptosis within the testis. However, sex steroid (plasma 11-ketotestosterone) levels were significantly reduced in both male and female fish exposed to the low CBZ concentration, but not in the high concentration. No change in estradiol was observed in either sex exposed to both low and high CBZ. Embryos collected from these exposed adults displayed no adverse impact on survival, hatching success, or rate of developmental abnormalities. In contrast, embryos that were directly exposed to CBZ showed increased mortality at the low concentration, but surprisingly showed a significant decrease in mortality (compared to control) at the high concentration (see Section 8.3.1). No effects on developmental abnormalities were observed in the exposed embryos. Overall, this study showed that chronic exposure to environmentally relevant concentrations of CBZ can lead to effects on reproduction.

A follow-up study to Galus *et al.* (2013a) involved assessment of trans-generational impacts of CBZ exposure on Zebrafish offspring (Galus *et al.* 2014). Reproductively mature adult (six-eight month old) zebrafish were exposed to either a control, or 10 µg/L CBZ for a period of 4 weeks. After four weeks, eggs were collected, embryos were reared to sexual maturity (six-eight months), followed with pairwise and reciprocal breeding of the first filial (F1) generation. Interestingly, when a CBZ exposed F1 female was crossed with a control male no difference was observed in embryo production compared to controls. When CBZ exposed F1 males were crossed with either a control female or a CBZ exposed F1 female, 50 per cent fewer embryos were produced. CBZ exposure in parents also resulted in reduced courtship behavior displays in F1 males. No histological changes in liver, kidney or gonads were observed in the CBZ exposed F1 fish.

The study by Madureira *et al.* (2011) investigated whether non-steroidal classes of pharmaceuticals (such as CBZ) could interfere with reproductive homeostasis in the Zebrafish. The 21-day exposure to one concentration of CBZ (1,780 µg/L) resulted in no significant difference in gonadosomatic index or total weight of gonads in either males or females, when compared to control (Madureira *et al.* 2011). Male fish did display a significant decrease in spermatocyte percentage (47.19±5.30 per cent) compared to controls (53.25±7.13 per cent) in the CBZ exposure.

A study assessing the impact of CBZ exposure on gene expression in 12 month old Atlantic salmon (*Salmo salar* L.) was assessed by Hampel *et al.* (2014). Fish were exposed for five days to 7.85±0.13 µg/L CBZ, a concentration representative of the upper limit of measurements made in hospital and STP effluents. Following the five-day exposure, brain tissue transcriptomics were analyzed (male fish were selected to minimize potential variation due to sex and developmental stage). Out of a total of 16,432 genes, 373 were differentially expressed compared to controls (t-test, p-value<0.05), with 26 of the 373 showing a greater than two-fold change compared to controls (24 were up-regulated and two down-regulated). Seven of the 26 have no known function (could not be identified). The microarray and qPCR showed revealed transcriptomal responses associated with pituitary activity. The authors note that these mRNA increases on hormone release have yet to be determined, in addition to how this impacts critical life stages of the Atlantic salmon.

A second study which conducted microarray experiments examining brain tissue of fathead minnow (*Pimephales promelas*) exposed to 100 µg/L CBZ (alone and in a three pharmaceutical mixture) showed enrichment of gene sets (highly conserved human genes) associated with idiopathic autism spectrum disorders (Thomas and Klaper 2012). It was noted that the high

exposure concentration was selected to account for conservative concentration estimates and the potential presence of bioactive transformation products.

Unlike with the acute studies, chronic effects at environmentally relevant concentrations are possible.

7.4 Toxicity to Algae and Aquatic Plants

The studies that were considered for guideline derivation are listed in Tables 7.5 and 7.6. All reviewed studies are listed in the appendix.

Table 7.5. Toxicity data points for aquatic plants and algae considered for the derivation of a carbamazepine short-term benchmark concentration.

Species	Common Name	Life Stage	Endpoint	Effect Conc. (µg/L)	Study Ranking	Reference
Chlorella vulgaris	Green algae		24 hour EC50 (growth)	110,929	2	Jos *et al.* 2003

Table 7.6. Toxicity data points for aquatic plants and algae considered for the derivation of a carbamazepine long-term water quality guideline.

Species	Common Name	Life Stage	Endpoint	Effect Conc. (µg/L)	Study Ranking	Reference
Chlorella pyrenoidosa	green algae		72 hour NOEC (growth rate)	1,000	2	Zhang *et al.* 2012
Chlorella pyrenoidosa	green algae		96 hour NOEC (growth rate)	500	2	Zhang *et al.* 2012
Chlorella pyrenoidosa	green algae		72 hour NOEC (Chlorophyll a)	1,000	2	Zhang *et al.* 2012
Chlorella pyrenoidosa	green algae		96 hour NOEC (Chlorophyll a)	1,000	2	Zhang *et al.* 2012
Chlorella vulgaris	green algae		48 hour NOEC (abundance)	>2,363 (but <23,627)	2	Jos *et al.* 2003
Chlorella vulgaris	green algae		48 hour LOEC (abundance)	23,627	2	Jos *et al.* 2003
Chlorella vulgaris	Green algae		48 hour EC50 (growth)	36,622	2	Jos *et al.* 2003
Cyclotella meneghiniana	diatom		96 hour EC50 (growth)	31,600	2	Ferrari *et al.* 2004
Cyclotella meneghiniana	diatom		96 hour NOEC (growth)	10,000	2	Ferrari *et al.* 2004
*Desmodesmus subspicatus (*formerly *Scenedesmus subspicatus)*	green algae		72 hour EC50 (inhbition of average growth rate)	74,000	2	Cleuvers 2003
Lemna minor	duckweed	plants with two or three fronds	7 day EC50 (growth inhibition)	25,500	2	Cleuvers 2003
Pseudokirchneriella subcapitata	green algae		96 hour NOEC (growth inhibition)	521	2	Harada *et al.* 2008

Species	Common Name	Life Stage	Endpoint	Effect Conc. (µg/L)	Study Ranking	Reference
Pseudokirchneriella subcapitata	Green algae		96 hour EC50 (growth inhibition)	48,900	2	Harada *et al.* 2008
Synechococcus leopolensis	Blue-green algae		96 hour EC50 (growth)	33,600	2	Ferrari *et al.* 2004
Synechococcus leopolensis	Blue-green algae		96 hour NOEC (growth)	17,500	2	Ferrari *et al.* 2004

The most sensitive receptor was the green algae *Chlorella pyrenoidosa* with a 96-hour NOEC for inhibition of growth of 500 µg/L (Zhang *et al.* 2012).

Jos *et al.* (2003) investigated if growth of the unicellular algae *Chlorella vulgaris* was inhibited by CBZ. In contrast to findings by Andreozzi *et al.* (2002) (where impact to growth was tested using *Ankistrodemus braunii* and *Selenastrum capricornutum*), a clear dose-response for growth inhibition was observed. The 24 and 48-hour EC50 (growth) concentrations were 110,929 and 36,622 µg/L.

Studies that were reviewed, but not used for long-term guideline derivation (with accompanying rationale) are listed in the appendix. The following describes a selection of the studies that were not used; two aquatic macrophytes (*Lemna gibba* and *Myriophyllum sibiricum)* were exposed to an eight (commonly used) pharmaceutical mixture (atorvastatin, acetaminophen, caffeine, sulfamethoxazole, carbamazepine, levofloxacin, sertraline, and trimethoprim) using a mecocosm exposure for a period of 35 days (Brain *et al.* 2004). Although no significant hazard was identified in this assessment at an effect size of >20 per cent, it was still noted that in a real world scenario, aquatic life is potentially exposed to thousands of pharmaceutical active ingredients, hundreds of pesticide active ingredients, as well as numerous other chemicals. Therefore potential for significant mixture effects does exist in real aquatic exposures.

Overall, the data indicated that toxicity to plants was not found to occur at environmentally-relevant concentrations[4].

7.5 Toxicity to Amphibians

Only one study was located that investigated the toxicity of CBZ to an amphibian species. Richards and Cole (2006) used the frog embryo teratogenesis assay-Xenopus (FETAX) using Stage 9 *Xenopus* blastulae. The highest nominal test concentration of 100,000 µg/L did not produce any signs of malformations or mortality.

7.6 Field Studies

Many field studies have assessed the potential environmental impact of CBZ to aquatic receptors through the computation of hazard quotients. A hazard quotient is computed by dividing the predicted or measured environmental concentration (PEC or MEC) of a substance (e.g., CBZ) by

[4] Highest measured CBZ concentration in Canadian surface waters is 0.988 ug/L (Kormos 2007). Highest measured CBZ concentration in Canadian STP effluent is 2.3 ug/L (Metcalfe *et al.* 2003b).

the predicted no-effect concentration (PNEC – depending on how they are derived, PNECs may be, or may not be, equivalent to the Canadian Water Quality Guideline). If the PEC is used, it is often based on the maximum measured concentration in effluent divided by a dilution factor (usually 10) to predict a worst-case scenario environmental concentration. The PNEC is based on either laboratory derived toxicity tests, or modeling. Hazard quotient values greater than or equal to one are flagged as having potential to be high risk to aquatic receptors, and result in more detailed evaluation.

Most studies predicted hazard quotients (based on a worst case set of assumptions) of less than one for carbamzepine, indicating low risk to aquatic receptors (Al Aukidy *et al.* 2012; Damásio *et al.* 2011; De Lange *et al.* 2006; Gros *et al.* 2010; Han *et al.* 2006; Kim *et al.* 2007). Some studies did calculate a hazard quotient for CBZ that was greater than one. This includes Dussault *et al.* (2008) who argued that there may be risk to benthic invertebrates. Ferrari *et al.* (2003) also stated that CBZ may cause adverse effects if effluents are discharged with no dilution occurring. The caveat for all these studies is that the hazard quotient evaluation is only related to the individual substance, and not to the mixture of compounds that aquatic organisms are actually exposed to in a real-world scenario (which also applies to all CWQGs that are developed on a chemical by chemical basis). It must be noted that mixtures of pharmaceuticals do show toxicity, which was not observed when these substances have been tested as a single compound (Cleuvers 2003). Note that toxicity studies examining mixtures were determined not fit for guideline derivation, as noted in the sections above.

A field study by Damásio *et al.* (2011) was conducted in the Llobregat river basin in north-east Spain, a Mediterranean river under the influence of sewage treatment plant effluent discharges, where pharmaceutical levels are among the highest reported worldwide. In the case of CBZ, reported concentrations ranged from less than limit of detection (0.3 ng/L) to 34.3 ng/L. Whole organism and biochemical responses to pharmaceuticals and other pollutants present in the river were assessed in two field-collected benthic macroinvertebrates (the caddisfly larvae of the species *Hydropsyche exocellata* and the amphipod *Echinogammarus longisetosus*) and in lab-reared *Daphnia magna*. All three species were deployed in cages along a pollution gradient (upstream and downstream of sewage treatment plant influence). A combination of hazard quotients/indices, as well as individual and biochemical responses allowed for the conclusion that pesticides, salinity, and ammonia had a greater effect on the studied species than the pharmaceuticals. The caveat was that the selected biochemical markers were not specific to assessing impacts from pharmaceuticals, as these were not available at the time for species such as aquatic insects and cladoceran-amphipod crustaceans. The authors noted that these results contradict previously reported information for the same study area (Ginebreda *et al.* 2010; Muñoz *et al.* 2009), where pharmaceuticals were noted to pose a higher hazard. Muñoz *et al.* (2009) did not consider the contribution from pesticides and metals, and Ginebreda *et al.* (2010) derived PNECs using much higher uncertainty factors.

7.7 Marine Aquatic Toxicity

At present only two long-term studies were located that investigated the effects of CBZ on marine organisms and conducted in salt water conditions (see appendix). No short-term marine exposures were found in the literature.

A long-term exposure (seven days) to environmentally relevant concentrations of carbamazepine (0, 0.1, and 10 µg/L) using the field-collected Mediterranean mussel *Mytilus galloprovincialis* was conducted to investigate whether CBZ interacts in mussels with the same cellular targets through which its therapeutic effects are thought to occur in human patients (Martin-Diaz *et al.* 2009). The action of CBZ involves voltage-dependent inhibition of Na^+ and Ca^{2+} channel currents, where CBZ interacts with the adenylyl cyclase system and lowers the intracellular levels of cAMP in the mammalian brain (Chen *et al.* 1996; Montezinho *et al.* 2007). Cyclic AMP is well known for regulating several functions in molluscs (Valbonesi *et al.* 2004). The effects of CBZ on the cAMP pathway indicate that the specific molecular target of the drug found in mammals is conserved also in mussels. Post-CBZ exposure analysis included: (a) six consolidated biomarkers related primarily to oxidative stress; (b) cAMP levels and protein kinase A (PKA) activities; and (c) mRNA expression of MXR-related genes (MXR proteins are involved both in the cAMP pathway and in the protective response of organisms towards xenobiotics). The biomarkers analysed indicate that a seven-day exposure to CBZ at environmentally relevant concentrations reduced the health status of mussels and induced oxidative stress. No mortality occurred during the exposures.

A second long-term (96-hour) exposure with the marine phytoplankton *Dunaliella tertiolecta* was conducted by DeLorenzo and Fleming (2008). A 96-hour EC50- could not be calculated for the highest CBZ concentration tested (80,000 µg/L), although cell density was reduced by 42 per cent at this concentration.

7.8 Mode of Toxic Action

Overall, there does not seem to be consensus regarding the mode of action of CBZ to aquatic receptors.

CBZ likely causes effects by a non-specific mode of action which is assumed to be non-polar narcosis (also known as baseline toxicity), associated with hydrophobicity (log K_{ow}) (De Lange *et al.* 2006). Binary mixture toxicity tests were conducted using both *Daphnia* and algae by Cleuvers (2003), where no specific mode of action could be identified using a mixture of CBZ and clofibrinic acid (see Section 8.11). For both *Daphnia* and algae, baseline toxicity (e.g., substance toxicity as a function of hydrophobicity and no specific mode of action) predicted using QSARs (log K_{ow} values) for non-polar narcosis was higher when compared to measured toxicity (predicted EC50s were lower than measured EC50s). However, the algal test showed independent action, which is not expected for substances acting by non-polar narcosis (Cleuvers 2003).

Narcosis was suggested as the main mechanism of action (Cleuvers 2003). Exposures with *Hyalella azteca* and *Chironomus dilutus* also support the likelihood that toxicity is due to non-polar narcosis (Dussault *et al.* 2008). Measured EC50 values for CBZ (Ferrari *et al.* 2003) indicate that these are in the same range as QSAR estimated baseline toxicity EC50 values using log K_{ow}. The work conducted by Gagné *et al.* (2006) - assessing immunotoxicity of CBZ to *Elliptio complananta* - provided some indication that due to the lipophilic nature of this pharmaceutical, it acts on membranes (narcosis-induced membrane depolarization) which led to the observed loss of cell adherence (total proteins in adhered cells).

Other modes of toxic action in addition to non-polar narcosis/baseline toxicity have been proposed. In CBZ-spiked sediment toxicity tests conducted with *Chironomus riparius* (28-day life cycle exposure), a consistent negative impact on emergence was observed at sediment concentrations of >70 ug/kg (Oetken *et al.* 2005). Larvae survived up to 28 days, but without undergoing pupation. This suppression of pupation led Oetken *et al.* (2005) to believe that there is an underlying specific mode of action, either interference with a physiological pathway first active at the larval life stage, or some impact to endocrine functions.

The six-week exposure with Zebrafish *(Danio rerio)* to two environmentally relevant CBZ concentrations (low of 0.5 µg/L and high of 10 µg/L) resulted in significantly decreased plasma concentrations of the teleost androgen 11-ketotestosterone at the low CBZ concentration, but no difference from control at the high concentration, for both males and females (Galus *et al.* 2013a). The author did not provide an explanation for this observation. However, it was stated that caution when interpreting hormone data is advised, since many fish were pooled, resulting in a decrease in the numbers of samples available for these analyses. That being stated, the assumption made is that the mode of action of CBZ in fish may be similar to the mode of action observed in mammals. CBZ affects the central nerve system by reducing overall neuronal activity. This is achieved by either reducing the excitability of neurons by blocking voltage gated sodium channels (Galus *et al.* 2013a), or by enhancing the inhibitory effects of GABA (gamma-aminobutyric acid) by binding to gamma receptors (Kim *et al.* 2009). GABA receptor homoloy has been found to exist between humans, fish (Zebrafish *Danio rerio*, Japanese medaka *Oryzias latipes*, Fathead minnow *Pimephales promelas*) and amphibians (Western clawed frog *Silurana tropicalis*) (Christen *et al.* 2010). A reduction in neuron excitability may result in reduced gonadal steroid synthesis by way of reducing neuronal stimulation to reproductive organs (Galus *et al.* 2013a).

7.9 Toxicity Modifying Factors

Limited information was located with respect to the influence of toxicity modifying factors such as pH and temperature on the toxicity of CBZ to aquatic receptors. One study by Meredith-Williams *et al.* (2012) discussed that many pharmaceuticals are ionizable, and therefore log Kow values may not be a useful descriptor for uptake potential. Various studies on fish, daphnids, and plants with a variety of pharmaceuticals have shown that uptake and resulting toxicity of ionizable pharmaceuticals can be easily affected by pH changes in the environment. However, the pKa of CBZ is very high which indicates that CBZ is not expected to ionize at environmentally relevant pH ranges (see Table 2.1).

7.10 Toxicity of Transformation Products

With respect to the toxicity of CBZ transformation products, many uncertainties still exist (Han *et al.* 2006). One study has been located that investigated the toxicity of the transformation product 10,11-dihydro-10,11-*trans*-dihydroxycarbamazepine to two *Daphnia* species (Bernhard 2010 In: SMDS 2011). The 28-day LOEC (reproduction) value for both *Daphnia magna* and *Daphnia cucullata* was ≤0.5 µg/L (only one concentration tested), where observations were a weak stimulatory effect on reproductive performance and size changes in offspring. This study

provides evidence that the transformation product may have a similar toxicity to CBZ, at least for *Daphnia*.

Recent work by Ortiz de Garcia *et al.* (2013) employed the use of quantitative structure activity relationships (QSARS) to estimate the risk of several CBZ transformation products to aquatic receptors. Based on this assessment, EP-CBZ was found to have a similar toxicity to the parent compound, while higher toxicity was expected for DiOH-CBZ, 2-OH-CBZ and 3-OH-CBZ (interestingly, DiOH-CBZ has been noted as being biologically inactive [Leclercq *et al.* 2009; Miao and Metcalfe 2003]). Preliminary toxicity test results suggest a higher acute toxicity of 2-OH-CBZ and 3-OH-CBZ for *Vibrio fischeri* compared to the toxicity of CBZ (Kaiser *et al.* 2013).

There is potential for CBZ transformation products, which make up the majority of the WWTP effluent (Bahlmann *et al.* 2014), to transform back to the parent compound, or they may be just as or more toxic as the parent compound. A study of the degradation kinetics and metabolites of CBZ in spiked soil was conducted by Li *et al.* (2013). The major transformation products were found to be 10,11-dihydro-10-hydroxycarbamazepine, carbamazepine-10,11-epoxide, acridone-N-carbaldehyde, 4-aldehyde-9-acridone, and acridine. Mineralization of CBZ did not exceed 2 per cent of the spiked rate in different soils. This type of transformation could occurr in surface water as well.

7.11 Mixture Effects

Considering that measured environmental CBZ concentrations in Canadian surface waters have been found to range from non-detect to 0.988 µg/L (Section 6.3), acute toxicity in the aquatic environment is very unlikely. However, combination effects of low levels of CBZ and other PPCPs in the aquatic environment can occur, with outcomes different than those predicted from single compounds (Cleuvers 2003).

The conservative default assumption when assessing mixtures is to assume a worst-case concentration addition mixture model (Escher *et al.* 2005; Price *et al.* 2012). Escher *et al.* (2005) tested this hypothesis by evaluating the toxicity of five pharmaceuticals (including CBZ) alone and in a mixture using a bioluminescence inhibition test. A chlorophyll fluorescence test was also used, but the mixture did not contain CBZ. Pharmaceuticals were mixed in the ratio of their individual EC50 values when conducted in these same tests. Overall, both tests provided results within a factor of three as predicted by concentration addition.

CBZ was tested along with clofibrinic acid using both *Daphnia* and algal tests (Cleuvers 2003). Both of these compounds have different modes of action in humans, where CBZ is an anti-epileptic drug and clofibrinic acid is a lipid lowering agent. The two pharmaceuticals were tested with *Daphnia* at half of respective 48-hour EC90/2 concentrations. In the case of CBZ, this was the concentration that resulted in 16 per cent immobilization in daphnids. For clofibrinic acid, this was the concentration that resulted in 1 per cent immobilization in daphnids. When tested as a mixture in a 48-hour exposure, the resulting effect was 95 per cent immobilization of daphnids. This observation can be explained by concentration addition (e.g., substances present at NOEC concentrations can contribute to the total effect of the mixture) and is based on the idea of similar mode of action (the toxicity of a single substance could increase strongly in combination with

other similar acting substances). This shows that the use of individual NOECs may not be applicable for the assessment of mixture toxicities. In the case of the algal test with *D. subspicatus*, the mixture effect was explained by independent action mixture toxicity with combination effects much lower than concentration addition (e.g., no mixture toxicity when single substances are present at respective NOEC concentrations).

A multi-generational exposure to one concentration of CBZ compared with a mixture of four pharmaceuticals (CBZ, diclofenac, 17α-ethinylestradiol, metoprolol) was conducted using *Daphnia magna* (experiment was conducted over six continuous generations) (Dietrich *et al.* 2010). The selected exposure concentration for CBZ alone and in the mixture was 0.50 µg/L, representing the maximum concentration measured in southern Germany rivers and streams. Nominal concentrations for diclofenac and metoprolol were 0.36 and 1.20 µg/L (also representing maximum measured concentrations). For 17α-ethinylestradiol, the median measured concentration of 0.10 ng/L was used. CBZ effects on life-history and morphological parameters were not evident in more than one generation (e.g., F3, F4 and F5 generations showed no change in age at first reproduction), indicating the potential for the onset of resistance. The mixture did not provoke stronger effects when compared to CBZ alone. As with the mixture study conducted by Cleuvers (2003), the drug mixture affected *D. magna* in a way contrary to CBZ alone emphasizing that toxicity of drug mixtures is difficult to predict.

Andreozzi *et al.* (2004) investigated the effects of three oxidative techniques (ozonation, H_2O_2/UV photolysis and TiO_2 photocatalysis) at an STP on the toxicity of a six pharmaceutical mixture to the blue-green algae *Synechococcus leopoliensis* and rotifer *Brachionus calyciflorus*. The selected six pharmaceuticals were those found at highest concentrations in STP effluent, and included (along with test concentrations) CBZ (7,070 µg/L), propranolol (325.5 µg/L), clofibric acid (11,200 µg/L), diclofenac (2,800 µg/L), ofloxacin (560 µg/L), and sulfamethoxazole (2,240 µg/L). Oxidation resulted in complete removal of mixture toxicity for both organisms.

Galus *et al.* (2013b) tested the effects of a mixture of pharmaceuticals (equal concentrations of acetaminophen, CBZ, gemfibrozil, venlafaxine) to the adult Zebrafish *(Danio rerio)*, previously tested individually in Galus *et al.* (2013a). Exposures were conducted at 28°C and conducted in artificial seawater (Instant Ocean) but with final salinity not reported. It was therefore decided that this study is not suitable for direct comparison to mixture effects in Canadian freshwater systems. The results are presented here nevertheless. The decline in embryo production in the four-pharmaceutical mixture (0.5 µg/L concentration of each compound) was 74 per cent, whereas when exposed to 0.5 µg/L CBZ alone, decrease in embryo production was 34 per cent. The incidence of kidney damage was similar in the mixture (96 per cent) and CBZ alone (89 per cent) exposures, however the changes were more severe in the mixture. The change in plasma androgen steroid (11-ketotestosterone) levels observed with CBZ alone was not observed in the mixture. Overall, results led authors to assume that observed mixture effects may be additive.

It has been suggested that the immune system is a major target for pharmaceuticals in mollusks. A study by Gust *et al.* (2013) assessed the effect of four environmentally relevant pharmaceutical mixtures (psychiatric drugs which included CBZ, antibiotic drugs, hypolipemic drugs and antihypertensive drugs), as well as the effect of the global mixture of all the pharmaceuticals (which included CBZ), on immunological endpoints in the freshwater gastropod *Lymnaea stagnalis* in a three-day exposure. Environmentally relevant concentrations were used. Results were then compared to a previous exposure using municipal wastewater effluent that had been

characterized for pharmaceutical constituents. Observed immunotoxic responses were comparable for the municipal effluent, the global pharmaceutical mixture and the antibiotic mixture (ciprofloxacin 100 ng/L, erythromycin 50 ng/L, novobiocin 100 ng/L, oxytetracycline 200 ng/L, sulfamethoxazole 50 ng/L, trimethoprim 50 ng/L).

Exposure of Zebrafish (*Danio rerio*) to a mixture of pharmaceuticals commonly detected in the Douro River estuary in Portugal on the maturation dynamics of fish ovaries and testes was investigated by Madureira *et al.* (2011). The hypothesis is that reproductive toxicology effects may be caused by pharmaceuticals which are not primarily identified as endocrine-disrupting compounds. Mixture A was made up of pharmaceuticals at maximum measured environmental concentrations for each compound in the Douro River (CBZ concentration was 178 ng/L). Mixture B was made up of pharmaceuticals at concentrations 10,000 times the maximum concentration found for each compound in the Douro River (CBZ concentration in the mixture test was 1,780 µg/L). Adult Zebrafish were used in the 21-day exposures, and no dose-response could be determined since only two mixture concentrations were used (high – Mixture B, and low – Mixture A). Overall, females showed effects in oocytes in the mixture exposures (significant switch between the volume densities of late/mature oocytes versus primary oocytes), whereas males showed significant effects in the single pharmaceutical exposures (see Section 8.4.2, decreased spermatocyte percentage compared to controls). This indicates that it is important to study effects in both sexes, and assessing both single compound and mixture exposures since the data obtained in one manner (single pharmaceutical exposure) may not correctly predict the second scenario (pharmaceutical mixture exposure).

An additional point of consideration when considering mixtures is the potential for non-monotonic dose responses. Chemicals identified as xenoestrogens can produce non-monotonic dose-responses at low concentrations when present as a mixture (Vandenberg *et al.* 2012). This makes assessment of mixture effects all the more challenging.

Although there are notable adverse effects from pharmaceutical mixtures involving CBZ, the interpretation of these studies make it difficult to allow them to be used in the derivation of the guideline.

8.0 GUIDELINES FROM OTHER JURISDICTIONS

The following provides a summary of currently available guidelines developed by various jurisdictions, as well as benchmarks found in the literature (Table 8.1). Each jurisdiction applies an independent methodology for guideline derivation (e.g., quality and quantity of toxicity data, use of a no- or low-effect toxicity data for criteria derivation, value of safety factors, etc.). Therefore, in some cases, two jurisdications may use the same critical effect study and endpoint, and derive final guideline values that are not equivalent.

Table 8.1. Water quality criteria for CBZ available in other jurisdictions.

Jurisdiction	Criteria[1,2,3]	Value (µg/L)	Rationale	Reference
European Union	Draft AA-EQS	0.5	7 day NOEC (reproduction) of 25 µg/L (nominal) for the water flea *C. dubia* (Ferrari *et al.* 2003) to which an assessment factor of 50 was applied	EC 2010 (cited in RIVM 2014)
Netherlands	MAC-EQS	1,600	EC50 (morphology, growth, feeding) of 15,520 µg/L to which an assessment factor of 10 was applied	RIVM 2014
Netherlands	AA-EQS	0.5	7 day NOEC (reproduction) of 25 µg/L (nominal) for the water flea *C. dubia* (Ferrari *et al.* 2003) to which an assessment factor of 50 was applied	RIVM 2014
Norway	PNEC	4.92	PNEC	Grung *et al.* 2007 (cited in RIVM 2014)
Sweden	PNEC	17	PNEC industry Material Safety Data Sheet	FASS 2013 (cited in RIVM 2014)
Sweden	Target Value	0.1	Pharmaceutical concentration not to be exceeded in surface water used for drinking water abstraction	IAWR/IAWD/RIWA 2008 (cited in RIVM 2014)
Switzerland	MAC-EQS	2,550	7 day EC50 (growth inhibition) of 25,500 µg/L for the duckweed *Lemna minor* (Cleuvers 2003), to which an assessment factor of 10 was applied	Kase *et al.* 2011; Swiss Ecotox Centre, 2013
Switzerland	AA-EQS	0.5	7 day NOEC (reproduction) of 25 µg/L (nominal) for the water flea *C. dubia* (Ferrari *et al.* 2003, 2004) to which an assessment factor of 50 was applied	Kase *et al.* 2011

[1] Maximum Acceptable Concentration Environmental Quality Standard (MAC-EQS) for aquatic ecosystems – the concentration protecting aquatic ecosystems from effects due to short-term exposure or concentration peaks (based on direct eco-toxicity only) (RIVM 2014).
[2] Annual Average Environmental Quality Standard (AA-EQS) – the concentration (expressed as an annual average) protecting aquatic ecosystems against adverse effects resulting from long-term exposures (normally based on chronic toxicity data) (RIVM 2014).
[3]PNEC = Predicted No Effect Concentration

Switzerland attempted to derive water quality criteria for the transformation product 10,11-dihydro-10,11-*trans*-dihydroxycarbamazepine, however only one study was located, a thesis examining the effects of the transformation product to *Daphnia* in a semi-static 28-day reproduction test (Bernhard 2010 in: SMDS 2011). Only one concentration was tested (0.5 µg/L) using two daphnid species (*Daphnia magna* and *Daphnia cucullata).* Observations at the one test concentration were a weak stimulatory effect on the reproductive performance (weakly significant increase in the number of offspring) and size changes (smaller) in the offspring (LOEC ≤0.5 µg/L).

An ecological risk assessment using exposure data from Germany and France included the evaluation of available acute and chronic toxicity data for CBZ and five other pharmaceuticals (Ferrari *et al.* 2004). Species sensitivity distributions were plotted for both acute and chronic data with all six pharmaceuticals in the same distribution (it is to be noted that combining data from six pharmaceuticals in one SSD to derive an HC5 value is not consistent with the approach used by CCME 2007). The resulting global acute HC5 (fifth percentile) value was determined to be 93.3 µg/L, and the global chronic HC5 value was 0.35 µg/L. These values appear to have been driven more by propranolol or ofloxacin than CBZ. A chronic predicted no-effect concentration (PNEC) was also derived for CBZ alone by dividing the lowest chronic effect concentration

(seven-day *C. dubia* nominal NOEC of 25 µg/L) by the largest recommended safety factor[5] of 50 to give a chronic PNEC of 0.5 µg/L.

Work conducted for the Ontario Ministry of the Environment, with respect to hazard screening of selected contaminants in the Great Lakes Basin, generated PNEC values based on both LOEC and NOEC CBZ benchmarks for water (Intrinsik 2012). The PNEC (LOEC) was derived by dividing the lowest chronic LOEC value of 100 µg/L (seven-day LOEC for reproduction) for *C. dubia* reported by Ferrari *et al.* (2003, 2004), by a safety factor of 100 to generate a final benchmark PNEC of 1 µg/L. The PNEC (NOEC) was derived by dividing the lowest chronic nominal NOEC value of 25 µg/L (seven-day NOEC for reproduction) for *C. dubia* reported by Ferrari *et al.* (2003, 2004), also by a safety factor of 100 to generate a final benchmark PNEC of 0.25 µg/L (Intrinsik 2012).

Overall, there is a noticeable variation in guideline/benchmark values and the safety factors used in their derivation. Each jurisdiction has rationale for the size of safety factor. Factors that are considered (but not limited to) when establishing the size of the safety factor include differences in (intra- and inter-) species sensitivity to a substance, exposure conditions (laboratory versus field, varying environmental conditions), test endpoints, paucity of toxicological data, cumulative exposures, and policy requirements (e.g., extrapolating from a low-effect toxicological threshold to a protective environmental management benchmark) (CCME 2007).

9.0 GUIDELINE DERIVATION

A CWQG for CBZ addresses its use in Canada and potential impacts to freshwater and marine aquatic systems. A CWQG provides guidance to risk assessors, risk managers and water managers in Canada on the level of CBZ in an aquatic system, below which protection of the most sensitive species and lifestage is expected to be maintained during indefinite exposures.

There are currently three options for developing a CWQG (CCME 2007). These consist of:

1. Statistical approach (Type A or SSD approach)
2. Lowest endpoint approach using only primary data (Type B1)
3. Lowest endpoint approach using primary and/or secondary data (Type B2).

The minimum data requirements for each of the three methods are presented in detail in CCME 2007. A species sensitivity distribution (SSD) is a statistical distribution that represents the variation in toxicological sensitivity to a contaminant among a given set of species. The SSD, which is a cumulative distribution function, is composed of effect concentrations obtained during toxicity testing (e.g., LC50, EC50, LOEL, or NOEL) on the horizontal axis and cumulative probability on the vertical axis (Posthuma *et al.* 2002). The number of data points used to construct the curve depends on the number of species tested for the endpoint of interest. Emphasis is placed on organism-level effects (e.g., survival, growth, reproduction) that can be more confidently used to predict ecologically-significant consequences at the population level

[5] Office for Official Publications of the European Communities.1996. Technical guidance document in support of council directive 93/67/EEC on risk assessment for new notified substances and commission regulation (EC) 1488/94 on risk assessment for existing substances. Luxembourg, Luxembourg.

(Forbes and Calow 1999; Meador 2000; Suter II *et al.* 2005). With the SSD method, the concentration of a substance in water that will be protective of at least 95 per cent of aquatic biota is estimated. For the purposes of a Canadian Water Quality Guideline, a short-term SSD is developed using acceptable short-term LC50 data. The guiding principle of a long-term SSD is to protect all of the species at all times, and is therefore based preferentially on long-term no-effect data. Data from both primary and secondary classified studies may be used to fill minimum data set requirements and for plotting on the SSD, for both short-term benchmark concentrations and long-term water water quality guidelines.

If insufficient data are available for deriving a CWQG using the statistical approach, the CWQG will be developed using the next tier method, the lowest endpoint approach. Depending on the quantity and quality of data a Type B1 or Type B2 approach is used. The Type B1 approach uses acceptable primary (obtained from high quality studies meeting criteria set out in CCME 2007) toxicity data only to fill minimum data requirements and to derive either the short-term benchmark concentration or the long-term water quality guideline. The Type B2 approach is somewhat different in that for a short-term benchmark concentration, minimum data requirements must be filled using primary data, but the value used to derive the benchmark may come from a secondary study. For a Type B2 long-term guideline, both primary and secondary data are used to fill mimum data requirements and used as the criterion for guideline derivation. A safety factor is applied in the derivation of a guideline using both Type B1 and B2 approaches. In every case, a CWQG must be developed using the most advanced method that the data allow.

The following sections describe the derivation of CWQGs for the protection of freshwater and marine life in surface water (not sediment) for the pharmaceutical CBZ. The derived CWQGs are pan-Canadian in scope and do not take into account watershed-specific conditions. The minimum data set requirements for the generation of short-term freshwater and marine CWQGs as well as long-term freshwater and marine CWQGs are found in the CCME 2007 protocol.

9.1 Protection of Freshwater Aquatic Life

The complete set of toxicity data considered for use in CWQG derivation (including data classified as primary, secondary), as well as studies which were reviewed and found to be unacceptable, are presented in the appendix.

A CWQG provides guidance separately for both short and long-term exposure. The short-term benchmark is not intended to protect all species indefinitely, but rather is to protect most species against lethality during severe, but transient events. Examples include an accidental spill or inappropriate disposal of the pharmaceutical (e.g., wastewater, biosolids) in question. The long-term exposure value of the CWQG is intended to protect against negative effects to all species and life stages during indefinite exposure. Aquatic life may be chronically exposed to a pharmaceutical as a result of continuous presence in the environment, including continuous release in effluent from sewage treatment plants, gradual release from soils/sediments and gradual entry through groundwater/runoff.

9.1.1 Short-term Freshwater Benchmark Concentration

To be considered for inclusion in development of a short-term benchmark concentration, the aquatic toxicity studies must meet minimum data quality requirements as specified in the water quality protocol (CCME 2007). In the case of CBZ there were insufficient primary data available to derive a short-term freshwater benchmark concentration (Table 9.1). For invertebrates, there is a paucity of acceptable studies, even for the derivation of a Type B2 benchmark concentration. The minimum data set requirement of endpoints for two aquatic or semi-aquatic species (which must come from primary studies) was not fulfilled (Section 8.3.1; appendix). A similar paucity of acceptable studies also holds true for fish. The minimum data set requirements of endpoints for two species of fish (at least one salmonid and one non-salmonid, both of which must come from primary studies) was not fulfilled (Section 8.4.1; appendix). Most published short-term toxicity studies with CBZ utilized solvent to keep CBZ dissolved in solution, resulting in effect concentrations that were above the compound's natural solubility in water. Based on this, CBZ is unlikely to have acute toxic effects on aquatic organisms. In addition, considering that short-term effect concentrations are well above any Canadian surface water concentrations measured to date (Section 6.3), short-term effects are highly unlikely to occur considering the sources of CBZ to the Canadian environment (Section 4.0).

Table 9.1. Minimum data set requirements for the derivation of a CBZ short-term exposure guideline for freshwater environments must be filled with primary data.

Short-term Type B2 Minimum Data Requirements were not met			
Fish	**Amphibians**	**Invertebrates**	**Plants/Algae**
2 species, incl. one salmonid	Desirable, but not necessary	2 species, including one planktonic crustacean	Desirable, but not necessary
1 (1º) 3 (2º)	0	3 (2º)	1 (2º)

9.1.2 Long-term Freshwater Canadian Water Quality Guideline

A long-term freshwater CWQG for CBZ for the protection of aquatic life was developed. The data requirements were not satisfied to derive a long-term freshwater CWQG using the SSD approach. Most types of long-term toxicity studies conducted with CBZ measured different endpoints (e.g., molecular markers, *in vitro* assays) than normally used in the development of CWQGs. Therefore, following the tiered approach, a long-term freshwater Type B2 CWQG was developed (Table 9.2). In the case of long-term Type B2 guidelines, secondary data are acceptable to meet the minimum data requirements, and the critical effect value used to set the guideline value can be from a study classified as secondary.

Table 9.2. Minimum data set requirements for the derivation of a CBZ long-term exposure guideline for freshwater environments may be filled using both primary and secondary data.

Long-term Type B2 Minimum Data Requirements were met			
Fish	**Amphibians**	**Invertebrates**	**Plants/Algae**
2 species, including one salmonid	Desirable, but not necessary	2 species, including one planktonic crustacean	Desirable, but not necessary
3 (2º)	0	3 (1º) 3 (2º)	7 (2º)

The most sensitive invertebrate was the water flea *C. dubia* with a seven-day NOEC and LOEC for reproduction inhibition of 25 µg/L and 100 µg/L, respectively (Ferrari *et al.* 2003; Ferrari *et*

al. 2004). The most sensitive fish study was the 42-day exposure with Rainbow trout *O. mykiss* by Li *et al.* (2009), with a 42-day NOEC and LOEC of 200 and 2,000 µg/L, respectively, for significant decrease in condition factor (growth effect) compared to controls. The most sensitive of the plant/algae receptors was the green algae *Chlorella pyrenoidosa* with a 96-hour NOEC (growth rate) of 500 µg/L (Zhang *et al.* 2012). When aquatic plants and algae are found to display a higher sensitivity to a substance when compared to invertebrates and fish, then the substance in question is considered to be phytotoxic. In this case, the order of sensitivity to long-term exposure of CBZ is *C. dubia* > *C. pyrenoidosa* > *O. mykiss*.

The lowest preferred or acceptable endpoint from a long-term exposure will be the critical study used in the derivation of the Type B2 long-term exposure guideline, to which a safety factor of 10 will be applied (CCME 2007). The seven-day exposure with *C. dubia* by Ferrari *et al.* (2003, 2004) was identified as the critical study, with a seven-day NOEC and LOEC of 25 and 100 µg/L, respectively, for significant decrease in reproduction compared to controls. As per CCME (2007) protocol, Type B2 long-term guidelines are to be derived using low effect data (not no effect data). Applying a safety factor of 10 to the LOEC (100 µg/L) results in a Canadian Water Quality Guideline of 10 µg/L. This Type B2 guideline may be further upgraded to a Type B1 or Type A guideline, when additional toxicity data become available.

9.2 Protection of Marine Aquatic Life

There were insufficient data to derive short- or long-term guidelines for the protection of marine life.

9.3 Guideline Summary

Table 9.3.Type B2 Canadian water quality guideline for CBZ (µg/L) for the protection of aquatic life, developed using the 2007 CCME derivation protocol.

	Long-Term Exposure	Short-Term Exposure
Freshwater	10[a]	NRG
Marine	NRG	NRG

NRG = no recommended guideline

[a] = Type B2 guideline – The minimum toxicological data to derive a Type A or Type B1 guideline were not available. The lowest acceptable endpoint, e.g., the most sensitive preferred low-effects endpoint, from a long-term exposure study is the critical study used in the derivation of the Type B2 long-term exposure guideline. The endpoint concentration from this critical study is divided by a safety factor of 10 to derive the long-term exposure guideline value. When more toxicity data become available, the Type B2 guideline can be upgraded to a Type B1 or Type A SSD-based value (CCME 2007)

9.4 Data Gaps and Research Recommendations

Additional high quality (that would qualify as primary or secondary) short-term studies with CBZ would be beneficial in order to generate a short-term benchmark concentration for Canadian surface waters. However, based on the use of solvents required to maintain the high CBZ concentrations used in short-term exposures in solution, acute toxicity is likely not of high concern. Of more importance would be to generate additional chronic studies in order to be able to generate a long-term Canadian water quality guideline derived using the SSD approach (CCME 2007). There are numerous published studies with chronic CBZ exposures, however, the

types of research (e.g., molecular markers, *in vitro* assays) conducted with CBZ resulted in different endpoints than normally used in the development of CWQGs. Standard chronic toxicity tests may not utilize endpoints adequate for assessing specific effects associated with low-level exposure to pharmaceuticals such as carbamazepine. Standard tests assess impacts on mortality, growth and reproduction. Pharmaceuticals can elicit low dose effects due to being designed for biological activity. Perhaps more sensitive and specific endpoints would be more useful, such as developmental abnormalities, sex ratios or metabolic perturbations (e.g., biomarkers), where testing may cover multiple generations, but the ecological relevance of these would need to be established and validated (Ferrari *et al.* 2003). There is growing evidence that environmentally relevant concentrations of pharmaceuticals can elicit changes in behavior. The European perch (*Perca fluviatilis*) was exposed to 1.8 µg/L of an anti-anxiety benzodiazepine drug (oxazepam) for seven days (Brodin *et al.* 2013). The concentration of oxazepam measured in river water (River Fyris) was 0.58 µg/L. Behavioural changes included increased activity, reduced sociality and higher feeding rate, all of which can lead to ecosystem-level consequences. Another example of behavioural impact following exposure to a benzodiazepinic drug (diazepam) was presented by Brandao *et al.* (2013). Pumpkin-seed sunfish (*Lepomis gibbosus*) exposed for 96 hours displayed a significant increase in activity at all concentrations tested (lowest was 266 µg/L). No dose-response was observed, rather it was an all or nothing effect. The highest concentration of diazepam reported in surface water is 0.0012 µg/L (Zuccato *et al.* 2000). This change in behavior following exposure to a single low level pharmaceutical is alarming, since aquatic organisms are exposed to a mixture of substances on a daily basis, and pharmaceutical use is only expected to increase (Brodin *et al.* 2013). In Section 8.2.1 Short-term Effects, a study by Quinn *et al.* (2008) did observe a behavioural change in *Hydra attenuata* (decreased feeding behavior), but this was a short-term exposure to an environmentally unrealistic CBZ concentration (3,760 µg/L). The short-term (96-hour) exposure of *L. gibbosus* to CBZ by Brandao *et al.* (2013) did not present significant differences in behavior endpoints between control and exposed organisms (lowest concentration was 62.5 µg/L). However, significant correlations were found between increasing CBZ and decreased anxiety (based on scototaxis) as well as increasing CBZ and increased activity. Further studies with behavioural endpoints using sensitive species may be warranted.

Due to the continuous presence of pharmaceuticals such as CBZ, chronic toxicity tests could underestimate the impacts of pharmaceuticals on aquatic organisms. They are relatively short when compared to the life span of tested organisms (e.g., 7-day *Ceriodaphnia dubia* exposure, 10-day *Danio rerio* early life stage exposure) (Ferrari *et al.* 2003). Standard test methods also do not take into consideration toxicant transfer from parent to progeny (Ferrari *et al.* 2003), although some studies are now being conducted to investigate this data gap (e.g., exposure of three successive generations of daphnids to CBZ) (Lamichhane *et al.* 2013) (it must be noted that in the case of the Lamichane *et al.* 2013 study, it was suggested that there is no difference between the results obtained from a seven-day test versus the follow through to the F3 generation multi-generational test. The authors also saw effects at two orders of magnitude higher than environmentally relevant concentrations).

With respect to freshwater exposures, Zebrafish embryos that were directly exposed to CBZ showed increased mortality at the low exposure concentration, but surprisingly showed a significant decrease in mortality (compared to control) at the high exposure concentration (Galus *et al.* 2013a). Results such as these may be an area to investigate further.

If monitoring in marine environments shows detection of CBZ, consideration should be given to generating additional marine toxicity data for the development of CWQGs for marine aquatic life protection.

Investigations on the assessment of impact that water quality parameters (e.g., toxicity modifying factors) have on CBZ toxicity are lacking.

Ecotoxicity data are available for CBZ, but data are lacking on the toxicity of the transformation product 10,11-dihydro-10,11-*trans*-dihydroxycarbamazepine (CBZ-DiOH), frequently measured in surface waters and often at higher concentrations than CBZ. This metabolite is not pharmaceutically active in humans (Duche *et al.* 1995), and therefore may not be an issue for aquatic receptors. The active metabolite, 10,11-dihydro-10,11-epoxycarbamazepine (CBZ-Ep) (Leclercq *et al.* 2009; Miao and Metcalfe, 2005) has not been detected in samples of surface water near an STP discharge in Canada, but has been detected in effluent (Miao and Metcalfe, 2003). Investigations into the toxicity of CBZ-Ep, and whether the toxicity of CBZ and CBZ-Ep are additive, or if there is a difference in magnitude of toxicity, may be considered. QSAR modelling of CBZ transformation products may be used to evaluate potential toxicity in comparison to CBZ (Ortiz de Garcia *et al.* 2013). However, for the purposes of guideline derivation, only the CBZ parent compound is taken into consideration. This is the same approach used for derivation of guideline values for pesticides (biologically active compounds also known to derive transformation products in the environment).

With respect to toxicity of mixtures, the relationships are not yet clear. Results such as those from Cleuvers (2003) (see Section 8.11 Mixture Effects), which showed that pharmaceutical mixtures prepared using individual NOEC concentrations produced significant effects in daphnids. The use of individual NOECs may not be applicable for the assessment of mixture toxicities and should be investigated further. The difficulty with mixtures is all of the nearly infinite number of permutations and combinations, making it virtually impossible to address using classic approaches.

In addition, the results from Madureira *et al.* (2011) (see Section 8.11 Mixture Effects) found that responses between male and female Zebrafish differed when exposed to a pharmaceutical mixture. Females showed significant effects in mixture exposures (significant switch between the volume densities of late/mature oocytes versus primary oocytes), whereas males displayed significant effects in single substance exposures (decreased spermatocyte percentage compared to controls). This indicates that it is important to study effects in both sexes, and assessing both single compound and mixture exposures since the data obtained in one manner (single pharmaceutical exposure) may not correctly predict the second scenario (pharmaceutical mixture exposure).

Of particular concern is the co-exposure to pharmaceuticals with similar modes of action (e.g., sedation, anxiety reduction) that alone may not be toxic, but when combined, may increase toxic potential (Brandao *et al.* 2013).

Sources of CBZ in the environment, such as landfill leachate, have not been thoroughly investigated. Landfills can be a potential source of CBZ release (Bonvin 2013), however, some limited monitoring in Ontario found no detection (Stafford 2008).

Another anti-convulsant pharmaceutical currently on the market, oxcarbazepine, is structurally similar to carbamazepine, the only difference being a ketone is in place of the carbon–carbon double bond on the dibenzazepine ring at the 10 position. This parent compound is know to derive transformation products common to carbamazepine (Leclercq *et al.* 2009). Approximately 1800 kg/yr of oxcarbazepine is used within Canada, with 96 per cent of distribution thorough retail pharmacies and the remaining 4 per cent is distributed within hospitals. These numbers are for 2011 and 2012 and the numbers are consistent between years (IMS AG 2013b). Consideration may be given to this parent compound as well.

9.5 Implementation and Other Considerations

The CWQG for CBZ is set to provide protection for long-term exposure periods. The guideline is based on generic environmental fate and behaviour and toxicity data. The guideline is a conservative value below which all forms of aquatic life, during all life stages and in all Canadian aquatic systems, should be protected. Because the guideline is not corrected for any toxicity modifying factors, it is a generic value that does not take into account any site-specific factors. If an exceedence of the guideline is observed, it does not necessarily suggest that toxic effects will be observed, but rather indicates the need to determine whether or not there is a potential for adverse environmental effects.

The guideline should be used as a screening and management tool to ensure that CBZ does not lead to the degradation of the aquatic environment. The CWQG for CBZ could, for example, be the basis for the derivation of site-specific guidelines and objectives (derived with site-specific data as well as consideration of technological, site-specific, socioeconomic or management factors) (CCME 2007).

The guideline does not take into consideration concentrations of transformation products detected in surface water. It is possible that transformation products may be contributing to toxicity, or may be converted back to the parent compound (CBZ) in the environment. The science related to toxicity and fate of transformation products is not yet developed and therefore cannot be included in derivation of the guideline to date. A similar consideration is given to the derivation of guideline values for pesticides, also known to generate transformation products in the environment. To date, no CWQG pesticide transformation products have been developed.

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APPENDIX
TOXICITY VALUES FOR FRESHWATER and MARINE AQUATIC SPECIES EXPOSED TO CARBAMAZEPINE

Acute Freshwater Toxicity Data – Carbamazepine

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{-1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{-1}$)	Hardness ($mg \cdot L^{-1}$)	Alkalinity ($mg \cdot L^{-1}$)	pH	Reference	Ranking (rationale)
Invertebrate	*Atyaephyra desmarestii*	water flea	field collected	No solvent added	96 hour LC50	94,300	Static-renewal	21.6				6.81	Nieto *et al.* 2013	U (technical grade product, no solvent control, 4 = Klimisch score from RIVM 2014)
Invertebrate	*Ceriodaphnia dubia*	water flea	<24 hour old	No solvent added	48 hour LC50	7,070	Static-renewal	25		80-100	64	7.4-7.8	Lamichhane *et al.* 2013	2 (loss of CBZ ranged from 51 to 74% after 24 hours of exposure, however the exposures were daily static renewal so exposed to the same level of nominal concentration even if CBZ loss existed, Klimisch score provided by RIVM was 4)
Invertebrate	*Ceriodaphnia dubia*	water flea	<24 hour old	No solvent added	48 hour EC50 (inhibition of mobility)	77,700	Static	25±1		MH synthetic water (80-100 as CaCO3)		7.0-8.5	Ferrari *et al.* 2003; Ferrari *et al.* 2004	2 (>99% purity, Klimisch score from RIVM 2014)
Invertebrate	*Daphnia magna*	water flea	<24 hour old	No solvent added	48 hour EC50 (inhibition of mobility)	>13,800	Static	20±1		90 MH synthetic water (80-100 as CaCO3)		7.0-8.5	Ferrari *et al.* 2003; Ferrari *et al.* 2004	2 (experiment conducted in darkness)
Invertebrate	*Daphnia magna*	water flea	<24 hour old	1% DMSO in stock solution	24 hour EC50 (inhibition of mobility)	112,228 (475 uM)	Static	20					Jos *et al.* 2003	U (exceeds solubility limit)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{-1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{-1}$)	Hardness ($mg \cdot L^{-1}$)	Alkalinity ($mg \cdot L^{-1}$)	pH	Reference	Ranking (rationale)
Invertebrate	*Daphnia magna*	water flea	<24 hour old	1% DMSO in stock solution	48 hour EC50 (inhibition of mobility)	97, 816 (414 uM)	Static	20		standard reference water			Jos *et al.* 2003	2 (Klimisch score from RIVM 2014)
Invertebrate	*Daphnia magna*	water flea	<24 hour old	No solvent added	48 hour EC50 (inhibition of mobility)	>100,000	Static	20±1		ADaM, culture medium imitating natural freshwater			Cleuvers 2003	2 (analytical grade product, Klimisch score from RIVM 2014)
Invertebrate	*Daphnia magna*	water flea	<24 hour old	<1% ethanol in final exposure	48 hour EC50 (inhibition of mobility)	111,000	Static	25		170 ± 5	110± 5	7.8 ± 0.2	Han *et al.* 2006	2 (Klimisch score from RIVM 2014)
Invertebrate	*Daphnia magna*	water flea	<24 hour old	≤0.5% DMSO in final exposure	48 hour EC50 (inhibition of mobility)	>100,000	Static	20 ± 1		MHW, recon as per EPA			Kim *et al.* 2007	2 (Klimisch score from RIVM 2014)
Invertebrate	*Daphnia magna*	water flea	<24 hour old		48 hour EC50 (inhibition of mobility)	67,500							Pfuger *et al.* 2000 in Kase 2010 in RIVM 2014	2 (Klimisch score from RIVM 2014)
Invertebrate	*Daphnia magna*	water flea			48 hour EC50 (inhibition of mobility)	>10,000							Harada *et al.* 2008 in RIVM 2014	2 (Daphtoxkit, Klimisch score from RIVM 2014)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{-1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{-1}$)	Hardness ($mg \cdot L^{-1}$)	Alkalinity ($mg \cdot L^{-1}$)	pH	Reference	Ranking (rationale)
Invertebrate	*Daphnia magna*	water flea	<24 hour old	≤0.5% DMSO in final exposure	96 hour EC50 (inhibition of mobility)	76,300 (64,400 – 88,100)	Static	20 ± 1		MHW, recon as per EPA			Kim *et al.* 2007	2 (Klimisch score from RIVM 2014)
Invertebrate	*Daphnia magna*	water flea	<24 hour old		48 hour NOEC (immobility)	30,000	Static	20		artificial medium		7.8-8	Donner *et al.* 2013 in RIVM 2014	2 (99% purity, Klimisch score from RIVM 2014)
Invertebrate	*Daphnia magna*	water flea	<24 hour old neonates	0.1 ug/L methanol in stock solution	24 hour NOEC (F0 generation) (Progeny counts/numbers)	0.5	Static renewal (weekly)	20±0.5					Dietrich *et al.* 2010	U (only one concentration tested, represented max conc measured in rivers & streams of southern Germany)
Invertebrate	*Daphnia magna*	water flea	<24 hour old neonates	0.1 ug/L methanol in stock solution	24 hour NOEC (F0 generation) (Time to first progeny)	0.5	Static renewal (weekly)	20±0.5					Dietrich *et al.* 2010	U (only one concentration tested, represented max conc measured in rivers & streams of southern Germany)
Invertebrate	*Daphnia magna*	water flea	<24 hour old neonates	0.1 ug/L methanol in stock solution	24 hour NOEC (F0 generation) (growth)	0.5	Static renewal (weekly)	20±0.5					Dietrich *et al.* 2010	U (only one concentration tested, represented max conc measured in rivers & streams of southern Germany)
Invertebrate	*Daphnia magna*	water flea	<24 hour old neonates	0.1 ug/L methanol in stock solution	24 hour NOEC (F0 generation) (mortality)	0.5	Static renewal (weekly)	20±0.5					Dietrich *et al.* 2010	U (only one concentration tested, represented max conc measured in rivers & streams of southern Germany)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{-1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{-1}$)	Hardness ($mg \cdot L^{-1}$)	Alkalinity ($mg \cdot L^{-1}$)	pH	Reference	Ranking (rationale)
Invertebrate	*Daphnia magna*	water flea	<24 hour old neonates	0.1 ug/L methanol in stock solution	48 hour NOEC (F1 generation) (Progeny counts/numbers)	0.5	Static renewal (weekly)	20±0.5					Dietrich *et al.* 2010	U (only one concentration tested, represented max conc measured in rivers & streams of southern Germany)
Invertebrate	*Daphnia magna*	water flea	<24 hour old neonates	0.1 ug/L methanol in stock solution	48 hour NOEC (F1 generation) (Time to first progeny)	0.5	Static renewal (weekly)	20±0.5					Dietrich *et al.* 2010	U (only one concentration tested, represented max conc measured in rivers & streams of southern Germany)
Invertebrate	*Daphnia magna*	water flea	<24 hour old neonates	0.1 ug/L methanol in stock solution	48 hour NOEC (F1 generation) (growth)	0.5	Static renewal (weekly)	20±0.5					Dietrich *et al.* 2010	U (only one concentration tested, represented max conc measured in rivers & streams of southern Germany)
Invertebrate	*Daphnia magna*	water flea	<24 hour old neonates	0.1 ug/L methanol in stock solution	48 hour NOEC (F1 generation) (mortality)	0.5	Static renewal (weekly)	20±0.5					Dietrich *et al.* 2010	U (only one concentration tested, represented max conc measured in rivers & streams of southern Germany)
Invertebrate	*Daphnia magna*	water flea	<24 hour old neonates	0.1 ug/L methanol in stock solution	72 hour NOEC (F2 generation) (Progeny counts/numbers)	0.5	Static renewal (weekly)	20±0.5					Dietrich *et al.* 2010	U (only one concentration tested, represented max conc measured in rivers & streams of southern Germany)
Invertebrate	*Daphnia magna*	water flea	<24 hour old neonates	0.1 ug/L methanol in stock solution	72 hour NOEC (F2 generation) (Time to first progeny)	0.5	Static renewal (weekly)	20±0.5					Dietrich *et al.* 2010	U (only one concentration tested, represented max conc measured in rivers & streams of southern Germany)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{-1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{-1}$)	Hardness ($mg \cdot L^{-1}$)	Alkalinity ($mg \cdot L^{-1}$)	pH	Reference	Ranking (rationale)
Invertebrate	*Daphnia magna*	water flea	<24 hour old neonates	0.1 ug/L methanol in stock solution	72 hour NOEC (F2 generation) (growth)	0.5	Static renewal (weekly)	20±0.5					Dietrich *et al.* 2010	U (only one concentration tested, represented max conc measured in rivers & streams of southern Germany)
Invertebrate	*Daphnia magna*	water flea	<24 hour old neonates	0.1 ug/L methanol in stock solution	72 hour NOEC (F2 generation) (mortality)	0.5	Static renewal (weekly)	20±0.5					Dietrich *et al.* 2010	U (only one concentration tested, represented max conc measured in rivers & streams of southern Germany)
Invertebrate	*Daphnia magna*	water flea	<24 hour old neonates	0.1 ug/L methanol in stock solution	96 hour NOEC (F3 generation) (Progeny counts/numbers)	0.5	Static renewal (weekly)	20±0.5					Dietrich *et al.* 2010	U (only one concentration tested, represented max conc measured in rivers & streams of southern Germany)
Invertebrate	*Daphnia magna*	water flea	<24 hour old neonates	0.1 ug/L methanol in stock solution	96 hour NOEC (F3 generation) (Time to first progeny)	0.5	Static renewal (weekly)	20±0.5					Dietrich *et al.* 2010	U (only one concentration tested, represented max conc measured in rivers & streams of southern Germany)
Invertebrate	*Daphnia magna*	water flea	<24 hour old neonates	0.1 ug/L methanol in stock solution	96 hour NOEC (F3 generation) (growth)	0.5	Static renewal (weekly)	20±0.5					Dietrich *et al.* 2010	U (only one concentration tested, represented max conc measured in rivers & streams of southern Germany)
Invertebrate	*Daphnia magna*	water flea	<24 hour old neonates	0.1 ug/L methanol in stock solution	96 hour NOEC (F3 generation) (mortality)	0.5	Static renewal (weekly)	20±0.5					Dietrich *et al.* 2010	U (only one concentration tested, represented max conc measured in rivers & streams of southern Germany)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{1}$)	Hardness ($mg \cdot L^{1}$)	Alkalinity ($mg \cdot L^{1}$)	pH	Reference	Ranking (rationale)
Invertebrate	*Gammarus pulex*	amphipod	Adult (Individuals in similar intermoult stage, between 8&10 mm in length selected for the study)	25 uL/L Ethanol in final exposure	1.5 hour NOEC (decreased activity / locomotion; ventilating, locomotion, feeding)	>1,000	Static			Artificial pond water (Dutch standard water)			De Lange *et al.* 2006	2
Invertebrate	*Gammarus pulex*	amphipod	Adult (Individuals in similar intermoult stage, between 8&10 mm in length selected for the study)	25 uL/L Ethanol in final exposure	1.5 hour LOEC (reduction in amphipod activity; ventilating, locomotion, feeding)	0.01 (not statistially different from control)	Static			Artificial pond water (Dutch standard water)			De Lange *et al.* 2006	2
Invertebrate	*Gammarus pulex*	amphipod			48 hour LC50	>3,780 (16 uM)	Static	20		Filtered water collected from Bishop Wilton Beck			Meredith-Williams *et al.* 2012	U (concentration series was used, but no control survival or water quality parameters were reported)
Invertebrate	*Hydra attenuata*	hydra		0.31% Acetone in final exposure	96 hour EC50 (decreased feeding behaviour)	3,760 (170 – 85,400)		20 ± 1		147 mg/L $CaCl_2$-$2H_20$		7	Quinn *et al.* 2008	2 (technical grade product, Klimisch score from RIVM 2014)
Invertebrate	*Hydra attenuata*	hydra		0.31% Acetone in final exposure	96 hour LC50	29,400 (26,320 - 32,830)		20 ± 1		147 mg/L $CaCl_2$-$2H_20$		7	Quinn *et al.* 2008	2 (technical grade product, Klimisch score from RIVM 2014)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{1}$)	Hardness ($mg \cdot L^{1}$)	Alkalinity ($mg \cdot L^{1}$)	pH	Reference	Ranking (rationale)
Invertebrate	*Hydra attenuata*	hydra		0.31% Acetone in final exposure	96 hour EC50 (morphological changes)	15,520 (8,300 - 29,020)		20 ± 1		147 mg/L $CaCl_2$-$2H_20$		7	Quinn *et al.* 2008	2 (technical grade product, Klimisch score from RIVM 2014)
Invertebrate	*Hydra attenuata*	hydra		0.31% Acetone in final exposure	96 hour NOEC (morphological changes)	1,000		20 ± 1		147 mg/L $CaCl_2$-$2H_20$		7	Quinn *et al.* 2008	2 (technical grade product, Klimisch score from RIVM 2014)
Invertebrate	*Hydra attenuata*	hydra		0.31% Acetone in final exposure	96 hour LOEC (morphological changes)	5,000		20 ± 1		147 mg/L $CaCl_2$-$2H_20$		7	Quinn *et al.* 2008	2 (technical grade product, Klimisch score from RIVM 2014)
Invertebrate	*Hydra attenuata*	hydra		0.31% Acetone in final exposure	96 hour toxicity threshold (TT) (morphological changes)	2,240		20 ± 1		147 mg/L $CaCl_2$-$2H_20$		7	Quinn *et al.* 2008	2 (technical grade product, Klimisch score from RIVM 2014)
Invertebrate	*Hydra attenuata*	hydra		0.31% Acetone in final exposure	96 hour NOEC (decreased feeding response)	25,000		20 ± 1		147 mg/L $CaCl_2$-$2H_20$		7	Quinn *et al.* 2008	2 (technical grade product, Klimisch score from RIVM 2014)
Invertebrate	*Hydra attenuata*	hydra		0.31% Acetone in final exposure	96 hour NOEC (decreased ability to attach to substrate)	10,000		20 ± 1		147 mg/L $CaCl_2$-$2H_20$		7	Quinn *et al.* 2008	2 (technical grade product, Klimisch score from RIVM 2014)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{1}$)	Hardness ($mg \cdot L^{1}$)	Alkalinity ($mg \cdot L^{1}$)	pH	Reference	Ranking (rationale)
Invertebrate	*Hydra attenuata*	hydra		0.31% Acetone in final exposure	96 hour LOEC (decreased ability to attach to substrate)	25,000		20 ± 1		147 mg/L $CaCl_2$-$2H_20$		7	Quinn *et al.* 2008	2 (technical grade product, Klimisch score from RIVM 2014)
Invertebrate	*Hydra attenuata*	hydra		1.25% DMSO in stock solution	48 hour LOEC (increased heme oxidase activity)	>1,418		18-20					Quinn *et al.* 2004	U (molecular markers are not considered for guideline derivaiton unless they are directly linked to survival, growth or reproduction)
Invertebrate	*Hydra attenuata*	hydra		1.25% DMSO in stock solution	48 hour NOEC (increased lipid peroxidation)	14,176							Quinn *et al.* 2004	U (molecular markers are not considered for guideline derivaiton unless they are directly linked to survival, growth or reproduction)
Invertebrate	*Hydra attenuata*	hydra		1.25% DMSO in stock solution	48 hour LOEC (increased lipid peroxidation)	141,762							Quinn *et al.* 2004	U (molecular markers are not considered for guideline derivaiton unless they are directly linked to survival, growth or reproduction; exceeds solubility limit)
Invertebrate	*Hydra attenuata*	hydra		1.25% DMSO in stock solution	48 hour NOEC (CYP1A1 activity)	1,417,620							Quinn *et al.* 2004	U (molecular markers are not considered for guideline derivaiton unless they are directly linked to survival, growth or reproduction; exceeds solubility limit)
Invertebrate	*Hydra attenuata*	hydra		1.25% DMSO in stock solution	48 hour LOEC (increased benzyloxyresorufin de-alkylase activity)	>1,420							Quinn *et al.* 2004	U (molecular markers are not considered for guideline derivaiton unless they are directly linked to survival, growth or reproduction)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{-1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{-1}$)	Hardness ($mg \cdot L^{-1}$)	Alkalinity ($mg \cdot L^{-1}$)	pH	Reference	Ranking (rationale)
Invertebrate	*Hydra attenuata*	hydra		1.25% DMSO in stock solution	48 hour LOEC (increased glutathione S-transferase activity)	>1,420							Quinn *et al.* 2004	U (molecular markers are not considered for guideline derivaiton unless they are directly linked to survival, growth or reproduction)
Invertebrate	*Hydra attenuata*	hydra		1.25% DMSO in stock solution	48 hour NOEC (increased sulfotransferase activity)	142,000							Quinn *et al.* 2004	U (molecular markers are not considered for guideline derivaiton unless they are directly linked to survival, growth or reproduction; exceeds solubility limit)
Invertebrate	*Hydra attenuata*	hydra		1.25% DMSO in stock solution	48 hour LOEC (increased sulfotransferase activity)	1,420,000							Quinn *et al.* 2004	U (molecular markers are not considered for guideline derivaiton unless they are directly linked to survival, growth or reproduction; exceeds solubility limit)
Invertebrate	*Hydra attenuata*	hydra		0.31% Acetone in final exposure	96 hour LOEC (decreased feeding response)	50,000		20 ± 1		147 mg/L $CaCl_2$-$2H_20$		7	Quinn *et al.* 2008	2
Invertebrate	*Hydra attenuata*	hydra		0.31% Acetone in final exposure	96 hour LOEC (decreased hydranth number)	>50,000		20 ± 1		147 mg/L $CaCl_2$-$2H_20$		7	Quinn *et al.* 2008	2
Invertebrate	*Lumbriculus variegatus*	oligochaete	Juvenile (14 day old posterior fragments grown to complete worms)	Not available	96 hour NOEC (mortality; lysis and lack of blood circulation)	>4,000	Static	20 ± 1					Nentwig *et al.* 2004; Oetken *et al.* 2005	U (organism loading density too high - 20 test animals in 2 mL of solution)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{-1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{-1}$)	Hardness ($mg \cdot L^{-1}$)	Alkalinity ($mg \cdot L^{-1}$)	pH	Reference	Ranking (rationale)
Invertebrate	*Chironomus riparius*	midge	Juvenile (1st instar)	Not available	24 hour NOEC (mortality; immobility and/or lack of reaction to touch)	>4,000	Static renewal	20 ± 1					Nentwig *et al.* 2004; Oetken *et al.* 2005	U (organism loading density too high – 20 test animals in 2 mL of solution)
Invertebrate	*Spirostomum ambiguum*	ciliate, protozoa		No solvent used	24 hour EC50 (morphological changes; spherical deformation and autolysis)	70,700						Maintained within 5.5 and 8.0	Nalecz-Jawecki and Sawicki 2003	U (rapid bioassay Spirotox, no info on control, water quality parameters not reported)
Invertebrate	*Spirostomum ambiguum*	ciliate, protozoa		No solvent used	25 hour LC50 (lethality)	70,700						Maintained within 5.5 and 8.0	Nalecz-Jawecki and Sawicki 2003	U (rapid bioassay Spirotox, no info on control, water quality parameters not reported)
Invertebrate	*Tetrahymena termophila*	ciliate, protozoa		No solvent used	24 hour EC50 (growth inhibition)	40,500						Maintained within 5.5 and 8.0	Nalecz-Jawecki and Sawicki 2003	U (rapid bioassay Protoxkit, no info on control, water quality parameters not reported)
Invertebrate	*Elliptio complanata*	freshwater mussel	Field collected adults	Not available	24 hour LOEC (immunotoxic effect : decreased cell or hemocyte adherence)	14,000 (61 uM)	Static	15					Gagne *et al.* 2006	U (hemolymph samples treated in vitro)
Invertebrate	*Elliptio complanata*	freshwater mussel	Field collected adults	Not available	24 hour LOEC (immunotoxic effect : increased cell viability; intracellular esterase activity)	709 (3 uM)	Static	15					Gagne *et al.* 2006	U (hemolymph samples treated in vitro)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{-1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{-1}$)	Hardness ($mg \cdot L^{-1}$)	Alkalinity ($mg \cdot L^{-1}$)	pH	Reference	Ranking (rationale)
Invertebrate	*Elliptio complanata*	freshwater mussel	Field collected adults	Not available	24 hour LOEC (immunotoxic effect: induction of phagocytosis; increased amount of ingested fluorescent bacteria)	14,000 (61 uM)	Static	15					Gagne *et al.* 2006	U (hemolymph samples treated in vitro)
Invertebrate	*Thamnocephalus platyurus*	beaver-tail fairy shrimp	Larvae	No solvent used	24 hour LC50	140,000		25					Nalecz-Jawecki and Persoone 2006	U (rapid toxicity tests Rapidtoxkit (1 hour) and Thamnotoxkit (24 hour) both employing larvae hatched from dormant eggs, no reporting of WQ parameters or control, exceeds solubility limit)
Invertebrate	*Thamnocephalus platyurus*	beaver-tail fairy shrimp	Larvae	No solvent used	1 hour EC50 (food ingestion)	43,000		25					Nalecz-Jawecki and Persoone 2006	U (rapid toxicity tests Rapidtoxkit (1 hour) and Thamnotoxkit (24 hour) both employing larvae hatched from dormant eggs, no reporting of WQ parameters or control)
Invertebrate	*Thamnocephalus platyurus*	beaver-tail fairy shrimp	Nauplii	≤0.1% DMSO in final exposure	24 hour LC50	>100,000		25					Kim *et al.*, 2009	U (incubated for 24 hours in darkness, rapid bioassay, no WQ paramaters reported)
Invertebrate	*Notonecta glauca*	water boatman			48 hour LC50	>945 (4 uM)	Static	20		Artificial pond water			Meredith-Williams *et al.* 2012	U (only one exposure concentration was used, no control information reported)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{-1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{-1}$)	Hardness ($mg \cdot L^{-1}$)	Alkalinity ($mg \cdot L^{-1}$)	pH	Reference	Ranking (rationale)
fish	*Cyprinus carpio*	Common carp	Sperm collected from 3 year old male fish (n=5)	DMSO conc. in final exposure <0.05% v/v	2 hour NOEC (sperm motility)	200							Li *et al.*, 2010b	U (assessment of sperm quality parameters in vitro)
fish	*Cyprinus carpio*	Common carp	Sperm collected from 3 year old male fish (n=5)	DMSO conc. in final exposure <0.05% v/v	2 hour LOEC (sperm motility)	2,000							Li *et al.*, 2010b	U (assessment of sperm quality parameters in vitro)
fish	*Cyprinus carpio*	Common carp	Sperm collected from 3 year old male fish (n=5)	DMSO conc. in final exposure <0.05% v/v	2 hour NOEC (sperm velocity)	200							Li *et al.*, 2010b	U (assessment of sperm quality parameters in vitro)
fish	*Cyprinus carpio*	Common carp	Sperm collected from 3 year old male fish (n=5)	DMSO conc. in final exposure <0.05% v/v	2 hour LOEC (sperm velocity)	2,000							Li *et al.*, 2010b	U (assessment of sperm quality parameters in vitro)
fish	*Cyprinus carpio*	Common carp	Sperm collected from 3 year old male fish (n=5)	DMSO conc. in final exposure <0.05% v/v	2 hour LOEC (sperm viability)	>2,000							Li *et al.*, 2010b	U (assessment of sperm quality parameters in vitro)
fish	*Cyprinus carpio*	Common carp	Sperm collected from 3 year old male fish (n=5)	DMSO conc. in final exposure <0.05% v/v	2 hour NOEC (increased spermatozoa TBARS level - to evaluate sperm LPO; oxidative stress indice)	<200							Li *et al.*, 2010b	U (marker of oxidative stress in vitro)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration (µg·L^{1})	Test Type	Temp (°C)	DO (mg·L^{1})	Hardness (mg·L^{1})	Alkalinity (mg·L^{1})	pH	Reference	Ranking (rationale)
fish	*Cyprinus carpio*	Common carp	Sperm collected from 3 year old male fish (n=5)	DMSO conc. in final exposure <0.05% v/v	2 hour NOEC (increase in CP; oxidative stress indice)	2,000							Li *et al.*, 2010b	U (marker of oxidative stress in vitro)
fish	*Cyprinus carpio*	Common carp	Sperm collected from 3 year old male fish (n=5)	DMSO conc. in final exposure <0.05% v/v	2 hour LOEC (increase in CP; oxidative stress indice)	20,000							Li *et al.*, 2010b	U (marker of oxidative stress in vitro)
fish	*Cyprinus carpio*	Common carp	Sperm collected from 3 year old male fish (n=5)	DMSO conc. in final exposure <0.05% v/v	2 hour NOEC (decrease in SOD; inhibition of antioxidant enzymes)	200							Li *et al.*, 2010b	U (marker of oxidative stress in vitro)
fish	*Cyprinus carpio*	Common carp	Sperm collected from 3 year old male fish (n=5)	DMSO conc. in final exposure <0.05% v/v	2 hour LOEC (decrease in SOD; inhibition of antioxidant enzymes)	2,000							Li *et al.*, 2010b	U (marker of oxidative stress in vitro)
fish	*Cyprinus carpio*	Common carp	Sperm collected from 3 year old male fish (n=5)	DMSO conc. in final exposure <0.05% v/v	2 hour NOEC (decrease in GR; inhibition of antioxidant enzymes)	2,000							Li *et al.*, 2010b	U (marker of oxidative stress in vitro)
fish	*Cyprinus carpio*	Common carp	Sperm collected from 3 year old male fish (n=5)	DMSO conc. in final exposure <0.05% v/v	2 hour LOEC (decrease in GR; inhibition of antioxidant enzymes)	20,000							Li *et al.*, 2010b	U (marker of oxidative stress in vitro)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration (µg·L^{1})	Test Type	Temp (°C)	DO (mg·L^{1})	Hardness (mg·L^{1})	Alkalinity (mg·L^{1})	pH	Reference	Ranking (rationale)
fish	*Cyprinus carpio*	Common carp	Sperm collected from 3 year old male fish (n=5)	DMSO conc. in final exposure <0.05% v/v	2 hour NOEC (decrease inGPx; inhibition of antioxidant enzymes)	200							Li *et al.*, 2010b	U (marker of oxidative stress in vitro)
fish	*Cyprinus carpio*	Common carp	Sperm collected from 3 year old male fish (n=5)	DMSO conc. in final exposure <0.05% v/v	2 hour LOEC (decrease in GPxD; inhibition of antioxidant enzymes)	2,000							Li *et al.*, 2010b	U (marker of oxidative stress in vitro)
fish	*Cyprinus carpio*	Common carp	average length 7.5 cm, average weight 15.0 g	0.01% DMSO	24 hour LC50	59,700 ug/L	Static	27.4± 1.2	6.4± 0.04	18.4± 0.5	18.6 ±8.0	7.2 ± 0.09	Malarvizhi *et al.*, 2012	2
fish	*Cyprinus carpio*	Common carp	average length 7.5 cm, average weight 15.0 g	0.01% DMSO	Activity of enzymological parameter GOT (glutamate oxaloacetate transaminase) in the gill	Significant decrease when exposed to 59,700 ug/L for 24 hours	Static	27.4± 1.2	6.4± 0.04	18.4± 0.5	18.6 ±8.0	7.2 ± 0.09	Malarvizhi *et al.*, 2012	U (molecular markers are not considered for guideline derivaiton unless they are directly linked to survival, growth or reproduction)
fish	*Cyprinus carpio*	Common carp	average length 7.5 cm, average weight 15.0 g	0.01% DMSO	Activity of enzymological parameter GOT (glutamate oxaloacetate transaminase) in the liver	Significant decrease when exposed to 59,700 ug/L for 24 hours	Static	27.4± 1.2	6.4± 0.04	18.4± 0.5	18.6 ±8.0	7.2 ± 0.09	Malarvizhi *et al.*, 2012	U (molecular markers are not considered for guideline derivaiton unless they are directly linked to survival, growth or reproduction)
fish	*Cyprinus carpio*	Common carp	average length 7.5 cm, average weight 15.0 g	0.01% DMSO	Activity of enzymological parameter GOT (glutamate oxaloacetate transaminase) in the muscle	Significant decrease when exposed to 59,700 ug/L for 24 hours	Static	27.4± 1.2	6.4± 0.04	18.4± 0.5	18.6 ±8.0	7.2 ± 0.09	Malarvizhi *et al.*, 2012	U (molecular markers are not considered for guideline derivaiton unless they are directly linked to survival, growth or reproduction)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration (µg·L^{1})	Test Type	Temp (°C)	DO (mg·L^{1})	Hardness (mg·L^{1})	Alkalinity (mg·L^{1})	pH	Reference	Ranking (rationale)
fish	*Cyprinus carpio*	Common carp	average length 7.5 cm, average weight 15.0 g	0.01% DMSO	Activity of enzymological parameter GPT (glutamate pyruvate transaminase) in the gill	Significant decrease when exposed to 59,700 ug/L for 24 hours	Static	27.4± 1.2	6.4± 0.04	18.4± 0.5	18.6 ±8.0	7.2 ± 0.09	Malarvizhi *et al.*, 2012	U (molecular markers are not considered for guideline derivaiton unless they are directly linked to survival, growth or reproduction)
fish	*Cyprinus carpio*	Common carp	average length 7.5 cm, average weight 15.0 g	0.01% DMSO	Activity of enzymological parameter GPT (glutamate pyruvate transaminase) in the liver	Significant increase when exposed to 59,700 ug/L for 24 hours	Static	27.4± 1.2	6.4± 0.04	18.4± 0.5	18.6 ±8.0	7.2 ± 0.09	Malarvizhi *et al.*, 2012	U (molecular markers are not considered for guideline derivaiton unless they are directly linked to survival, growth or reproduction)
fish	*Cyprinus carpio*	Common carp	average length 7.5 cm, average weight 15.0 g	0.01% DMSO	Activity of enzymological parameter GPT (glutamate pyruvate transaminase) in the muscle	Significant increase when exposed to 59,700 ug/L for 24 hours	Static	27.4± 1.2	6.4± 0.04	18.4± 0.5	18.6 ±8.0	7.2 ± 0.09	Malarvizhi *et al.*, 2012	U (molecular markers are not considered for guideline derivaiton unless they are directly linked to survival, growth or reproduction)
fish	*Cyprinus carpio*	Common carp	average length 7.5 cm, average weight 15.0 g	0.01% DMSO	Activity of enzymological parameter LDH (lactate dehydrogenase) in the gill	Significant decrease when exposed to 59,700 ug/L for 24 hours	Static	27.4± 1.2	6.4± 0.04	18.4± 0.5	18.6 ±8.0	7.2 ± 0.09	Malarvizhi *et al.*, 2012	U (molecular markers are not considered for guideline derivaiton unless they are directly linked to survival, growth or reproduction)
fish	*Cyprinus carpio*	Common carp	average length 7.5 cm, average weight 15.0 g	0.01% DMSO	Activity of enzymological parameter LDH (lactate dehydrogenase) in the liver	Significant increase when exposed to 59,700 ug/L for 24 hours	Static	27.4± 1.2	6.4± 0.04	18.4± 0.5	18.6 ±8.0	7.2 ± 0.09	Malarvizhi *et al.*, 2012	U (molecular markers are not considered for guideline derivaiton unless they are directly linked to survival, growth or reproduction)
fish	*Cyprinus carpio*	Common carp	average length 7.5 cm, average weight 15.0 g	0.01% DMSO	Activity of enzymological parameter LDH (lactate dehydrogenase) in the muscle	Significant increase when exposed to 59,700 ug/L for 24 hours	Static	27.4± 1.2	6.4± 0.04	18.4± 0.5	18.6 ±8.0	7.2 ± 0.09	Malarvizhi *et al.*, 2012	U (molecular markers are not considered for guideline derivaiton unless they are directly linked to survival, growth or reproduction)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{-1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{-1}$)	Hardness ($mg \cdot L^{-1}$)	Alkalinity ($mg \cdot L^{-1}$)	pH	Reference	Ranking (rationale)
fish	*Danio rerio*	Zebrafish	2 hour old embryos (fish embryo tox test an alternate to acute fish test)	No solvent	72 hour NOEC (tail deformation) (effect at 15,300 ug/L was not considered dose related since at 30,600 ug/L no effects were found)	30,600	Static	26±1	□6.6	196		8.07 (7.7-8.1)	van den Brandhof and Montforts 2010	2
fish	*Danio rerio*	Zebrafish	Fertilized embryos (2-2.5 hour post fertilization)	0.5% DMSO	72 hour post hatch EC20 (teratogenicity)	17,531 (74.2 uM)	Static	26				7.4	Weigt *et al.* 2011	2 (developing teratogenicity assay)
fish	*Danio rerio*	Zebrafish	2 hour old embryos (fish embryo tox test an alternate to acute fish test)	No solvent	72 hour EC50 (growth retardation)	86,500 (81,500 – 91,700 95% CI)	Static	26±1	□6.6	196		8.07 (7.7-8.1)	van den Brandhof and Montforts 2010	2
fish	*Danio rerio*	Zebrafish	2 hour old embryos (fish embryo tox test an alternate to acute fish test)	No solvent	72 hour LC50	□□245,000	Static	26±1	□6.6	196		8.07	van den Brandhof and Montforts 2010	U (exceeds solubility limit of 112,000 mg/L; stock solution was made up to be 200,000 ug/L & allowed to precipitate before use)
fish	*Danio rerio*	Zebrafish	16 weeks old (3.0 cm, 132 mg)		96 hour LC50	35,400	Static	26	□			7.0-7.8	Liebig 2005 cited in RIVM 2014	1 (Klimisch score provided in RIVM 2014)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration (µg·L^{1})	Test Type	Temp (°C)	DO (mg·L^{1})	Hardness (mg·L^{1})	Alkalinity (mg·L^{1})	pH	Reference	Ranking (rationale)
fish	*Danio rerio*	Zebrafish	Fertilized embryos (collected daily 1.5 hour after first light)	DMSO - 0.004% in control and exposure tanks	96 hour (post hatch) LOEC (increased % mortality compared to control)	0.5	Static	28.5		Distilled water + NaHCO3 + sea salts (instant ocean) [final conductivity of 455–470 uS/cm]			Galus *et al.*, 2013a	U (conducted at environmentally relevant concentrations, but only two exposure concentrations were tested – low (0.5 ug/L) and high (10 ug/L), and exposures were conducted using sea salts (Instant Ocean) which has been shown to contain trace contaminants)
fish	*Danio rerio*	Zebrafish	Fertilized embryos (collected daily 1.5 hour after first light)	DMSO - 0.004% in control and exposure tanks	96 hour (post hatch) LOEC (decreased % mortality compared to control)	10	Static	28.5		Distilled water + NaHCO3 + sea salts (instant ocean) [final conductivity of 455–470 uS/cm]			Galus *et al.*, 2013a	U (conducted at environmentally relevant concentrations, but only two exposure concentrations were tested – low (0.5 ug/L) and high (10 ug/L), and exposures were conducted using sea salts (Instant Ocean) which has been shown to contain trace contaminants)
fish	*Danio rerio*	Zebrafish	Fertilized embryos (collected daily 1.5 hour after first light)	DMSO - 0.004% in control and exposure tanks	96 hour (post hatch) LOEC (% abnormality)	>10	Static	28.5		Distilled water + NaHCO3 + sea salts (instant ocean) [final conductivity of 455–470 uS/cm]			Galus *et al.*, 2013a	U (conducted at environmentally relevant concentrations, but only two exposure concentrations were tested – low (0.5 ug/L) and high (10 ug/L), and exposures were conducted using sea salts (Instant Ocean) which has been shown to contain trace contaminants)
fish	*Danio rerio*	Zebrafish	Fertilized embryos (2-2.5 hour post fertilization)	0.5% DMSO	72 hour post hatch LC50	>118,135 (500 uM)	Static	26				7.4	Weigt *et al.* 2011	U (focus of study was development of a teratogenicity assay, exceeds solubility limit)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{1}$)	Hardness ($mg \cdot L^{1}$)	Alkalinity ($mg \cdot L^{1}$)	pH	Reference	Ranking (rationale)
fish	*Danio rerio*	Zebrafish	Fertilized embryos (2-2.5 hour post fertilization)	0.5% DMSO	72 hour post hatch EC50 (teratogenicity)	52,452 (222 uM)	Static	26				7.4	Weigt *et al.* 2011	U (focus of study was development of a teratogenicity assay)
fish	*Lepomis gibbosus*	Pumpkin-seed fish	Field-collected unsexed juveniles (1-2g, 4-5 cm)	ethanol	72 hour NOEC (behavioural, percentage of time in black compartment [scototaxis])	>1,000 (highest concentration tested)	Static renewal (at 48 hour)	20±1					Brandao *et al.* 2013	2 (behavioural endpoints assessed are ecologically relevant as they may increase predation risk of test organisms)
fish	*Lepomis gibbosus*	Pumpkin-seed fish	Field-collected unsexed juveniles (1-2g, 4-5 cm)	ethanol	72 hour NOEC (behavioural, percentage of time in the periphery of the aquarium)	>1,000 (highest concentration tested)	Static renewal (at 48 hour)	20±1					Brandao *et al.* 2013	2 (behavioural endpoints assessed are ecologically relevant as they may increase predation risk of test organisms)
fish	*Lepomis gibbosus*	Pumpkin-seed fish	Field-collected unsexed juveniles (1-2g, 4-5 cm)	ethanol	72 hour NOEC (behavioural, percentage of time in motion)	>1,000 (highest concentration tested)	Static renewal (at 48 hour)	20±1					Brandao *et al.* 2013	2 (behavioural endpoints assessed are ecologically relevant as they may increase predation risk of test organisms)
fish	*Lepomis gibbosus*	Pumpkin-seed fish	Field-collected unsexed juveniles (1-2g, 4-5 cm)	ethanol	96 hour NOEC (lipid peroxidation in gills)	>1,000 (highest concentration tested)	Static renewal (at 48 hour)	20±1					Brandao *et al.* 2013	U (enzymatic biomarker of oxidative stress)
fish	*Lepomis gibbosus*	Pumpkin-seed fish	Field-collected unsexed juveniles (1-2g, 4-5 cm)	ethanol	96 hour NOEC (lipid peroxidation in liver)	>1,000 (highest concentration tested)	Static renewal (at 48 hour)	20±1					Brandao *et al.* 2013	U (enzymatic biomarker of oxidative stress)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{1}$)	Hardness ($mg \cdot L^{1}$)	Alkalinity ($mg \cdot L^{1}$)	pH	Reference	Ranking (rationale)
fish	*Lepomis gibbosus*	Pumpkin-seed fish	Field-collected unsexed juveniles (1-2g, 4-5 cm)	ethanol	96 hour LOEC (increase in glutathione reductase activity in gills)	62.5 (lowest concentration tested)	Static renewal (at 48 hour)	20±1					Brandao *et al.* 2013	U (enzymatic biomarker of oxidative stress; no significant dose-response relationship; significant effect observed only at lowest test concentration)
fish	*Lepomis gibbosus*	Pumpkin-seed fish	Field-collected unsexed juveniles (1-2g, 4-5 cm)	ethanol	96 hour LOEC (increase in glutathione reductase activity in digestive tract)	62.5 (lowest concentration tested)	Static renewal (at 48 hour)	20±1					Brandao *et al.* 2013	U (enzymatic biomarker of oxidative stress; statistically significant increase enzyme activity at all test concentrations with exception of 250 ug/L)
fish	*Lepomis gibbosus*	Pumpkin-seed fish	Field-collected unsexed juveniles (1-2g, 4-5 cm)	ethanol	96 hour NOEC (glutathione S-transferase activity in gills)	>1,000 (highest concentration tested)	Static renewal (at 48 hour)	20±1					Brandao *et al.* 2013	U (enzymatic biomarker of oxidative stress)
fish	*Lepomis gibbosus*	Pumpkin-seed fish	Field-collected unsexed juveniles (1-2g, 4-5 cm)	ethanol	96 hour NOEC (glutathione S-transferase activity in liver)	>1,000 (highest concentration tested)	Static renewal (at 48 hour)	20±1					Brandao *et al.* 2013	U (enzymatic biomarker of oxidative stress)
fish	*Lepomis gibbosus*	Pumpkin-seed fish	Field-collected unsexed juveniles (1-2g, 4-5 cm)	ethanol	96 hour NOEC (glutathione S-transferase activity in digestive tract)	125	Static renewal (at 48 hour)	20±1					Brandao *et al.* 2013	U (enzymatic biomarker of oxidative stress)
fish	*Lepomis gibbosus*	Pumpkin-seed fish	Field-collected unsexed juveniles (1-2g, 4-5 cm)	ethanol	96 hour LOEC (glutathione S-transferase activity in digestive tract)	250	Static renewal (at 48 hour)	20±1					Brandao *et al.* 2013	U (enzymatic biomarker of oxidative stress)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{-1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{-1}$)	Hardness ($mg \cdot L^{-1}$)	Alkalinity ($mg \cdot L^{-1}$)	pH	Reference	Ranking (rationale)
fish	*Oryzias latipes*	Japaese medaka	Length 2.0 ± 1.0 cm	DMSO	48 hour LC50	35,400 (nominal)	Static renewal (at 48 hour exposure)	25±1	>80% saturation	Dechlorinated tap water			Kim *et al.* 2007	2 (Klimisch score from RIVM 2014)
fish	*Oryzias latipes*	Japaese medaka	Length 2.0 ± 1.0 cm	DMSO	96 hour LC50	45,870 (42,490-50,100) (nominal)	Static renewal (at 48 hour exposure)	25±1	>80% saturation	Dechlorinated tap water			Kim *et al.* 2009	2 (Klimisch score from RIVM 2014)
fish	*Oryzias latipes*	Japaese medaka	Adult (avg body length of 3.25±0.34 cm, average body weight of 366±30 mg)	DMSO	96 hour LC50	61,500	Static renewal (at 48 hour exposure)	25±1					Nassef *et al.* 2009	U (artifical sewater was used to generate the test dilution water, for a final salinity of 0.035 psu. The salt product used was not identified.)
fish	*Oryzias latipes*	Japanese medaka	Adult fish - average body length 3.25±0.34 cm, average body weight of 366±30 mg	DMSO, 0.05 mL/L (solvent DMSO was never in excess of 0.005 (v/v) in the final exposure solutions)	24 hour LC50	72,150±0.00	Static renewal (at 48 hours exposure)	25±1		artificial seawater (salinity, 0.035 psu)			Nassef *et al.* 2009	U (artifical sewater was used to generate the test dilution water, for a final salinity of 0.035 psu. The salt product used was not identified.)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration (µg·L^{1})	Test Type	Temp (°C)	DO (mg·L^{1})	Hardness (mg·L^{1})	Alkalinity (mg·L^{1})	pH	Reference	Ranking (rationale)
fish	*Oryzias latipes*	Japanese medaka	Adult fish - average body length 3.25±0.34 cm, average body weight of 366±30 mg	DMSO, 0.05 mL/L (solvent DMSO was never in excess of 0.005 (v/v) in the final exposure solutions)	48 hour LC50	65,300±40	Static renewal (at 48 hours exposure)	25±1		artificial seawater (salinity, 0.035 psu)			Nassef *et al.* 2009	U (artifical sewater was used to generate the test dilution water, for a final salinity of 0.035 psu. The salt product used was not identified.)
fish	*Oryzias latipes*	Japanese medaka	Adult fish - average body length 3.25±0.34 cm, average body weight of 366±30 mg	DMSO, 0.05 mL/L (solvent DMSO was never in excess of 0.005 (v/v) in the final exposure solutions)	72 hour LC50	63,200±30	Static renewal (at 48 hours exposure)	25±1		artificial seawater (salinity, 0.035 psu)			Nassef *et al.* 2009	U (artifical sewater was used to generate the test dilution water, for a final salinity of 0.035 psu. The salt product used was not identified.)
fish	*Oryzias latipes*	Japanese medaka	Adult fish - average body length 3.25±0.34 cm, average body weight of 366±30 mg	DMSO, 0.05 mL/L (solvent DMSO was never in excess of 0.005 (v/v) in the final exposure solutions)	96 hour LC50	61,500±0.00	Static renewal (at 48 hours exposure)	25±1		artificial seawater (salinity, 0.035 psu)			Nassef *et al.* 2009	U (artifical sewater was used to generate the test dilution water, for a final salinity of 0.035 psu. The salt product used was not identified.)
fish	*Poeciliopsis lucida*	Topminnow	hepatocytes	DMSO may have been used, not to exceed 1% (v/v)	3 hour EC50 (cytotoxicity, MTT reduction test)	153,812 (81,277-502,546) (651 uM)							Laville *et al.* 2004	U (hepatocyte assay - not considered for guideline derivation unless directly linked to survival, growth or reproduction, exceeds solubility limit)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration (µg·L^{1})	Test Type	Temp (°C)	DO (mg·L^{1})	Hardness (mg·L^{1})	Alkalinity (mg·L^{1})	pH	Reference	Ranking (rationale)
fish	*Oncorhynchus mykiss*	Rainbow trout	RTG-2 cell line derived from gonad	1% DMSO in stock solution	24 hour EC50 (lactate dehydrogenase [LDH] leakage)	>283,524 (>1200 uM)	Static	20					Jos *et al.* 2003	U (cell assay - not considered for guideline derivation unless directly linked to survival, exceeds solubility limit)
fish	*Oncorhynchus mykiss*	Rainbow trout	RTG-2 cell line derived from gonad	1% DMSO in stock solution	24 hour EC50 (lactate dehydrogenase [LDH] activity)	>283,524 (>1200 uM)	Static	20					Jos *et al.* 2003	U (cell assay - not considered for guideline derivation unless directly linked to survival, exceeds solubility limit)
fish	*Oncorhynchus mykiss*	Rainbow trout	RTG-2 cell line derived from gonad	1% DMSO in stock solution	24 hour EC50 (total protein content)	196,577 (832 uM)	Static	20					Jos *et al.* 2003	U (cell assay - not considered for guideline derivation unless directly linked to survival, exceeds solubility limit)
fish	*Oncorhynchus mykiss*	Rainbow trout	RTG-2 cell line derived from gonad	1% DMSO in stock solution	48 hour EC50 (total protein content)	168,461 (713 uM)	Static	20					Jos *et al.* 2003	U (cell assay - not considered for guideline derivation unless directly linked to survival, exceeds solubility limit)
fish	*Oncorhynchus mykiss*	Rainbow trout	RTG-2 cell line derived from gonad	1% DMSO in stock solution	72 hour EC50 (total protein content)	220,440 (933 uM)	Static	20					Jos *et al.* 2003	U (cell assay - not considered for guideline derivation unless directly linked to survival, exceeds solubility limit)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration (µg·L^{1})	Test Type	Temp (°C)	DO (mg·L^{1})	Hardness (mg·L^{1})	Alkalinity (mg·L^{1})	pH	Reference	Ranking (rationale)
fish	*Oncorhynchus mykiss*	Rainbow trout	RTG-2 cell line derived from gonad	1% DMSO in stock solution	24 hour EC50 (neutral red uptake)	117,190 (496 uM)	Static	20					Jos *et al.* 2003	U (cell assay - not considered for guideline derivation unless directly linked to survival, exceeds solubility limit)
fish	*Oncorhynchus mykiss*	Rainbow trout	RTG-2 cell line derived from gonad	1% DMSO in stock solution	48 hour EC50 (neutral red uptake)	111,661 (472.6 uM)	Static	20					Jos *et al.* 2003	U (cell assay - not considered for guideline derivation unless directly linked to survival, approaching solubility limit)
fish	*Oncorhynchus mykiss*	Rainbow trout	RTG-2 cell line derived from gonad	1% DMSO in stock solution	72 hour EC50 (neutral red uptake)	116,954 (495 uM)	Static	20					Jos *et al.* 2003	U (cell assay - not considered for guideline derivation unless directly linked to survival, exceeds solubility limit)
fish	*Oncorhynchus mykiss*	Rainbow trout	RTG-2 cell line derived from gonad	1% DMSO in stock solution	24 hour EC50 (MTS metabolization)	164,917 (698 uM)	Static	20					Jos *et al.* 2003	U (cell assay - not considered for guideline derivation unless directly linked to survival, exceeds solubility limit)
fish	*Oncorhynchus mykiss*	Rainbow trout	RTG-2 cell line derived from gonad	1% DMSO in stock solution	48 hour EC50 (MTS metabolization)	143,794 (608.6 uM)	Static	20					Jos *et al.* 2003	U (cell assay - not considered for guideline derivation unless directly linked to survival, exceeds solubility limit)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration (µg·L^{1})	Test Type	Temp (°C)	DO (mg·L^{1})	Hardness (mg·L^{1})	Alkalinity (mg·L^{1})	pH	Reference	Ranking (rationale)
fish	*Oncorhynchus mykiss*	Rainbow trout	RTG-2 cell line derived from gonad	1% DMSO in stock solution	72 hour EC50 (MTS metabolization)	142,093 (601.4 uM)	Static	20					Jos *et al.* 2003	U (cell assay - not considered for guideline derivation unless directly linked to survival, exceeds solubility limit)
fish	*Oncorhynchus mykiss*	Rainbow trout	RTG-2 cell line derived from gonad	1% DMSO in stock solution	24 hour EC50 (G6PDH activity)	201,538 (853 uM)	Static	20					Jos *et al.* 2003	U (cell assay - not considered for guideline derivation unless directly linked to survival, exceeds solubility limit)
fish	*Oncorhynchus mykiss*	Rainbow trout	RTG-2 cell line derived from gonad	1% DMSO in stock solution	48 hour EC50 (G6PDH activity)	169,335 (716.7 uM)	Static	20					Jos *et al.* 2003	U (cell assay - not considered for guideline derivation unless directly linked to survival, exceeds solubility limit)
fish	*Oncorhynchus mykiss*	Rainbow trout	RTG-2 cell line derived from gonad	1% DMSO in stock solution	72 hour EC50 (G6PDH activity)	162,318	Static	20					Jos *et al.* 2003	U (cell assay - not considered for guideline derivation unless directly linked to survival, exceeds solubility limit)
fish	*Oncorhynchus mykiss*	Rainbow trout	Hepatocytes (immature)	DMSO may have been used, not to exceed 1% (v/v)	3 hour EC50 (cytotoxicity, MTT reduction test)	75,134 (59,776-94,744) (318 uM)							Laville *et al.* 2004	U (hepatocyte assay - not considered for guideline derivation unless directly linked to survival)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration (µg·L^{1})	Test Type	Temp (°C)	DO (mg·L^{1})	Hardness (mg·L^{1})	Alkalinity (mg·L^{1})	pH	Reference	Ranking (rationale)
fish	*Oncorhynchus mykiss*	Rainbow trout	EROD activity test (immature)	DMSO may have been used, not to exceed 1% (v/v)	15 min EC50 (EROD inhibition)	75,134 (59,776-94,744) (318 uM)							Laville *et al.* 2004	U (hepatocyte assay - not considered for guideline derivation unless directly linked to survival)
fish	*Oncorhynchus mykiss*	Rainbow trout	Brain homogenate collected from adults (weight 264.33±39.97 g)	0.05% v/v DMSO for highest test conc.	60 min LOEC (lipid peroxidation = oxidative stress response, as indicated by significant induction of tissue thiobarbituric acid-reactive substance [TBARS] levels) Oxidative Stress Indice	1.0		25					Li *et al.*, 2010a	U (brain homogenate in vitro assay)
fish	*Oncorhynchus mykiss*	Rainbow trout	Brain homogenate collected from adults (weight 264.33±39.97 g)	0.05% v/v DMSO for highest test conc.	120 min LOEC (lipid peroxidation = oxidative stress response, as indicated by significant induction of tissue thiobarbituric acid-reactive substance [TBARS] levels) Oxidative Stress Indice	1.0		25					Li *et al.*, 2010a	U (brain homogenate in vitro assay)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration (µg·L^{1})	Test Type	Temp (°C)	DO (mg·L^{1})	Hardness (mg·L^{1})	Alkalinity (mg·L^{1})	pH	Reference	Ranking (rationale)
fish	*Oncorhynchus mykiss*	Rainbow trout	Brain homogenate collected from adults (weight 264.33±39.97 g)	0.05% v/v DMSO for highest test conc.	60 minute NOEC (significant increase in carbonyl protein = oxidative stress response) Oxidative Stress Indice	1.0		25					Li *et al.*, 2010a	U (brain homogenate in vitro assay)
fish	*Oncorhynchus mykiss*	Rainbow trout	Brain homogenate collected from adults (weight 264.33±39.97 g)	0.05% v/v DMSO for highest test conc.	60 minute LOEC (significant increase in carbonyl protein = oxidative stress response) Oxidative Stress Indice	200		25					Li *et al.*, 2010a	U (brain homogenate in vitro assay)
fish	*Oncorhynchus mykiss*	Rainbow trout	Brain homogenate collected from adults (weight 264.33±39.97 g)	0.05% v/v DMSO for highest test conc.	120 minute LOEC (significant increase in carbonyl protein = oxidative stress response) Oxidative Stress Indice	1.0		25					Li *et al.*, 2010a	U (brain homogenate in vitro assay)
fish	*Oncorhynchus mykiss*	Rainbow trout	Brain homogenate collected from adults	0.05% v/v DMSO for highest test conc.	60 & 120 minute NOEC (significant inhibition of superoxide dimutase [SOD] = oxidative stress response) Antioxidant parameter	1.0		25					Li *et al.*, 2010a	U (brain homogenate in vitro assay)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration (µg·L^{1})	Test Type	Temp (°C)	DO (mg·L^{1})	Hardness (mg·L^{1})	Alkalinity (mg·L^{1})	pH	Reference	Ranking (rationale)
fish	*Oncorhynchus mykiss*	Rainbow trout	Brain homogenate collected from adults (weight 264.33±39.97 g)	0.05% v/v DMSO for highest test conc.	60 & 120 minute LOEC (significant inhibition of superoxide dimutase [SOD] = oxidative stress response) Antioxidant parameter	200		25					Li *et al.*, 2010a	U (brain homogenate in vitro assay)
fish	*Oncorhynchus mykiss*	Rainbow trout	Brain homogenate collected from adults (weight 264.33±39.97 g)	0.05% v/v DMSO for highest test conc.	60 minute NOEC (significant ihibition of glutathione reductase [GR]) Antioxidant parameter	200		25					Li *et al.*, 2010a	U (brain homogenate in vitro assay)
fish	*Oncorhynchus mykiss*	Rainbow trout	Brain homogenate collected from adults (weight 264.33±39.97 g)	0.05% v/v DMSO for highest test conc.	60 minute LOEC (significant ihibition of glutathione reductase [GR]) Antioxidant parameter	2,000		25					Li *et al.*, 2010a	U (brain homogenate in vitro assay)
fish	*Oncorhynchus mykiss*	Rainbow trout	Brain homogenate collected from adults (weight 264.33±39.97 g)	0.05% v/v DMSO for highest test conc.	120 minute NOEC (significant ihibition of glutathione reductase [GR]) Antioxidant parameter	1.0		25					Li *et al.*, 2010a	U (brain homogenate in vitro assay)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration (µg·L^{1})	Test Type	Temp (°C)	DO (mg·L^{1})	Hardness (mg·L^{1})	Alkalinity (mg·L^{1})	pH	Reference	Ranking (rationale)
fish	*Oncorhynchus mykiss*	Rainbow trout	Brain homogenate collected from adults (weight 264.33±39.97 g)	0.05% v/v DMSO for highest test conc.	120 minute LOEC (significant ihibition of glutathione reductase [GR]) Antioxidant parameter	200		25					Li *et al.*, 2010a	U (brain homogenate in vitro assay)
fish	*Oncorhynchus mykiss*	Rainbow trout	Brain homogenate collected from adults (weight 264.33±39.97 g)	0.05% v/v DMSO for highest test conc.	60 minute NOEC (significant ihibition of glutathione peroxidase [GPx]) Antioxidant parameter	200		25					Li *et al.*, 2010a	U (brain homogenate in vitro assay)
fish	*Oncorhynchus mykiss*	Rainbow trout	Brain homogenate collected from adults (weight 264.33±39.97 g)	0.05% v/v DMSO for highest test conc.	60 minute LOEC (significant ihibition of glutathione peroxidase [GPx]) Antioxidant parameter	2,000		25					Li *et al.*, 2010a	U (brain homogenate in vitro assay)
fish	*Oncorhynchus mykiss*	Rainbow trout	Brain homogenate collected from adults (weight 264.33±39.97 g)	0.05% v/v DMSO for highest test conc.	120 minute NOEC (significant ihibition of glutathione peroxidase [GPx]) Antioxidant parameter	1.0		25					Li *et al.*, 2010a	U (brain homogenate in vitro assay)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration (µg·L^{1})	Test Type	Temp (°C)	DO (mg·L^{1})	Hardness (mg·L^{1})	Alkalinity (mg·L^{1})	pH	Reference	Ranking (rationale)
fish	*Oncorhynchus mykiss*	Rainbow trout	Brain homogenate collected from adults (weight 264.33±39.97 g)	0.05% v/v DMSO for highest test conc.	120 minute LOEC (significant ihibition of glutathione peroxidase [GPx]) Antioxidant parameter	200		25					Li *et al.*, 2010a	U (brain homogenate in vitro assay)
fish	*Oncorhynchus mykiss*	Rainbow trout	Brain homogenate collected from adults (weight 264.33±39.97 g)	0.05% v/v DMSO for highest test conc.	60 & 120 minute NOEC (significant decrease in reduced glutathione [GSH]) Antioxidant parameter	1.0		25					Li *et al.*, 2010a	U (brain homogenate in vitro assay)
fish	*Oncorhynchus mykiss*	Rainbow trout	Brain homogenate collected from adults (weight 264.33±39.97 g)	0.05% v/v DMSO for highest test conc.	60 & 120 minute LOEC (significant decrease in reduced glutathione [GSH]) Antioxidant parameter	200		25					Li *et al.*, 2010a	U (brain homogenate in vitro assay)
fish	*Oncorhynch us mykiss*	Rainbow trout	juveniles (64.3 g)		96 hour LC50	19,900	Static renewal	15				7.4	Li *et al.* 2011	2 (Klimisch criteria provided by RIVM 2014)
amphibian	*Xenopus laevis*	African clawed frog	Blastulae, stage 9	DMSO did not exceed 1% v/v	96 hour EC10 (increased deformity)	>100,000	Static renewal	23±3		FETAX solution			Richards and Cole 2006	2 (true effect concentration may exceed solubility limit)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{1}$)	Hardness ($mg \cdot L^{1}$)	Alkalinity ($mg \cdot L^{1}$)	pH	Reference	Ranking (rationale)
amphibian	*Xenopus laevis*	African clawed frog	Blastulae, stage 9	DMSO did not exceed 1% v/v	96 hour EC16 (increased deformity)	>100,000	Static renewal	23±3		FETAX solution			Richards and Cole 2006	2 (true effect concentration may exceed solubility limit)
amphibian	*Xenopus laevis*	African clawed frog	Blastulae, stage 9	DMSO did not exceed 1% v/v	96 hour EC50 (increased deformity)	>100,000	Static renewal	23±3		FETAX solution			Richards and Cole 2006	2 (true effect concentration may exceed solubility limit)
amphibian	*Xenopus laevis*	African clawed frog	Blastulae, stage 9	DMSO did not exceed 1% v/v	96 hour EC84 (increased deformity)	>100,000	Static renewal	23±3		FETAX solution			Richards and Cole 2006	2 (true effect concentration may exceed solubility limit)
amphibian	*Xenopus laevis*	African clawed frog	Blastulae, stage 9	DMSO did not exceed 1% v/v	96 hour LC10 (increased mortality)	>100,000	Static renewal	23±3		FETAX solution			Richards and Cole 2006	2 (true effect concentration may exceed solubility limit)
amphibian	*Xenopus laevis*	African clawed frog	Blastulae, stage 9	DMSO did not exceed 1% v/v	96 hour LOEC (decreased growth)	>100,000	Static renewal	23±3		FETAX solution			Richards and Cole 2006	2 (true effect concentration may exceed solubility limit)

Chronic Freshwater Toxicity Data – Carbamazepine

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{-1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{-1}$)	Hardness ($mg \cdot L^{-1}$)	Alkalinity ($mg \cdot L^{-1}$)	pH	Reference	Ranking (rationale)
Invertebrate	*Brachionus calyciflorus*	Rotifer	Newly hatched	No solvent added	48 hour NOEC (reproduction)	377	Static	25±1		MHW for control		7.0-8.5	Ferrari *et al.* 2003; Ferrari *et al.* 2004	2 (test conducted in darkness)
Invertebrate	*Brachionus calyciflorus*	Rotifer	Newly hatched	No solvent added	48 hour LOEC (reproduction)	754	Static	25±1		MHW for control		7.0-8.5	Ferrari *et al.* 2003; Ferrari *et al.* 2004	2 (test conducted in darkness)
Invertebrate	*Brachionus calyciflorus*	Rotifer		No solvent added	48 hour LOEC (Number living females)	7,070							Anderozzi *et al.* 2004	U (six pharmaceutical mixture)
Invertebrate	*Ceriodaphnia dubia*	Water flea	<24 hours old	No solvent added	7 day NOEC (reproduction)	25	Static-renewal	25±1		MH synthetic water (80-100 as CaCO3)		7.0-8.5	Ferrari *et al.* 2003; Ferrari *et al.* 2004	2
Invertebrate	*Ceriodaphnia dubia*	Water flea	<24 hours old	No solvent added	7 day LOEC (reproduction)	100	Static-renewal	25±1		MH synthetic water (80-100 as CaCO3)		7.0-8.5	Ferrari *et al.* 2003; Ferrari *et al.* 2004	2

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{-1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{-1}$)	Hardness ($mg \cdot L^{-1}$)	Alkalinity ($mg \cdot L^{-1}$)	pH	Reference	Ranking (rationale)
Invertebrate	*Ceriodaphnia dubia*	Water flea	<24 hour old	No solvent added	7 day LOEC (fecundity, 3 broods, F0, F1, F2)	264.6	Static renewal	25		80-100	64	7.4-7.8	Lamichhane *et al.* 2013	2 (loss of CBZ ranged from 51 to 74% after 24 hours of exposure, however the exposures were daily static renewal so exposed to the same level of nominal concentration even if CBZ loss existed)
Invertebrate	*Ceriodaphnia dubia*	Water flea	<24 hour old	No solvent added	7 day NOEC (fecundity, 3 broods, F0, F1, F2)	**196.7**	Static renewal	25		80-100	64	7.4-7.8	Lamichhane *et al.* 2013	2 (loss of CBZ ranged from 51 to 74% after 24 hours of exposure, however the exposures were daily static renewal so exposed to the same level of nominal concentration even if CBZ loss existed)
Invertebrate	*Ceriodaphnia dubia*	Water flea	<24 hour old	No solvent added	14 day LOEC (fecundity, 1-7 broods, F0, F1)	196.7	Static renewal	25		80-100	64	7.4-7.8	Lamichhane *et al.* 2013	2 (loss of CBZ ranged from 51 to 74% after 24 hours of exposure, however the exposures were daily static renewal so exposed to the same level of nominal concentration even if CBZ loss existed)
Invertebrate	*Ceriodaphnia dubia*	Water flea	<24 hour old	No solvent added	14 day NOEC (fecundity, 1-7 broods, F0, F1)	**104**	Static renewal	25		80-100	64	7.4-7.8	Lamichhane *et al.* 2013	2 (loss of CBZ ranged from 51 to 74% after 24 hours of exposure, however the exposures were daily static renewal so exposed to the same level of nominal concentration even if CBZ loss existed)
Invertebrate	*Ceriodaphnia dubia*	Water flea	<24 hour old	No solvent added	14 day LOEC (fecundity, 1-7 broods, F2)	264.6	Static renewal	25		80-100	64	7.4-7.8	Lamichhane *et al.* 2013	2 (loss of CBZ ranged from 51 to 74% after 24 hours of exposure, however the exposures were daily static renewal so exposed to the same level of nominal concentration even if CBZ loss existed)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{1}$)	Hardness ($mg \cdot L^{1}$)	Alkalinity ($mg \cdot L^{1}$)	pH	Reference	Ranking (rationale)
Invertebrate	*Ceriodaphnia dubia*	Water flea	<24 hour old	No solvent added	14 day NOEC (fecundity, 1-7 broods, F2)	196.7	Static renewal	25		80-100	64	7.4-7.8	Lamichhane *et al.* 2013	2 (loss of CBZ ranged from 51 to 74% after 24 hours of exposure, however the exposures were daily static renewal so exposed to the same level of nominal concentration even if CBZ loss existed)
Invertebrate	*Ceriodaphnia dubia*	Water flea	<24 hour old	No solvent added	14 day LOEC (fecundity, age at first reproduction, F0, F1)	≥264.6	Static renewal	25		80-100	64	7.4-7.8	Lamichhane *et al.* 2013	2 (loss of CBZ ranged from 51 to 74% after 24 hours of exposure, however the exposures were daily static renewal so exposed to the same level of nominal concentration even if CBZ loss existed)
Invertebrate	*Ceriodaphnia dubia*	Water flea	<24 hour old	No solvent added	14 day NOEC (fecundity, age at first reproduction, F0, F1)	264.6	Static renewal	25		80-100	64	7.4-7.8	Lamichhane *et al.* 2013	2 (loss of CBZ ranged from 51 to 74% after 24 hours of exposure, however the exposures were daily static renewal so exposed to the same level of nominal concentration even if CBZ loss existed)
Invertebrate	*Ceriodaphnia dubia*	Water flea	<24 hour old	No solvent added	14 day LOEC (fecundity, age at first reproduction, F2)	264.6	Static renewal	25		80-100	64	7.4-7.8	Lamichhane *et al.* 2013	2 (loss of CBZ ranged from 51 to 74% after 24 hours of exposure, however the exposures were daily static renewal so exposed to the same level of nominal concentration even if CBZ loss existed)
Invertebrate	*Ceriodaphnia dubia*	Water flea	<24 hour old	No solvent added	14 day NOEC (fecundity, age at first reproduction, F2)	196.7	Static renewal	25		80-100	64	7.4-7.8	Lamichhane *et al.* 2013	2 (loss of CBZ ranged from 51 to 74% after 24 hours of exposure, however the exposures were daily static renewal so exposed to the same level of nominal concentration even if CBZ loss existed)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{1}$)	Hardness ($mg \cdot L^{1}$)	Alkalinity ($mg \cdot L^{1}$)	pH	Reference	Ranking (rationale)
Invertebrate	*Ceriodaphnia dubia*	Water flea	<24 hour old	No solvent added	14 day LOEC (growth, neonate body length (mm), F0, F1, F2)	≥264.6	Static renewal	25		80-100	64	7.4-7.8	Lamichhane *et al.* 2013	2 (loss of CBZ ranged from 51 to 74% after 24 hours of exposure, however the exposures were daily static renewal so exposed to the same level of nominal concentration even if CBZ loss existed)
Invertebrate	*Ceriodaphnia dubia*	Water flea	<24 hour old	No solvent added	14 day NOEC (growth, neonate body length (mm), F0, F1, F2)	264.6	Static renewal	25		80-100	64	7.4-7.8	Lamichhane *et al.* 2013	2 (loss of CBZ ranged from 51 to 74% after 24 hours of exposure, however the exposures were daily static renewal so exposed to the same level of nominal concentration even if CBZ loss existed)
Invertebrate	*Ceriodaphnia dubia*	Water flea	<24 hour old	No solvent added	14 day LOEC (growth, adult body length (mm), F0, F1)	≥264.6	Static renewal	25		80-100	64	7.4-7.8	Lamichhane *et al.* 2013	2 (loss of CBZ ranged from 51 to 74% after 24 hours of exposure, however the exposures were daily static renewal so exposed to the same level of nominal concentration even if CBZ loss existed)
Invertebrate	*Ceriodaphnia dubia*	Water flea	<24 hour old	No solvent added	14 day NOEC (growth, adult body length (mm), F0, F1)	264.6	Static renewal	25		80-100	64	7.4-7.8	Lamichhane *et al.* 2013	2 (loss of CBZ ranged from 51 to 74% after 24 hours of exposure, however the exposures were daily static renewal so exposed to the same level of nominal concentration even if CBZ loss existed)
Invertebrate	*Ceriodaphnia dubia*	Water flea	<24 hour old	No solvent added	14 day LOEC (growth, adult body length (mm), F2)	264.6	Static renewal	25		80-100	64	7.4-7.8	Lamichhane *et al.* 2013	2 (loss of CBZ ranged from 51 to 74% after 24 hours of exposure, however the exposures were daily static renewal so exposed to the same level of nominal concentration even if CBZ loss existed)

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Invertebrate	*Ceriodaphnia dubia*	Water flea	<24 hour old	No solvent added	14 day NOEC (growth, adult body length (mm), F2)	196.7	Static renewal	25		80-100	64	7.4-7.8	Lamichhane *et al.* 2013	2 (loss of CBZ ranged from 51 to 74% after 24 hours of exposure, however the exposures were daily static renewal so exposed to the same level of nominal concentration even if CBZ loss existed)
Invertebrate	*Ceriodaphnia dubia*	Water flea	<24 hour old	No solvent added	14 day LOEC (growth, neonate dry weight (ug), F0)	≥264.6	Static renewal	25		80-100	64	7.4-7.8	Lamichhane *et al.* 2013	2 (loss of CBZ ranged from 51 to 74% after 24 hours of exposure, however the exposures were daily static renewal so exposed to the same level of nominal concentration even if CBZ loss existed)
Invertebrate	*Ceriodaphnia dubia*	Water flea	<24 hour old	No solvent added	14 day NOEC (growth, neonate dry weight (ug), F0)	264.6	Static renewal	25		80-100	64	7.4-7.8	Lamichhane *et al.* 2013	2 (loss of CBZ ranged from 51 to 74% after 24 hours of exposure, however the exposures were daily static renewal so exposed to the same level of nominal concentration even if CBZ loss existed)
Invertebrate	*Ceriodaphnia dubia*	Water flea	<24 hours old	No solvent added	14 day LOEC (growth, neonate dry weight (ug), F1)	196.7	Static renewal	25		80-100	64	7.4-7.8	Lamichhane *et al.* 2013	2 (loss of CBZ ranged from 51 to 74% after 24 hours of exposure, however the exposures were daily static renewal so exposed to the same level of nominal concentration even if CBZ loss existed)
Invertebrate	*Ceriodaphnia dubia*	Water flea	<24 hour old	No solvent added	14 day NOEC (growth, neonate dry weight (ug), F1)	104	Static renewal	25		80-100	64	7.4-7.8	Lamichhane *et al.* 2013	2 (loss of CBZ ranged from 51 to 74% after 24 hours of exposure, however the exposures were daily static renewal so exposed to the same level of nominal concentration even if CBZ loss existed)

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Invertebrate	*Ceriodaphnia dubia*	Water flea	<24 hour old	No solvent added	14 day LOEC (growth, neonate dry weight (ug), F2)	264.6	Static renewal	25		80-100	64	7.4-7.8	Lamichhane *et al.* 2013	2 (loss of CBZ ranged from 51 to 74% after 24 hours of exposure, however the exposures were daily static renewal so exposed to the same level of nominal concentration even if CBZ loss existed)
Invertebrate	*Ceriodaphnia dubia*	Water flea	<24 hour old	No solvent added	14 day NOEC (growth, neonate dry weight (ug), F2)	196.7	Static renewal	25		80-100	64	7.4-7.8	Lamichhane *et al.* 2013	2 (loss of CBZ ranged from 51 to 74% after 24 hours of exposure, however the exposures were daily static renewal so exposed to the same level of nominal concentration even if CBZ loss existed)
Invertebrate	*Daphnia magna*	Water flea	<24 hour old neonates	0.1 ug/uL methanol in stock solution	5 day NOEC (F4 generation) (Progeny counts/numbers)	0.5	Static renewal (weekly)	20±0.5					Dietrich *et al.* 2010	U (only one concentration tested, represented max conc measured in rivers & streams of southern Germany)
Invertebrate	*Daphnia magna*	Water flea	<24 hour old neonates	0.1 ug/uL methanol in stock solution	5 day NOEC (F4 generation) (Time to first progeny)	0.5	Static renewal (weekly)	20±0.5					Dietrich *et al.* 2010	U (only one concentration tested)
Invertebrate	*Daphnia magna*	Water flea	<24 hour old neonates	0.1 ug/uL methanol in stock solution	5 day NOEC (F4 generation) (growth)	0.5	Static renewal (weekly)	20±0.5					Dietrich *et al.* 2010	U (only one concentration tested)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{1}$)	Hardness ($mg \cdot L^{1}$)	Alkalinity ($mg \cdot L^{1}$)	pH	Reference	Ranking (rationale)
Invertebrate	*Daphnia magna*	Water flea	<24 hour old neonates	0.1 ug/uL methanol in stock solution	5 day NOEC (F4 generation) (mortality)	0.5	Static renewal (weekly)	20±0.5					Dietrich *et al.* 2010	U (only one concentration tested)
Invertebrate	*Daphnia magna*	Water flea	<24 hour old neonates	0.1 ug/uL methanol in stock solution	6 day NOEC (F5 generation) (Progeny counts/numbers)	0.5	Static renewal (weekly)	20±0.5					Dietrich *et al.* 2010	U (only one concentration tested)
Invertebrate	*Daphnia magna*	Water flea	<24 hour old neonates	0.1 ug/uL methanol in stock solution	6 day NOEC (F5 generation) (Time to first progeny)	0.5	Static renewal (weekly)	20±0.5					Dietrich *et al.* 2010	U (only one concentration tested)
Invertebrate	*Daphnia magna*	Water flea	<24 hour old neonates	0.1 ug/uL methanol in stock solution	6 day NOEC (F5 generation) (growth)	0.5	Static renewal (weekly)	20±0.5					Dietrich *et al.* 2010	U (only one concentration tested)
Invertebrate	*Daphnia magna*	Water flea	<24 hour old neonates	0.1 ug/uL methanol in stock solution	6 day NOEC (F5 generation) (mortality)	0.5	Static renewal (weekly)	20±0.5					Dietrich *et al.* 2010	U (only one concentration tested)
Invertebrate	*Daphnia magna*	Water flea	<24 hour old neonates		21 day NOEC (mortality)	4,000	static renewal	20		artificial medium		7.6-8.7	Liebig 2005	1 (Klimisch score from RIVM 2014)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{-1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{-1}$)	Hardness ($mg \cdot L^{-1}$)	Alkalinity ($mg \cdot L^{-1}$)	pH	Reference	Ranking (rationale)
Invertebrate	*Daphnia magna*	Water flea	<24 hour old neonates		21 day NOEC (reproduction)	400	Static renewal	20		artificial medium		7.6-8.7	Liebig 2005	1 (Klimisch score from RIVM 2014)
Invertebrate	*Daphnia magna*	Water flea	<24 hour old neonates		21 day NOEC (growth)	400	Static renewal	20		artificial medium		7.6-8.7	Liebig 2005	1 (Klimisch score from RIVM 2014)
Invertebrate	*Daphnia magna*	Water flea	<24 hour old neonates		21 day LOEC (mortality)	12,600	Static renewal	20		artificial medium		7.6-8.7	Liebig 2005	1 (Klimisch score from RIVM 2014)
Invertebrate	*Daphnia magna*	Water flea	<24 hour old neonates		21 day LOEC (reproduction)	1,260	Static renewal	20		artificial medium		7.6-8.7	Liebig 2005	1 (Klimisch score from RIVM 2014)
Invertebrate	*Daphnia magna*	Water flea	<24 hour old neonates		21 day LOEC (growth)	1,260	Static renewal	20		artificial medium		7.6-8.7	Liebig 2005	1 (Klimisch score from RIVM 2014)
Invertebrate	*Daphnia magna*	Water flea	<24 hour old neonates		21 day EC50 (reproduction)	6,600	Static renewal	20		artificial medium		7.6-8.7	Liebig 2005	1 (Klimisch score from RIVM 2014)

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Invertebrate	*Daphnia magna*	Water flea	<24 hour old neonates		21 day EC50 (growth)	9,400	Static renewal	20		artificial medium		7.6-8.7	Liebig 2005	1 (Klimisch score from RIVM 2014)
Invertebrate	*Daphnia pulex*	Water flea	<24 hour old neonates	25 uL of acetone added to 100 mL exposure medium	5 day (juvenile period) LOEC for body length / somatic growth rate (delayed maturation)	200	Static renewal (daily)	20				7.5-8.0	Lurling *et al.* 2006	2
Invertebrate	*Daphnia pulex*	Water flea	<24 hour old neonates	25 uL of acetone added to 100 mL exposure medium	14 day (entire experimental period) LOEC for body length / somatic growth rate (delayed maturation)	>200	Static renewal (daily)	20				7.5-8.0	Lurling *et al.* 2006	2
Invertebrate	*Daphnia pulex*	Water flea	<24 hour old neonates	25 uL of acetone added to 100 mL exposure medium	14 day (entire experimental period) LOEC size at first reproduction (mm)	>200	Static renewal (daily)	20				7.5-8.0	Lurling *et al.* 2006	2
Invertebrate	*Daphnia pulex*	Water flea	<24 hour old neonates	25 uL of acetone added to 100 mL exposure medium	14 day (entire experimental period) LOEC survival (survival of 67%, compared to control & solvent control, both at 83%)	100	Static renewal (daily)	20				7.5-8.0	Lurling *et al.* 2006	2

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Invertebrate	*Daphnia pulex*	Water flea	<24 hour old neonates	25 uL of acetone added to 100 mL exposure medium	14 day (entire experimental period) LOEC spine length	>200	Static renewal (daily)	20				7.5-8.0	Lurling *et al.* 2006	2
Invertebrate	*Daphnia pulex*	Water flea	<24 hour old neonates	25 uL of acetone added to 100 mL exposure medium	14 day (entire experimental period) - Earlier maturation and production of more offspring compared to control group	1 (highest number of offspring produced in 1 and 10 ug/L, but this was not sig different from controls)	Static renewal (daily)	20				7.5-8.0	Lurling *et al.* 2006	2
Invertebrate	*Chironomus dilutus*	midge	12 day old	No solvent	10 day LC25 (survival)	23,700 (21,880 – 25,460)	Static renewal (every 48h), with sand	21.9 ± 0.73	7.9 ± 0.39	0.25 ± 0.081 mmol (Recon water)	0.35 ± 0.1 mEq	8.4 ± 0.12	Dussault *et al.* 2008	1
Invertebrate	*Chironomus dilutus*	midge	12 day old	No solvent	10 day LC10 (survival)	9,500 (8,750 – 10,180)	Static renewal (every 48h), with sand	21.9 ± 0.73	7.9 ± 0.39	0.25 ± 0.081 mmol (Recon water)	0.35 ± 0.1 mEq	8.4 ± 0.12	Dussault *et al.* 2008	1
Invertebrate	*Chironomus dilutus*	midge	12 day old	No solvent	10 day EC50 (growth)	9,500 (7,910 – 11,370)	Static renewal (every 48h), with sand	21.9 ± 0.73	7.9 ± 0.39	0.25 ± 0.081 mmol (Recon water)	0.35 ± 0.1 mEq	8.4 ± 0.12	Dussault *et al.* 2008	1
Invertebrate	*Chironomus dilutus*	midge	12 day old	No solvent	10 day EC25 (growth)	4,600 (3,610 – 5,850)	Static renewal (every 48h), with sand	21.9 ± 0.73	7.9 ± 0.39	0.25 ± 0.081 mmol (Recon water)	0.35 ± 0.1 mEq	8.4 ± 0.12	Dussault *et al.* 2008	1

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Invertebrate	*Chironomus dilutus*	midge	12 day old	No solvent	10 day EC10 (growth)	2,600 (1,690 – 9,410)	Static renewal (every 48h), with sand	21.9 ± 0.73	7.9 ± 0.39	0.25 ± 0.081 mmol (Recon water)	0.35 ± 0.1 mEq	8.4 ± 0.12	Dussault *et al.* 2008	1
Invertebrate	*Chironomus dilutus*	midge	12 day old	No solvent	10 day LC50 (survival)	47,300 (43,750 – 50,910)	Static renewal (every 48h), with sand	21.9 ± 0.73	7.9 ± 0.39	0.25 ± 0.081 mmol (Recon water)	0.35 ± 0.1 mEq	8.4 ± 0.12	Dussault *et al.* 2008	1
Invertebrate	*Chironomus riparius*	midge		Ethyl acetate	28 day NOEC (life cycle, emergence)	164 (0.140 mg/kg dw)	Static						Nentwig *et al.* 2004; Oetken *et al.* 2005	U (spiked sediment assay)
Invertebrate	*Chironomus riparius*	midge		Ethyl acetate	28 day LOEC (life cycle, emergence)	332 (0.234 mg/kg dw)	Static						Nentwig *et al.* 2004; Oetken *et al.* 2005	U (spiked sediment assay)
Invertebrate	*Chironomus riparius*	midge		Ethyl acetate	28 day EC50	385 (≈0.2 mg/kd dw)	Satic	20					Dussault *et al.* 2010; Oetken *et al.* 2005	U (spiked sediment assay)
Invertebrate	*Chironomus riparius*	midge		Ethyl acetate	28 day EC10 – Series I (emergence)	0.07 mg/kg dw	Static	20					Nentwig *et al.* 2004; Oetken *et al.* 2005	U (spiked sediment assay)

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Invertebrate	*Chironomus riparius*	midge		Ethyl acetate	28 day EC10 – Series II (emergence)	0.14 mg/kg dw	Static	20					Nentwig *et al.* 2004; Oetken *et al.* 2005	U (spiked sediment assay)
Invertebrate	*Chironomus riparius*	midge		Ethyl acetate	28 day EC10 – Series III (emergence)	0.13 mg/kg dw	Static	20					Nentwig *et al.* 2004; Oetken *et al.* 2005	U (spiked sediment assay)
Invertebrate	*Chironomus riparius*	midge		Ethyl acetate	28 day EC10 – Series IV (emergence)	0.21 mg/kg dw	Static	23					Nentwig *et al.* 2004; Oetken *et al.* 2005	U (spiked sediment assay)
Invertebrate	*Hyalella azteca*	amphipod	7-14 day old	No solvent	10 day LC50 (survival)	9,900 (6,530 – 14,820)	Static renewal (every 48h), cotton gauze	22.4 ± 1.13	6.5 ± 1.33	0.13 ± 0.004 mmol (Recon, SAM)	0.11 ± 0.008 mEq	7.7 ± 0.3	Dussault *et al.* 2008	1 (>99% purity, measured concentrations reported)
Invertebrate	*Hyalella azteca*	amphipod	7-14 day old	No solvent	10 day LC25 (survival)	2,300 (1,740 – 2,980)	Static renewal (every 48h), cotton gauze	22.4 ± 1.13	6.5 ± 1.33	0.13 ± 0.004 mmol (Recon, SAM)	0.11 ± 0.008 mEq	7.7 ± 0.3	Dussault *et al.* 2008	1 (>99% purity, measured concentrations reported)
Invertebrate	*Hyalella azteca*	amphipod	7-14 day old	No solvent	10 day LC10 (survival)	600 (500 - 740)	Static renewal (every 48h), cotton gauze	22.4 ± 1.13	6.5 ± 1.33	0.13 ± 0.004 mmol (Recon, SAM)	0.11 ± 0.008 mEq	7.7 ± 0.3	Dussault *et al.* 2008	1 (>99% purity, measured concentrations reported)

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Invertebrate	*Hyalella azteca*	amphipod	7-14 day old	No solvent	10 day EC50 (survival)	15,000 (10,370 – 19,640)	Static renewal (every 48h), cotton gauze	22.4 ± 1.13	6.5 ± 1.33	0.13 ± 0.004 mmol (Recon, SAM)	0.11 ± 0.008 mEq	7.7 ± 0.3	Dussault *et al.* 2008	1 (>99% purity, measured concentrations reported)
Invertebrate	*Hyalella azteca*	amphipod	7-14 day old	No solvent	10 day EC25 (survival)	6,100 (2,840 – 9,290)	Static renewal (every 48h), cotton gauze	22.4 ± 1.13	6.5 ± 1.33	0.13 ± 0.004 mmol (Recon, SAM)	0.11 ± 0.008 mEq	7.7 ± 0.3	Dussault *et al.* 2008	1 (>99% purity, measured concentrations reported)
Invertebrate	*Hyalella azteca*	amphipod	7-14 day old	No solvent	10 day EC10 (survival)	2,400 (140 – 4,760)	Static renewal (every 48h), cotton gauze	22.4 ± 1.13	6.5 ± 1.33	0.13 ± 0.004 mmol (Recon, SAM)	0.11 ± 0.008 mEq	7.7 ± 0.3	Dussault *et al.* 2008	1 (>99% purity, measured concentrations reported)
Invertebrate	*Lumbriculus variegatus*	oligochaete		Ethyl acetate	28 day NOEC (reproduction)	10 mg/dg dw	Static						Nentwig *et al.* 2004	U (spiked sediment assay)
Invertebrate	*Potamopyrgus antipodarum*	freshwater mudsnail	Adult (shell height >3.77 mm)	Ethyl acetate	28 day LOEC (reproduction: number of embryos without shell)	>250	Static renewal (every 48 hrs)	16 ± 1					Oetken *et al.* 2005; Nentwig *et al.*, 2004	U (concentration of solvent not specified, control survival not listed, no reporting of water quality parameters)
Invertebrate	*Potamopyrgus antipodarum*	freshwater mudsnail	Adult (shell height >3.77 mm)	Ethyl acetate	28 day LOEC (reproduction: total number of embryos)	>250	Static renewal (every 48 hrs)	16 ± 1					Oetken *et al.* 2005; Nentwig *et al.* 2004	U (concentration of solvent not specified, control survival not listed, no reporting of water quality parameters)

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bacteria	Nitrifying bacteria / Nitrate production			Not available	5 hour NOEC (inhibition of nitrate production)	>10,000		25				7.4-8.4 (7.6)	Dokianakis *et al.* 2004	U (mixed culture of nitrite oxidizing bacteria isolated from activate sludge)
bacteria	Methanogenic bacteria			Not available	5 day EC20 (20% inhibition of methane production)	41,000		35				7.2-7.5 (7.35)	Fountoulakis, M. *et al.* 2004	U (temp too high; anaerobic bacteria from STP, exceeds solubility limit)
bacteria	Methanogenic bacteria			Not available	5 day EC50 (50% inhibition of methane production)	220,000		35				7.2-7.5 (7.35)	Fountoulakis, M. *et al.* 2004	U (temp too high; anaerobic bacteria from STP, exceeds solubility limit)
fish	*Cyprinus carpio*	Common carp	1.5 year old (avg wt 370g, avg lt 27 cm), female and male	0.002% DMSO	28 day unbounded LOEC liver (total) (cytopathology in the liver)	>100	Flow through		70% saturation (control water)	378.6 (German well water, control water)			Triebskorn *et al.* 2007	U (assessment of impacts to ultrastructure of cells and tissue, ecological relevance of impact not explained)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration (µg·L^{1})	Test Type	Temp (°C)	DO (mg·L^{1})	Hardness (mg·L^{1})	Alkalinity (mg·L^{1})	pH	Reference	Ranking (rationale)
fish	*Cyprinus carpio*	Common carp	1.5 year old (avg wt 370g, avg lt 27 cm), female and male	0.002% DMSO	28 day unbounded LOEC kidney (total) (trunk kidney, symptoms observed in posterior portions of the proximal tubules [PII] and in distal tubules [DI].)	1.0	Flow through		70% saturation (control water)	378.6 (German well water, control water)			Triebskorn *et al.* 2007	U (assessment of impacts to ultrastructure of cells and tissue, ecological relevance of impact not explained)
fish	*Cyprinus carpio*	Common carp	1.5 year old (avg wt 370g, avg lt 27 cm), female and male	0.002% DMSO	28 day NOEC gills (total) (epitheial lifting and hypertrophy and hyperplasia of mucus cells)	5.0	Flow through		70% saturation (control water)	378.6 (German well water, control water)			Triebskorn *et al.* 2007	U (assessment of impacts to ultrastructure of cells and tissue, ecological relevance of impact not explained)
fish	*Cyprinus carpio*	Common carp	1.5 year old (avg wt 370g, avg lt 27 cm), female and male	0.002% DMSO	28 day LOEC gills (total) (epitheial lifting and hypertrophy and hyperplasia of mucus cells)	(20)* (no clear dose-effect response relationship)	Flow through		70% saturation (control water)	378.6 (German well water, control water)			Triebskorn *et al.* 2007	U (assessment of impacts to ultrastructure of cells and tissue, ecological relevance of impact not explained)
fish	*Cyprinus carpio*	Common carp	average length 7.5 cm, average weight 15.0 g	0.01% DMSO	Activity of enzymological parameter GOT (glutamate oxaloacetate transaminase) in the gill	No change measured at day 7, 14 and 35 when exposed to 5,970 ug/L	Static	27.4± 1.2	6.4± 0.04	18.4± 0.5	18.6 ±8.0	7.2 ± 0.09	Malarvizhi *et al.*, 2012	U (molecular markers are not considered for guideline derivaiton unless they are directly linked to survival, growth or reproduction)
fish	*Cyprinus carpio*	Common carp	average length 7.5 cm, average weight 15.0 g	0.01% DMSO	Activity of enzymological parameter GOT (glutamate oxaloacetate transaminase) in the gill	Significant decrease measured at day 21 and 28 when exposed to 5,970 ug/L	Static	27.4± 1.2	6.4± 0.04	18.4± 0.5	18.6 ±8.0	7.2 ± 0.09	Malarvizhi *et al.*, 2012	U (molecular markers are not considered for guideline derivaiton unless they are directly linked to survival, growth or reproduction)

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fish	*Cyprinus carpio*	Common carp	average length 7.5 cm, average weight 15.0 g	0.01% DMSO	Activity of enzymological parameter GOT (glutamate oxaloacetate transaminase) in the liver	Significant decrease measured at day 7, 14, 21, 28 and 35 when exposed to 5,970 ug/L	Static	27.4± 1.2	6.4± 0.04	18.4± 0.5	18.6 ±8.0	7.2 ± 0.09	Malarvizhi *et al.*, 2012	U (molecular markers are not considered for guideline derivaiton unless they are directly linked to survival, growth or reproduction)
fish	*Cyprinus carpio*	Common carp	average length 7.5 cm, average weight 15.0 g	0.01% DMSO	Activity of enzymological parameter GOT (glutamate oxaloacetate transaminase) in the muscle	Significant decrease measured at day 7, 14, 21, 28 and 35 when exposed to 5,970 ug/L	Static	27.4± 1.2	6.4± 0.04	18.4± 0.5	18.6 ±8.0	7.2 ± 0.09	Malarvizhi *et al.*, 2012	U (molecular markers are not considered for guideline derivaiton unless they are directly linked to survival, growth or reproduction)
fish	*Cyprinus carpio*	Common carp	average length 7.5 cm, average weight 15.0 g	0.01% DMSO	Activity of enzymological parameter GPT (glutamate pyruvate transaminase) in the gill	No significant difference for any sampling day when exposed to 5,970 ug/L for 35 days	Static	27.4± 1.2	6.4± 0.04	18.4± 0.5	18.6 ±8.0	7.2 ± 0.09	Malarvizhi *et al.*, 2012	U (molecular markers are not considered for guideline derivaiton unless they are directly linked to survival, growth or reproduction)
fish	*Cyprinus carpio*	Common carp	average length 7.5 cm, average weight 15.0 g	0.01% DMSO	Activity of enzymological parameter GPT (glutamate pyruvate transaminase) in the liver	Significant increase measured at day 14, 21, 28 when exposed to 5,970 ug/L	Static	27.4± 1.2	6.4± 0.04	18.4± 0.5	18.6 ±8.0	7.2 ± 0.09	Malarvizhi *et al.*, 2012	U (molecular markers are not considered for guideline derivaiton unless they are directly linked to survival, growth or reproduction)
fish	*Cyprinus carpio*	Common carp	average length 7.5 cm, average weight 15.0 g	0.01% DMSO	Activity of enzymological parameter GPT (glutamate pyruvate transaminase) in the liver	No change measured at day 7 and 35 when exposed to 5,970 ug/L	Static	27.4± 1.2	6.4± 0.04	18.4± 0.5	18.6 ±8.0	7.2 ± 0.09	Malarvizhi *et al.*, 2012	U (molecular markers are not considered for guideline derivaiton unless they are directly linked to survival, growth or reproduction)

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fish	*Cyprinus carpio*	Common carp	average length 7.5 cm, average weight 15.0 g	0.01% DMSO	Activity of enzymological parameter GPT (glutamate pyruvate transaminase) in the muscle	Significant increase measured at day 14, 21, 28 and 35 when exposed to 5,970 ug/L	Static	27.4± 1.2	6.4± 0.04	18.4± 0.5	18.6 ±8.0	7.2 ± 0.09	Malarvizhi *et al.*, 2012	U (molecular markers are not considered for guideline derivaiton unless they are directly linked to survival, growth or reproduction)
fish	*Cyprinus carpio*	Common carp	average length 7.5 cm, average weight 15.0 g	0.01% DMSO	Activity of enzymological parameter GPT (glutamate pyruvate transaminase) in the muscle	No change measured at day 7 when exposed to 5,970 ug/L	Static	27.4± 1.2	6.4± 0.04	18.4± 0.5	18.6 ±8.0	7.2 ± 0.09	Malarvizhi *et al.*, 2012	U (molecular markers are not considered for guideline derivaiton unless they are directly linked to survival, growth or reproduction)
fish	*Cyprinus carpio*	Common carp	average length 7.5 cm, average weight 15.0 g	0.01% DMSO	Activity of enzymological parameter LDH (lactate dehydrogenase) in the gill	Significant increase measured at day 7 and 14 when exposed to 5,970 ug/L	Static	27.4± 1.2	6.4± 0.04	18.4± 0.5	18.6 ±8.0	7.2 ± 0.09	Malarvizhi *et al.*, 2012	U (molecular markers are not considered for guideline derivaiton unless they are directly linked to survival, growth or reproduction)
fish	*Cyprinus carpio*	Common carp	average length 7.5 cm, average weight 15.0 g	0.01% DMSO	Activity of enzymological parameter LDH (lactate dehydrogenase) in the gill	Significant decrease measured at day 21, 28, 35 when exposed to 5,970 ug/L	Static	27.4± 1.2	6.4± 0.04	18.4± 0.5	18.6 ±8.0	7.2 ± 0.09	Malarvizhi *et al.*, 2012	U (molecular markers are not considered for guideline derivaiton unless they are directly linked to survival, growth or reproduction)
fish	*Cyprinus carpio*	Common carp	average length 7.5 cm, average weight 15.0 g	0.01% DMSO	Activity of enzymological parameter LDH (lactate dehydrogenase) in the liver	Significant increase measured at day 7, 14, 21, 28 when exposed to 5,970 ug/L	Static	27.4± 1.2	6.4± 0.04	18.4± 0.5	18.6 ±8.0	7.2 ± 0.09	Malarvizhi *et al.*, 2012	U (molecular markers are not considered for guideline derivaiton unless they are directly linked to survival, growth or reproduction)
fish	*Cyprinus carpio*	Common carp	average length 7.5 cm, average weight 15.0 g	0.01% DMSO	Activity of enzymological parameter LDH (lactate dehydrogenase) in the liver	Significant decrease measured at day 35 when exposed to 5,970 ug/L	Static	27.4± 1.2	6.4± 0.04	18.4± 0.5	18.6 ±8.0	7.2 ± 0.09	Malarvizhi *et al.*, 2012	U (molecular markers are not considered for guideline derivaiton unless they are directly linked to survival, growth or reproduction)

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fish	*Cyprinus carpio*	Common carp	average length 7.5 cm, average weight 15.0 g	0.01% DMSO	Activity of enzymological parameter LDH (lactate dehydrogenase) in the muscle	Significant increase measured at day 7, 14, 21, 28 when exposed to 5,970 ug/L	Static	27.4± 1.2	6.4± 0.04	18.4± 0.5	18.6 ±8.0	7.2 ± 0.09	Malarvizhi *et al.*, 2012	U (molecular markers are not considered for guideline derivaiton unless they are directly linked to survival, growth or reproduction)
fish	*Cyprinus carpio*	Common carp	average length 7.5 cm, average weight 15.0 g	0.01% DMSO	Activity of enzymological parameter LDH (lactate dehydrogenase) in the muscle	Significant decrease measured at day 35 when exposed to 5,970 ug/L	Static	27.4± 1.2	6.4± 0.04	18.4± 0.5	18.6 ±8.0	7.2 ± 0.09	Malarvizhi *et al.*, 2012	U (molecular markers are not considered for guideline derivaiton unless they are directly linked to survival, growth or reproduction)
fish	*Danio rerio*	Zebrafish	Embryos, 3 hours post fertilization	No solvent added	10 day NOEC (fish embryo and larvae mortality)	25,000	Flow-through	26±1		100		7.5-8.5	Ferrari *et al.* 2003; Ferrari *et al.* 2004	2 (nominal concentration)
fish	*Danio rerio*	Zebrafish	Embryos, 3 hours post fertilization	No solvent added	10 day LOEC (fish embryo and larvae mortality)	50,000	Flow-through	26±1		100		7.5-8.5	Ferrari *et al.* 2003; Ferrari *et al.* 2004	2
fish	*Danio rerio*	Zebrafish	Adult (females : 431-445 mg; males : 514-518 mg)	0.004% DMSO	6 week LOEC (reduced embryo production in females)	0.5	Recirculating, 90% renewal every 3 days	28-29	□87%	Distilled water + NaHCO3 + sea salts (instant ocean) [final conductivity of 455–470 uS/cm]		7.0-7.7	Galus *et al.*, 2013a	U (conducted at environmentally relevant concentrations, but only two exposure levels were tested – low (0.5 ug/L) and high (10 ug/L), exposures were conducted using sea salts (Instant Ocean) which have been shown to contain trace contaminants (Atkinson and Bingman 1997))

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration (µg·L^{1})	Test Type	Temp (°C)	DO (mg·L^{1})	Hardness (mg·L^{1})	Alkalinity (mg·L^{1})	pH	Reference	Ranking (rationale)
fish	*Danio rerio*	Zebrafish	Adult (females : 431-445 mg; males : 514-518 mg)	0.004% DMSO	6 week LOEC (atretic oocytes [indicating elevated apoptosis]and significantly altered ovarian histology)	0.5	Recirculating, 90% renewal every 3 days	28-29	□87%	Distilled water + NaHCO3 + sea salts (instant ocean) [final conductivity of 455–470 uS/cm]		7.0-7.7	Galus *et al.*, 2013a	U (conducted at environmentally relevant concentrations, but only two exposure levels were tested – low (0.5 ug/L) and high (10 ug/L), exposures were conducted using sea salts (Instant Ocean) which have been shown to contain trace contaminants (Atkinson and Bingman 1997))
fish	*Danio rerio*	Zebrafish	Adult (females : 431-445 mg; males : 514-518 mg)	0.004% DMSO	6 week LOEC (fertilization rate of embryos)	>10	Recirculating, 90% renewal every 3 days	28-29	□87%	Distilled water + NaHCO3 + sea salts (instant ocean) [final conductivity of 455–470 uS/cm]		7.0-7.7	Galus *et al.*, 2013a	U (conducted at environmentally relevant concentrations, but only two exposure levels were tested – low (0.5 ug/L) and high (10 ug/L), exposures were conducted using sea salts (Instant Ocean) which have been shown to contain trace contaminants (Atkinson and Bingman 1997))
fish	*Danio rerio*	Zebrafish	Adult (females : 431-445 mg; males : 514-518 mg)	0.004% DMSO	6 week LOEC (plasma estradiol concentrations in both male and female fish)	>10	Recirculating, 90% renewal every 3 days	28-29	□87%	Distilled water + NaHCO3 + sea salts (instant ocean) [final conductivity of 455–470 uS/cm]		7.0-7.7	Galus *et al.*, 2013a	U (conducted at environmentally relevant concentrations, but only two exposure levels were tested – low (0.5 ug/L) and high (10 ug/L), exposures were conducted using sea salts (Instant Ocean) which have been shown to contain trace contaminants (Atkinson and Bingman 1997))

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fish	*Danio rerio*	Zebrafish	Adult (females : 431-445 mg; males : 514-518 mg)	0.004% DMSO	6 week LOEC (decreased 11-ketotestosterone in both male and female fish)	0.5*	Recirculating, 90% renewal every 3 days	28-29	87%	Distilled water + NaHCO3 + sea salts (instant ocean) [final conductivity of 455–470 uS/cm]		7.0-7.7	Galus *et al.*, 2013a	U (conducted at environmentally relevant concentrations, but only two exposure levels were tested – low (0.5 ug/L) and high (10 ug/L), exposures were conducted using sea salts (Instant Ocean) which have been shown to contain trace contaminants (Atkinson and Bingman 1997))
fish	*Danio rerio*	Zebrafish	Adult (female wt and lt = 1.39±0.16 g and 4.69±0.21 cm; male wt and lt = 0.89±0.14 g and 4.37±0.18 cm)	Ethanol control 0.01% (v/v)	21 day LOEC (Change in Gonadosomatic index (GSI) for female and male fish)	>1,780	Semi-static (100% renewal daily)	27±1		Dechlorinated carbon filtered tap water			Madureira *et al.*, 2011	U (only one concentration tested, nominal concentration is 10,000 times the maximum found in the reference system, Douro River estuary)
fish	*Danio rerio*	Zebrafish	Adult (female wt and lt = 1.39±0.16 g and 4.69±0.21 cm; male wt and lt = 0.89±0.14 g and 4.37±0.18 cm)	Ethanol control 0.01% (v/v)	21 day effect conc. (significant decrease in spermatocyte percentage in male fish compared to control)	1,780	Semi-static (100% renewal daily)	27±1		Dechlorinated carbon filtered tap water			Madureira *et al.*, 2011	U (only one concentration tested, nominal concentration is 10,000 times the maximum found in the reference system, Douro River estuary)
fish	*Danio rerio*	Zebrafish	F0 Adult (8-9 months of age, reproductively mature)	final DMSO conc in exposure tanks 0.004%	4 week LOEC (significant decline in fecundity, e.g. total embryos produced)	10	Recirculating (90% renewal every 3 days, for duration of exposure)	28-29		Medium made using distilled water with 12 mg/L sodium bicarbonate and 60 mg/L sea salts (Instant Ocean)			Galus *et al.* 2014	U (only one concentraiton tested, nominal concentrations were not verified [relied on identical method used in Galus *et al.* 2013a], exposures were conducted using sea salts (Instant Ocean) which have been shown to contain trace contaminants (Atkinson and Bingman 1997))

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fish	*Danio rerio*	Zebrafish	F1 control males and F1 CBZ exposed females [from F0 10 ug/L CBZ exposed parents] (8-9 months of age)		1.5 hour LOEC (decrease in % breeding success [fewer clutches] compared to controls, decreased fecundity)	>10 (F0 exposed concentration)	Semi-recirculating system (≥10% daily renewal)	28.5	≥87%	Medium made using distilled water with 12 mg/L sodium bicarbonate and 60 mg/L sea salts (Instant Ocean)		7-8	Galus *et al.* 2014	U (only one concentraiton tested, nominal concentrations were not verified [relied on identical method used in Galus *et al.* 2013a], exposures were conducted using sea salts (Instant Ocean) which have been shown to contain trace contaminants (Atkinson and Bingman 1997))
fish	*Danio rerio*	Zebrafish	F1 CBZ exposed males [from F0 10 ug/L CBZ exposed parents] and F1 control females (8-9 months of age)		1.5 hour LOEC (decrease in % breeding success [fewer clutches] compared to controls, decreased fecundity)	10 (F0 exposed concentration)	Semi-recirculating system (≥10% daily renewal)	28.5	≥87%	Medium made using distilled water with 12 mg/L sodium bicarbonate and 60 mg/L sea salts (Instant Ocean)		7-8	Galus *et al.* 2014	U (only one concentraiton tested, nominal concentrations were not verified [relied on identical method used in Galus *et al.* 2013a], exposures were conducted using sea salts (Instant Ocean) which have been shown to contain trace contaminants (Atkinson and Bingman 1997))
fish	*Danio rerio*	Zebrafish	F1 CBZ exposed males [from F0 10 ug/L CBZ exposed parents] and F1 CBZ exposed females [from F0 10 ug/L CBZ exposed parents] (8-9 months of age)		1.5 hour LOEC (decrease in % breeding success [fewer clutches] compared to controls, decreased fecundity)	10 (F0 exposed concentration)	Semi-recirculating system (≥10% daily renewal)	28.5	≥87%	Medium made using distilled water with 12 mg/L sodium bicarbonate and 60 mg/L sea salts (Instant Ocean)		7-8	Galus *et al.* 2014	U (only one concentraiton tested, nominal concentrations were not verified [relied on identical method used in Galus *et al.* 2013a], exposures were conducted using sea salts (Instant Ocean) which have been shown to contain trace contaminants (Atkinson and Bingman 1997))

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fish	*Danio rerio*	Zebrafish	F1 control males and F1 CBZ exposed females [from F0 10 ug/L CBZ exposed parents] (8-9 months of age)		1.5 hour LOEC (decrease in clutch size [number of embryos produced in a single breeding event] compared to controls)	>10 (F0 exposed concentration)	Semi-recirculating system (≥10% daily renewal)	28.5	≥87%	Medium made using distilled water with 12 mg/L sodium bicarbonate and 60 mg/L sea salts (Instant Ocean)		7-8	Galus *et al.* 2014	U (only one concentraiton tested, nominal concentrations were not verified [relied on identical method used in Galus *et al.* 2013a], exposures were conducted using sea salts (Instant Ocean) which have been shown to contain trace contaminants (Atkinson and Bingman 1997))
fish	*Danio rerio*	Zebrafish	F1 CBZ exposed males [from F0 10 ug/L CBZ exposed parents] and F1 control females (8-9 months of age)		1.5 hour LOEC (decrease in clutch size [number of embryos produced in a single breeding event] compared to controls)	>10 (F0 exposed concentration)	Semi-recirculating system (≥10% daily renewal)	28.5	≥87%	Medium made using distilled water with 12 mg/L sodium bicarbonate and 60 mg/L sea salts (Instant Ocean)		7-8	Galus *et al.* 2014	U (only one concentraiton tested, nominal concentrations were not verified [relied on identical method used in Galus *et al.* 2013a], exposures were conducted using sea salts (Instant Ocean) which have been shown to contain trace contaminants (Atkinson and Bingman 1997))
fish	*Danio rerio*	Zebrafish	F1 CBZ exposed males [from F0 10 ug/L CBZ exposed parents] and F1 CBZ exposed females [from F0 10 ug/L CBZ exposed parents] (8-9 months of age)		1.5 hour LOEC (decrease in clutch size [number of embryos produced in a single breeding event] compared to controls)	>10 (F0 exposed concentration)	Semi-recirculating system (≥10% daily renewal)	28.5	≥87%	Medium made using distilled water with 12 mg/L sodium bicarbonate and 60 mg/L sea salts (Instant Ocean)		7-8	Galus *et al.* 2014	U (only one concentraiton tested, nominal concentrations were not verified [relied on identical method used in Galus *et al.* 2013a], exposures were conducted using sea salts (Instant Ocean) which have been shown to contain trace contaminants (Atkinson and Bingman 1997))

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fish	*Danio rerio*	Zebrafish	F1 control males and F1 CBZ exposed females [from F0 10 ug/L CBZ exposed parents] (8-9 months of age)		10 minute LOEC (duration of male courtship behaviour - approach, leading, lateral, nudge, quiver)	>10 (F0 exposed concentration)	Semi-recirculating system (≥10% daily renewal)	28.5	≥87%	Medium made using distilled water with 12 mg/L sodium bicarbonate and 60 mg/L sea salts (Instant Ocean)		7-8	Galus *et al.* 2014	U (only one concentraiton tested, nominal concentrations were not verified [relied on identical method used in Galus *et al.* 2013a], exposures were conducted using sea salts (Instant Ocean) which have been shown to contain trace contaminants (Atkinson and Bingman 1997))
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fish	*Danio rerio*	Zebrafish	F1 control males and F1 CBZ exposed females [from F0 10 ug/L CBZ exposed parents] (8-9 months of age)		10 minute LOEC (frequency of male courtship behaviour - decrease in lateral display)	>10 (F0 exposed concentration)	Semi-recirculating system (≥10% daily renewal)	28.5	≥87%	Medium made using distilled water with 12 mg/L sodium bicarbonate and 60 mg/L sea salts (Instant Ocean)		7-8	Galus *et al.* 2014	U (only one concentraiton tested, nominal concentrations were not verified [relied on identical method used in Galus *et al.* 2013a], exposures were conducted using sea salts (Instant Ocean) which have been shown to contain trace contaminants (Atkinson and Bingman 1997))
fish	*Danio rerio*	Zebrafish	F1 CBZ exposed males [from F0 10 ug/L CBZ exposed parents] and F1 control females (8-9 months of age)		10 minute LOEC (frequency of male courtship behaviour - decrease in lateral display)	>10 (F0 exposed concentration)	Semi-recirculating system (≥10% daily renewal)	28.5	≥87%	Medium made using distilled water with 12 mg/L sodium bicarbonate and 60 mg/L sea salts (Instant Ocean)		7-8	Galus *et al.* 2014	U (only one concentraiton tested, nominal concentrations were not verified [relied on identical method used in Galus *et al.* 2013a], exposures were conducted using sea salts (Instant Ocean) which have been shown to contain trace contaminants (Atkinson and Bingman 1997))
fish	*Danio rerio*	Zebrafish	F1 CBZ exposed males [from F0 10 ug/L CBZ exposed parents] and F1 CBZ exposed females [from F0 10 ug/L CBZ exposed parents] (8-9 months of age)		10 minute LOEC (frequency of male courtship behaviour - decrease in lateral display)	10 (F0 exposed concentration)	Semi-recirculating system (≥10% daily renewal)	28.5	≥87%	Medium made using distilled water with 12 mg/L sodium bicarbonate and 60 mg/L sea salts (Instant Ocean)		7-8	Galus *et al.* 2014	U (only one concentraiton tested, nominal concentrations were not verified [relied on identical method used in Galus *et al.* 2013a], exposures were conducted using sea salts (Instant Ocean) which have been shown to contain trace contaminants (Atkinson and Bingman 1997))

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fish	*Danio rerio*	Zebrafish	F1 control males and F1 CBZ exposed females [from F0 10 ug/L CBZ exposed parents] (8-9 months of age)		10 minute LOEC (frequency of male courtship behaviour - increase in nudge rates)	>10 (F0 exposed concentration)	Semi-recirculating system (≥10% daily renewal)	28.5	≥87%	Medium made using distilled water with 12 mg/L sodium bicarbonate and 60 mg/L sea salts (Instant Ocean)		7-8	Galus *et al.* 2014	U (only one concentraiton tested, nominal concentrations were not verified [relied on identical method used in Galus *et al.* 2013a], exposures were conducted using sea salts (Instant Ocean) which have been shown to contain trace contaminants (Atkinson and Bingman 1997))
fish	*Danio rerio*	Zebrafish	F1 CBZ exposed males [from F0 10 ug/L CBZ exposed parents] and F1 control females (8-9 months of age)		10 minute LOEC (frequency of male courtship behaviour - increase in nudge rates)	>10 (F0 exposed concentration)	Semi-recirculating system (≥10% daily renewal)	28.5	≥87%	Medium made using distilled water with 12 mg/L sodium bicarbonate and 60 mg/L sea salts (Instant Ocean)		7-8	Galus *et al.* 2014	U (only one concentraiton tested, nominal concentrations were not verified [relied on identical method used in Galus *et al.* 2013a], exposures were conducted using sea salts (Instant Ocean) which have been shown to contain trace contaminants (Atkinson and Bingman 1997))
fish	*Danio rerio*	Zebrafish	F1 CBZ exposed males [from F0 10 ug/L CBZ exposed parents] and F1 CBZ exposed females [from F0 10 ug/L CBZ exposed parents] (8-9 months of age)		10 minute LOEC (frequency of male courtship behaviour - increase in nudge rates)	10 (F0 exposed concentration)	Semi-recirculating system (≥10% daily renewal)	28.5	≥87%	Medium made using distilled water with 12 mg/L sodium bicarbonate and 60 mg/L sea salts (Instant Ocean)		7-8	Galus *et al.* 2014	U (only one concentraiton tested, nominal concentrations were not verified [relied on identical method used in Galus *et al.* 2013a], exposures were conducted using sea salts (Instant Ocean) which have been shown to contain trace contaminants (Atkinson and Bingman 1997))

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fish	*Danio rerio*	Zebrafish	F1 CBZ exposed males [from F0 10 ug/L CBZ exposed parents] (8-9 months of age)		LOEC - Change in sperm velocity (increase) at 20 seconds post activation (time after activating spermatozoa with tank water)	10 (F0 exposed concentration)	Semi-recirculating system (≥10% daily renewal)	28.5	≥87%	Medium made using distilled water with 12 mg/L sodium bicarbonate and 60 mg/L sea salts (Instant Ocean)		7-8	Galus *et al.* 2014	U (only one concentraiton tested, nominal concentrations were not verified [relied on identical method used in Galus *et al.* 2013a], exposures were conducted using sea salts (Instant Ocean) which have been shown to contain trace contaminants (Atkinson and Bingman 1997))
fish	*Danio rerio*	Zebrafish	F1 CBZ exposed males [from F0 10 ug/L CBZ exposed parents] (8-9 months of age)		LOEC - Change in sperm velocity (increase) at 30 seconds post activation (time after activating spermatozoa with tank water)	10 (F0 exposed concentration)	Semi-recirculating system (≥10% daily renewal)	28.5	≥87%	Medium made using distilled water with 12 mg/L sodium bicarbonate and 60 mg/L sea salts (Instant Ocean)		7-8	Galus *et al.* 2014	U (only one concentraiton tested, nominal concentrations were not verified [relied on identical method used in Galus *et al.* 2013a], exposures were conducted using sea salts (Instant Ocean) which have been shown to contain trace contaminants (Atkinson and Bingman 1997))
fish	*Danio rerio*	Zebrafish	F1 CBZ exposed males [from F0 10 ug/L CBZ exposed parents] (8-9 months of age)		LOEC - Change in sperm velocity at 40 seconds post activation (time after activating spermatozoa with tank water)	>10 (F0 exposed concentration)	Semi-recirculating system (≥10% daily renewal)	28.5	≥87%	Medium made using distilled water with 12 mg/L sodium bicarbonate and 60 mg/L sea salts (Instant Ocean)		7-8	Galus *et al.* 2014	U (only one concentraiton tested, nominal concentrations were not verified [relied on identical method used in Galus *et al.* 2013a], exposures were conducted using sea salts (Instant Ocean) which have been shown to contain trace contaminants (Atkinson and Bingman 1997))

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fish	*Danio rerio*	Zebrafish	F1 CBZ exposed males [from F0 10 ug/L CBZ exposed parents] (8-9 months of age)		LOEC - Change in sperm velocity at 50 seconds post activation (time after activating spermatozoa with tank water)	>10 (F0 exposed concentration)	Semi-recirculating system (≥10% daily renewal)	28.5	≥87%	Medium made using distilled water with 12 mg/L sodium bicarbonate and 60 mg/L sea salts (Instant Ocean)		7-8	Galus *et al.* 2014	U (only one concentraiton tested, nominal concentrations were not verified [relied on identical method used in Galus *et al.* 2013a], exposures were conducted using sea salts (Instant Ocean) which have been shown to contain trace contaminants (Atkinson and Bingman 1997))
fish	*Danio rerio*	Zebrafish	F1 CBZ exposed males [from F0 10 ug/L CBZ exposed parents] and F1 CBZ exposed females [from F0 10 ug/L CBZ exposed parents] (8-9 months of age)		LOEC histopathological damage (kidneys, livers, reproductive organs)	>10 (F0 exposed concentration)	Semi-recirculating system (≥10% daily renewal)	28.5	≥87%	Medium made using distilled water with 12 mg/L sodium bicarbonate and 60 mg/L sea salts (Instant Ocean)		7-8	Galus *et al.* 2014	U (only one concentraiton tested, nominal concentrations were not verified [relied on identical method used in Galus *et al.* 2013a], exposures were conducted using sea salts (Instant Ocean) which have been shown to contain trace contaminants (Atkinson and Bingman 1997))
fish	*Oncorhynchus mykiss*	Rainbow trout	10-12 months old (264.33 ± 39.97g weight), female and male	<0.05% DMSO in highest test conc.	42 day LOEC (lower condition factor compared to controls; CF = body weight(g) / fork length3 (cm) x 103) Gross morphometric response - CF	2,000 (1,780 ± 170 measured)	Static renewal (80% of solution renewed daily)	15±1 (culture aquaria)	7.5-8.0 (culture aquaria)	Freshwater (Czech Republic) (culture aquaria)		7.4±0.2	Li *et al.* 2009	2

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fish	*Oncorhynchus mykiss*	Rainbow trout	10-12 months old (264.33 ± 39.97g weight) , female and male	<0.05% DMSO in highest test conc.	42 day NOEC (lower condition factor compared to controls; CF = body weight(g) / fork length3 (cm) x 103) Gross morphometric response - CF	200 (180 ± 10 measured)	Static renewal (80% of solution renewed daily)	15±1 (culture aquaria)	7.5-8.0 (culture aquaria)	Freshwater (Czech Republic) (culture aquaria)		7.4±0.2	Li *et al.* 2009	2
fish	*Oncorhynchus mykiss*	Rainbow trout	10-12 months old (264.33 ± 39.97g weight) , female and male	<0.05% DMSO in highest test conc.	42 day LOEC (hepatosomatic index; HSI = liver weight(g) / fork weight (g) x 100) Gross morphometric response - HIS	> 2,000 (1,780 ± 170 measured)	Static renewal (80% of solution renewed daily)	15±1 (culture aquaria)	7.5-8.0 (culture aquaria)	Freshwater (Czech Republic) (culture aquaria)		7.4±0.2	Li *et al.* 2009	2
fish	*Oncorhynchus mykiss*	Rainbow trout	10-12 months old (264.33 ± 39.97g weight) , female and male	<0.05% DMSO in highest test conc.	42 day LOEC (higher lipid peroxidation levels, LPO) Antioxidant response	2,000 (1,780 ± 170 measured)	Static renewal (80% of solution renewed daily)	15±1 (culture aquaria)	7.5-8.0 (culture aquaria)	Freshwater (Czech Republic) (culture aquaria)		7.4±0.2	Li *et al.* 2009	U (endpoint not ecologically relevant - not considered for guideline derivation unless directly linked to survival)
fish	*Oncorhynchus mykiss*	Rainbow trout	10-12 months old (264.33 ± 39.97g weight) , female and male	<0.05% DMSO in highest test conc.	42 day NOEC (higher lipid peroxidation levels, LPO) Antioxidant response	200 (180 ± 10 measured)	Static renewal (80% of solution renewed daily)	15±1 (culture aquaria)	7.5-8.0 (culture aquaria)	Freshwater (Czech Republic) (culture aquaria)		7.4±0.2	Li *et al.* 2009	U (endpoint not ecologically relevant - not considered for guideline derivation unless directly linked to survival)
fish	*Oncorhynchus mykiss*	Rainbow trout	10-12 months old (264.33 ± 39.97g weight) , female and male	<0.05% DMSO in highest test conc.	42 day LOEC (higher carbonyl protein levels, CP) Antioxidant response in muscle	200 (180 ± 10 measured)	Static renewal (80% of solution renewed daily)	15±1 (culture aquaria)	7.5-8.0 (culture aquaria)	Freshwater (Czech Republic) (culture aquaria)		7.4±0.2	Li *et al.* 2009	U (endpoint not ecologically relevant - not considered for guideline derivation unless directly linked to survival)

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fish	*Oncorhynchus mykiss*	Rainbow trout	10-12 months old (264.33 ± 39.97g weight) , female and male	<0.05% DMSO in highest test conc.	42 day NOEC (higher carbonyl protein levels, CP) Antioxidant response in muscle	1.0 (0.89 ± 0.09 measured)	Static renewal (80% of solution renewed daily)	15±1 (culture aquaria)	7.5-8.0 (culture aquaria)	Freshwater (Czech Republic) (culture aquaria)		7.4±0.2	Li *et al.* 2009	U (endpoint not ecologically relevant - not considered for guideline derivation unless directly linked to survival)
fish	*Oncorhynchus mykiss*	Rainbow trout	10-12 months old (264.33 ± 39.97g weight) , female and male	<0.05% DMSO in highest test conc.	42 day LOEC (induction of superoxide dismutase activity, SOD) Antioxidant enzyme assay	200 (180 ± 10 measured)	Static renewal (80% of solution renewed daily)	15±1 (culture aquaria)	7.5-8.0 (culture aquaria)	Freshwater (Czech Republic) (culture aquaria)		7.4±0.2	Li *et al.* 2009	U (endpoint not ecologically relevant - not considered for guideline derivation unless directly linked to survival)
fish	*Oncorhynchus mykiss*	Rainbow trout	10-12 months old (264.33 ± 39.97g weight) , female and male	<0.05% DMSO in highest test conc.	42 day NOEC (induction of superoxide dismutase activity, SOD) Antioxidant enzyme assay	1.0 (0.89 ± 0.09 measured)	Static renewal (80% of solution renewed daily)	15±1 (culture aquaria)	7.5-8.0 (culture aquaria)	Freshwater (Czech Republic) (culture aquaria)		7.4±0.2	Li *et al.* 2009	U (endpoint not ecologically relevant - not considered for guideline derivation unless directly linked to survival)
fish	*Oncorhynchus mykiss*	Rainbow trout	10-12 months old (264.33 ± 39.97g weight) , female and male	<0.05% DMSO in highest test conc.	42 day LOEC (inhibition of superoxide dismutase activity, SOD) Antioxidant enzyme assay	2,000 (1,780 ± 170 measured)	Static renewal (80% of solution renewed daily)	15±1 (culture aquaria)	7.5-8.0 (culture aquaria)	Freshwater (Czech Republic) (culture aquaria)		7.4±0.2	Li *et al.* 2009	U (endpoint not ecologically relevant - not considered for guideline derivation unless directly linked to survival)
fish	*Oncorhynchus mykiss*	Rainbow trout	10-12 months old (264.33 ± 39.97g weight) , female and male	<0.05% DMSO in highest test conc.	42 day LOEC (induction of catalase activity, CAT) Antioxidant enzyme assay	200 (180 ± 10 measured)	Static renewal (80% of solution renewed daily)	15±1 (culture aquaria)	7.5-8.0 (culture aquaria)	Freshwater (Czech Republic) (culture aquaria)		7.4±0.2	Li *et al.* 2009	U (endpoint not ecologically relevant - not considered for guideline derivation unless directly linked to survival)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration (µg·L^{1})	Test Type	Temp (°C)	DO (mg·L^{1})	Hardness (mg·L^{1})	Alkalinity (mg·L^{1})	pH	Reference	Ranking (rationale)
fish	*Oncorhynchus mykiss*	Rainbow trout	10-12 months old (264.33 ± 39.97g weight) , female and male	<0.05% DMSO in highest test conc.	42 day NOEC (induction of catalase activity, CAT) Antioxidant enzyme assay	1.0 (0.89 ± 0.09 measured)	Static renewal (80% of solution renewed daily)	15±1 (culture aquaria)	7.5-8.0 (culture aquaria)	Freshwater (Czech Republic) (culture aquaria)		7.4±0.2	Li *et al.* 2009	U (endpoint not ecologically relevant - not considered for guideline derivation unless directly linked to survival)
fish	*Oncorhynchus mykiss*	Rainbow trout	10-12 months old (264.33 ± 39.97g weight) , female and male	<0.05% DMSO in highest test conc.	42 day LOEC (inhibition of catalase activity, CAT) Antioxidant enzyme assay	2,000 (1,780 ± 170 measured)	Static renewal (80% of solution renewed daily)	15±1 (culture aquaria)	7.5-8.0 (culture aquaria)	Freshwater (Czech Republic) (culture aquaria)		7.4±0.2	Li *et al.* 2009	U (endpoint not ecologically relevant - not considered for guideline derivation unless directly linked to survival)
fish	*Oncorhynchus mykiss*	Rainbow trout	10-12 months old (264.33 ± 39.97g weight) , female and male	<0.05% DMSO in highest test conc.	42 day LOEC (inhibition of glutathione reductase activity,GR) Antioxidant enzyme assay	1.0 (0.89 ± 0.09 measured) (at 200 ug/L no difference from control, at 2,000 ug/L inhibition observed – no dose response)	Static renewal (80% of solution renewed daily)	15±1 (culture aquaria)	7.5-8.0 (culture aquaria)	Freshwater (Czech Republic) (culture aquaria)		7.4±0.2	Li *et al.* 2009	U (endpoint not ecologically relevant - not considered for guideline derivation unless directly linked to survival)
fish	*Oncorhynchus mykiss*	Rainbow trout	10-12 months old (264.33 ± 39.97g weight) , female and male	<0.05% DMSO in highest test conc.	42 day LOEC (induction of glutathione peroxidase activity, GPx) Antioxidant enzyme assay	200 (180 ± 10 measured)	Static renewal (80% of solution renewed daily)	15±1 (culture aquaria)	7.5-8.0 (culture aquaria)	Freshwater (Czech Republic) (culture aquaria)		7.4±0.2	Li *et al.* 2009	U (endpoint not ecologically relevant - not considered for guideline derivation unless directly linked to survival)
fish	*Oncorhynchus mykiss*	Rainbow trout	10-12 months old (264.33 ± 39.97g weight) , female and male	<0.05% DMSO in highest test conc.	42 day NOEC (induction of glutathione peroxidase activity, GPx) Antioxidant enzyme assay	1.0 (0.89 ± 0.09 measured)	Static renewal (80% of solution renewed daily)	15±1 (culture aquaria)	7.5-8.0 (culture aquaria)	Freshwater (Czech Republic) (culture aquaria)		7.4±0.2	Li *et al.* 2009	U (endpoint not ecologically relevant - not considered for guideline derivation unless directly linked to survival)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{-1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{-1}$)	Hardness ($mg \cdot L^{-1}$)	Alkalinity ($mg \cdot L^{-1}$)	pH	Reference	Ranking (rationale)
fish	*Oncorhynchus mykiss*	Rainbow trout	10-12 months old (264.33 ± 39.97g weight) , female and male	<0.05% DMSO in highest test conc.	42 day LOEC (inhibition of glutathione peroxidase activity, GPx) Antioxidant enzyme assay	2,000 (1,780 ± 170 measured)	Static renewal (80% of solution renewed daily)	15±1 (culture aquaria)	7.5-8.0 (culture aquaria)	Freshwater (Czech Republic) (culture aquaria)		7.4±0.2	Li *et al.* 2009	U (endpoint not ecologically relevant - not considered for guideline derivation unless directly linked to survival)
fish	*Oncorhynchus mykiss*	Rainbow trout	10-12 months old (264.33 ± 39.97g weight) , female and male	<0.05% DMSO in highest test conc.	42 day LOEC (depression in muscle RNA-DNA ratio) Energy metabolic parameter; Changes in muscle RNA-DNA ratio	2,000 (1,780 ± 170 measured)	Static renewal (80% of solution renewed daily)	15±1 (culture aquaria)	7.5-8.0 (culture aquaria)	Freshwater (Czech Republic) (culture aquaria)		7.4±0.2	Li *et al.* 2009	U (endpoint not ecologically relevant - not considered for guideline derivation unless directly linked to survival)
fish	*Oncorhynchus mykiss*	Rainbow trout	10-12 months old (264.33 ± 39.97g weight) , female and male	<0.05% DMSO in highest test conc.	42 day NOEC (depression in muscle RNA-DNA ratio) Energy metabolic parameter; Changes in muscle RNA-DNA ratio	200 (180 ± 10 measured)	Static renewal (80% of solution renewed daily)	15±1 (culture aquaria)	7.5-8.0 (culture aquaria)	Freshwater (Czech Republic) (culture aquaria)		7.4±0.2	Li *et al.* 2009	U (endpoint not ecologically relevant - not considered for guideline derivation unless directly linked to survival)
fish	*Oncorhynchus mykiss*	Rainbow trout	10-12 months old (264.33 ± 39.97g weight) , female and male	<0.05% DMSO in highest test conc.	42 day LOEC (inhibition of Na+-K+-ATPase) Energy metabolic parameter	200 (180 ± 10 measured)	Static renewal (80% of solution renewed daily)	15±1 (culture aquaria)	7.5-8.0 (culture aquaria)	Freshwater (Czech Republic) (culture aquaria)		7.4±0.2	Li *et al.* 2009	U (endpoint not ecologically relevant - not considered for guideline derivation unless directly linked to survival)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{-1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{-1}$)	Hardness ($mg \cdot L^{-1}$)	Alkalinity ($mg \cdot L^{-1}$)	pH	Reference	Ranking (rationale)
fish	*Oncorhynchus mykiss*	Rainbow trout	10-12 months old (264.33 ± 39.97g weight) , female and male	<0.05% DMSO in highest test conc.	42 day NOEC (inhibition of Na+-K+-ATPase) Energy metabolic parameter	1.0 (0.89 ± 0.09 measured)	Static renewal (80% of solution renewed daily)	15±1 (culture aquaria)	7.5-8.0 (culture aquaria)	Freshwater (Czech Republic) (culture aquaria)		7.4±0.2	Li *et al.* 2009	U (endpoint not ecologically relevant - not considered for guideline derivation unless directly linked to survival)
fish	*Oryzias latipes*	Japanese medaka	Eggs, 8 hours post-fertilization (late blastula stage)	Ethanol, with triolein as the injection vehicle	EC50 (survival at hatching)	13.1±1.0 ng/egg	Static	27					Nassef *et al.* 2010	U (nanoinjection of CBZ into the embryonic yolk, to mimic maternal transfer without the need to chronically expose adult fish - no information provided on what the associated water concentration would be)
fish	*Pimephales promelas*	Fathead minnow	juvenile		18 day NOEC	<100	Static renewal (every 2 days)						Thomas and Klaper 2012	U (only one concentration tested; determination of gene-class analysis expression patterns)
fish	*Pimephales promelas*	Fathead minnow	juvenile		18 day LOEC	100	Static renewal (every 2 days)						Thomas and Klaper 2012	U (only one concentration tested; determination of gene-class analysis expression patterns)
fish	*Pimephales promelas*	Fathead minnow	eggs <48 hours old	DMF conc in test vessels ≤0.001%	28 day NOEC (survival)	862	Static (daily) renewal	22-24	7.5-8.0	110-150	100-130	8.3-8.5	Overturf *et al.* 2012	2 (Klimisch score in RIVM 2014; at daily 24 hour solution renewal mean CBZ conc was 93% of nominal, hatching success in control ≥85%, effect conc based on mean measured conc)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration (µg·L^{1})	Test Type	Temp (°C)	DO (mg·L^{1})	Hardness (mg·L^{1})	Alkalinity (mg·L^{1})	pH	Reference	Ranking (rationale)
fish	*Pimephales promelas*	Fathead minnow	eggs <48 hours old	DMF conc in test vessels ≤0.001%	28 day LOEC (survival)	>862	Static (daily) renewal	22-24	7.5-8.0	110-150	100-130	8.3-8.5	Overturf *et al.* 2012	2 (Klimisch score in RIVM 2014; at daily 24 hour solution renewal mean CBZ conc was 93% of nominal, hatching success in control ≥85%, effect conc based on mean measured conc)
fish	*Pimephales promelas*	Fathead minnow	eggs <48 hours old	DMF conc in test vessels ≤0.001%	28 day LC50	>862	Static (daily) renewal	22-24	7.5-8.0	110-150	100-130	8.3-8.5	Overturf *et al.* 2012	2 (Klimisch score in RIVM 2014; at daily 24 hour solution renewal mean CBZ conc was 93% of nominal, hatching success in control ≥85%, effect conc based on mean measured conc)
fish	*Pimephales promelas*	Fathead minnow	eggs <48 hours old	DMF conc in test vessels ≤0.001%	28 day NOEC (growth)	862	Static (daily) renewal	22-24	7.5-8.0	110-150	100-130	8.3-8.5	Overturf *et al.* 2012	2 (Klimisch score in RIVM 2014; at daily 24 hour solution renewal mean CBZ conc was 93% of nominal, hatching success in control ≥85%, effect conc based on mean measured conc)
fish	*Pimephales promelas*	Fathead minnow	eggs <48 hours old	DMF conc in test vessels ≤0.001%	28 day LOEC (survival)	>862	Static (daily) renewal	22-24	7.5-8.0	110-150	100-130	8.3-8.5	Overturf *et al.* 2012	2 (Klimisch score in RIVM 2014; at daily 24 hour solution renewal mean CBZ conc was 93% of nominal, hatching success in control ≥85%, effect conc based on mean measured conc)
fish	*Salmo salar* L.	Atlantic salmon	12 month old (160.11±13.57 mm; 40.44±10.29g)	Methonol, final conc. 0.001% (v/v)	5 day NOEC (differential transcriptome expression)	<7.85	Flow through (0.25 L/min)	12±1					Hampel *et al.* 2014	U (only one exposure concentration was used [7.85±0.13 ug/L], similar to upper level measured in hospital and STP effluent; endpoint not ecologically relevant - not considered for guideline derivation unless directly linked to survival)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{1}$)	Hardness ($mg \cdot L^{1}$)	Alkalinity ($mg \cdot L^{1}$)	pH	Reference	Ranking (rationale)
aquatic plants and algae	*Ankistrodesmus braunii*	algae		No solvent added	60 day NOEC (growth)	>18,900	Static	23					Andreozzi *et al.* 2002	U (over 50% loss of CBZ over time)
aquatic plants and algae	*Ankistrodesmus braunii*	algae		No solvent added	96 hour NOEC (growth)	>20,000	Static	23					Andreozzi *et al.* 2002	U (over 50% loss of CBZ over time)
aquatic plants and algae	*Chlorella pyrenoidosa*	green algae		<1% ethanol including solvent controls	72 hour EC50 (growth rate)	167,330	Static	24		artificial medium (HB4)			Zhang *et al.* 2012	U-(>99% purity, 3 = Klimisch criteria ranking in RIVM 2014; extrapolated effect conc., highest conc tested was 10 mg/L)
aquatic plants and algae	*Chlorella pyrenoidosa*	green algae		<1% ethanol including solvent controls	96 hour EC50 (growth rate)	49,400	Static	24		artificial medium (HB4)			Zhang *et al.* 2012	U (>99% purity, 3 = Klimisch criteria ranking in RIVM 2014; extrapolated effect conc., highest conc tested was 10 mg/L)
aquatic plants and algae	*Chlorella pyrenoidosa*	green algae		<1% ethanol including solvent controls	72 hour NOEC (growth rate)	1,000	Static	24		artificial medium (HB4)			Zhang *et al.* 2012	2 (>99% purity, Klimisch criteria ranking in RIVM 2014)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration (µg·L^{1})	Test Type	Temp (°C)	DO (mg·L^{1})	Hardness (mg·L^{1})	Alkalinity (mg·L^{1})	pH	Reference	Ranking (rationale)
aquatic plants and algae	*Chlorella pyrenoidosa*	green algae		<1% ethanol including solvent controls	96 hour NOEC (growth rate)	500	Static	24		artificial medium (HB4)			Zhang *et al.* 2012	2 (>99% purity, Klimisch criteria ranking in RIVM 2014)
aquatic plants and algae	*Chlorella pyrenoidosa*	green algae		<1% ethanol including solvent controls	72 hour NOEC (Chlorohyll a)	1,000	Static	24		artificial medium (HB4)			Zhang *et al.* 2012	2 (>99% purity, Klimisch criteria ranking in RIVM 2014)
aquatic plants and algae	*Chlorella pyrenoidosa*	green algae		<1% ethanol including solvent controls	96 hour NOEC (Chlorophyll a)	1,000	Static	24		artificial medium (HB4)			Zhang *et al.* 2012	2 (>99% purity, Klimisch criteria ranking in RIVM 2014)
aquatic plants and algae	*Chlorella vulgaris*	green algae		1% DMSO in stock solution	24 hour NOEC (abundance)	23,627 (100 uM)	Static	22		filtered water (not specified)			Jos *et al.* 2003	2 (purity of product not specified, Klimisch score from RIVM 2014)
aquatic plants and algae	*Chlorella vulgaris*	green algae		1% DMSO in stock solution	24 hour LOEC (abundance)	>23,627 but <236,270 (>100 but <1,000 uM)	Static	22		filtered water (not specified)			Jos *et al.* 2003	2 (purity of product not specified, Klimisch score from RIVM 2014)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration (µg·L^{1})	Test Type	Temp (°C)	DO (mg·L^{1})	Hardness (mg·L^{1})	Alkalinity (mg·L^{1})	pH	Reference	Ranking (rationale)
aquatic plants and algae	*Chlorella vulgaris*	green algae		1% DMSO in stock solution	48 hour NOEC (abundance)	>2,363 but <23,627 (>10 but <100 uM)	Static	22		filtered water (not specified)			Jos *et al.* 2003	2 (purity of product not specified, Klimisch score from RIVM 2014)
aquatic plants and algae	*Chlorella vulgaris*	green algae		1% DMSO in stock solution	48 hour LOEC (abundance)	23,627 (100 uM)	Static	22		filtered water (not specified)			Jos *et al.* 2003	2 (purity of product not specified, Klimisch score from RIVM 2014)
aquatic plants and algae	*Chlorella vulgaris*	Green algae		1% DMSO in stock solution	24 hour EC50 (growth)	110,929 (469.5 uM)	Static	22		filtered water (not specified)			Jos *et al.* 2003	2 (purity of product not specified, Klimisch score from RIVM 2014)
aquatic plants and algae	*Chlorella vulgaris*	Green algae		1% DMSO in stock solution	48 hour EC50 (growth)	36,622 (155 uM)	Static	22		filtered water (not specified)			Jos *et al.* 2003	2 (purity of product not specified, Klimisch score from RIVM 2014)
aquatic plants and algae	*Cyclotella meneghiniana*	diatom		No solvent added	96 hour EC50 (growth)	31,600	Static	28±1		artificial medium			Ferrari *et al.* 2004	2 (>99% purity, Klimisch score from RIVM 2014)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{-1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{-1}$)	Hardness ($mg \cdot L^{-1}$)	Alkalinity ($mg \cdot L^{-1}$)	pH	Reference	Ranking (rationale)
aquatic plants and algae	*Cyclotella meneghiniana*	diatom		No solvent added	96 hour NOEC (growth)	10,000	Static	28±1		artificial medium			Ferrari *et al.* 2004	2 (>99% purity, Klimisch score from RIVM 2014)
aquatic plants and algae	*Desmodesmus subspicatus* (formerly *Scenedesmus subspicatus)*	planktonic chlorococcale green algae		No solvent added	72 hour EC50 (inhibition of average growth rate)	74,000	Static	23±2		Medium prepared using deionized water and analytical grade chemicals			Cleuvers 2003	2 (analytical grade product, Klimisch score from RIVM 2014)
aquatic plants and algae	*Desmodesmus subspicatus* (formerly *Scenedesmus subspicatus)*	planktonic chlorococcale green algae		0.1M ethanol in stock solution	96 hour NOEC (chlorophyll concentration)	236,270							Escher *et al.* 2005	U (exceeds solubility limit)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration (µg·L^{1})	Test Type	Temp (°C)	DO (mg·L^{1})	Hardness (mg·L^{1})	Alkalinity (mg·L^{1})	pH	Reference	Ranking (rationale)
aquatic plants and algae	*Desmodesmus subspicatus* (formerly *Scenedesmus subspicatus*)	planktonic chlorococcale green algae		0.1M ethanol in stock solution	24 hour EC50 (inhibition of photosynthesis)	236,270				Medium used			Escher *et al.* 2005	U (exceeds solubility limit)
aquatic plants and algae	*Dunaliella tertiolecta*	phytoplankton		0.4% acetone in exposure medium	96 hour EC50 (cell density)	>80,000							DeLorenzo and Fleming 2008	U (exceeds solubility limit)
aquatic plants and algae	*Lemna gibba*	macrophyte		No solvent added	35 day NOEC (Frond Number Decrease)	3.31		23.05				7.9	Brain *et al.* 2004	U (mixture)
aquatic plants and algae	*Lemna gibba*	macrophyte		No solvent added	35 day LOEC (Frond Number Decrease)	33.9		23.15				8	Brain *et al.* 2004	U (mixture)
aquatic plants and algae	*Lemna gibba*	macrophyte		No solvent added	35 day NOEC (Growth decrease)	3.31		23.05				7.9	Brain *et al.* 2004	U (mixture)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{1}$)	Hardness ($mg \cdot L^{1}$)	Alkalinity ($mg \cdot L^{1}$)	pH	Reference	Ranking (rationale)
aquatic plants and algae	*Lemna gibba*	macrophyte		No solvent added	35 day LOEC (Growth decrease)	33.9		23.15				8	Brain *et al.* 2004	U (mixture)
aquatic plants and algae	*Lemna gibba*	macrophyte		No solvent added	35 day NOEC (Chlorophyll-a decrease)	179.47		23.35				7.7	Brain *et al.* 2004	U (mixture)
aquatic plants and algae	*Lemna gibba*	macrophyte		No solvent added	35 day LOEC (Chlorophyll-a decrease)	617.34		23.3				7.5	Brain *et al.* 2004	U (mixture)
aquatic plants and algae	*Lemna gibba*	macrophyte		No solvent added	35 day NOEC (Chlorophyll-b decrease)	179.47		23.35				7.7	Brain *et al.* 2004	U (mixture)
aquatic plants and algae	*Lemna gibba*	macrophyte		No solvent added	35 day LOEC (Chlorophyll-b decrease)	617.34		23.3				7.5	Brain *et al.* 2004	U (mixture)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{1}$)	Hardness ($mg \cdot L^{1}$)	Alkalinity ($mg \cdot L^{1}$)	pH	Reference	Ranking (rationale)
aquatic plants and algae	*Lemna gibba*	macrophyte		No solvent added	35 day NOEC (Carotenooids decrease)	179.47		23.35				7.7	Brain *et al.* 2004	U (mixture)
aquatic plants and algae	*Lemna gibba*	macrophyte		No solvent added	35 day LOEC (Carotenoids decrease)	617.34		23.3				7.5	Brain *et al.* 2004	U (mixture)
aquatic plants and algae	*Lemna gibba*	macrophyte		No solvent added	7 day NOEC (frond number)	>1,000		25					Brain *et al.* 2004	U (mixture)
aquatic plants and algae	*Lemna gibba*	macrophyte		No solvent added	7 day NOEC (total plant wet mass)	>1,000		25					Brain *et al.* 2004	U (mixture)
aquatic plants and algae	*Lemna gibba*	macrophyte		No solvent added	7 day NOEC (Chlorophyll a)	>1,000		25					Brain *et al.* 2004	U (mixture)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration (µg·L^{1})	Test Type	Temp (°C)	DO (mg·L^{1})	Hardness (mg·L^{1})	Alkalinity (mg·L^{1})	pH	Reference	Ranking (rationale)
aquatic plants and algae	*Lemna gibba*	macrophyte		No solvent added	7 day NOEC (Chlorophyll b)	>1,000		25					Brain *et al.* 2004	U (mixture)
aquatic plants and algae	*Lemna gibba*	macrophyte		No solvent added	7 day NOEC (carotenoids)	>1,000		25					Brain *et al.* 2004	U (mixture)
aquatic plants and algae	*Lemna minor*	duckweed	plants with two or three fronds	No solvent added	7 day EC50 (growth inhibition)	25,500	Static	25±2		Steinberg-medium with addition of 2 phosphate species			Cleuvers 2003	2 (analytical grade product, Klimisch score from RIVM 2014)
aquatic plants and algae	*Myriophyllum sibiricum*	macrophyte		No solvent added	35 day NOEC (plant length)	179.47		23.35				7.7	Brain *et al.* 2004	U (mixture)
aquatic plants and algae	*Myriophyllum sibiricum*	macrophyte		No solvent added	35 day LOEC (plant length)	617.34		23.3				7.5	Brain *et al.* 2004	U (mixture)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{1}$)	Hardness ($mg \cdot L^{1}$)	Alkalinity ($mg \cdot L^{1}$)	pH	Reference	Ranking (rationale)
aquatic plants and algae	*Myriophyllum sibiricum*	macrophyte		No solvent added	35 day NOEC (root number)	33.9		23.15				8	Brain *et al.* 2004	U (mixture)
aquatic plants and algae	*Myriophyllum sibiricum*	macrophyte		No solvent added	35 day LOEC (root number)	179.47		23.35				7.7	Brain *et al.* 2004	U (mixture)
aquatic plants and algae	*Myriophyllum sibiricum*	macrophyte		No solvent added	35 day NOEC (longest root)	179.47		23.35				7.7	Brain *et al.* 2004	U (mixture)
aquatic plants and algae	*Myriophyllum sibiricum*	macrophyte		No solvent added	35 day LOEC (longest root)	617.34		23.3				7.5	Brain *et al.* 2004	U (mixture)
aquatic plants and algae	*Myriophyllum sibiricum*	macrophyte		No solvent added	35 day NOEC (node number)	179.47		23.35				7.7	Brain *et al.* 2004	U (mixture)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{1}$)	Hardness ($mg \cdot L^{1}$)	Alkalinity ($mg \cdot L^{1}$)	pH	Reference	Ranking (rationale)
aquatic plants and algae	*Myriophyllum sibiricum*	macrophyte		No solvent added	35 day LOEC (node number)	617.34		23.3				7.5	Brain *et al.* 2004	U (mixture)
aquatic plants and algae	*Myriophyllum sibiricum*	macrophyte		No solvent added	35 day NOEC (wet mass, g)	179.47		23.35				7.7	Brain *et al.* 2004	U (mixture)
aquatic plants and algae	*Myriophyllum sibiricum*	macrophyte		No solvent added	35 day LOEC (wet mass, g)	617.34		23.3				7.5	Brain *et al.* 2004	U (mixture)
aquatic plants and algae	*Myriophyllum sibiricum*	macrophyte		No solvent added	35 day NOEC (dry mass, g)	33.9		23.15				8	Brain *et al.* 2004	U (mixture)
aquatic plants and algae	*Myriophyllum sibiricum*	macrophyte		No solvent added	35 day LOEC (dry mass, g)	179.47		23.35				7.7	Brain *et al.* 2004	U (mixture)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration (µg·L^{1})	Test Type	Temp (°C)	DO (mg·L^{1})	Hardness (mg·L^{1})	Alkalinity (mg·L^{1})	pH	Reference	Ranking (rationale)
aquatic plants and algae	*Pseudokirchneriella subcapitata*	green algae		No solvent added	96 hour NOEC (growth inhibition)	521	Static						Harada *et al.* 2008	2
aquatic plants and algae	*Pseudokirchneriella subcapitata*	Green algae		No solvent added	96 hour NOEC (growth)	>100,000	Static	23±1				7.0-8.5	Ferrari *et al.* 2003; Ferrari *et al.* 2004	2 (>99% purity, Klimisch score from RIVM 2014)
aquatic plants and algae	*Pseudokirchneriella subcapitata*	Green algae		No solvent added	96 hour LOEC (growth)	>100,000	Static	23±1				7.0-8.5	Ferrari *et al.* 2003; Ferrari *et al.* 2004	2
aquatic plants and algae	*Pseudokirchneriella subcapitata*	Green algae		No solvent added	96 hour EC50 (growth inhibition)	48,900	Static						Harada *et al.* 2008	2
aquatic plants and algae	*Selenastrum capricornutum*	algae		No solvent added	4 day NOEC (growth)	>20,000	Static			Bolds basal medium			Andreozzi *et al.* 2002	U (loss of CBZ over time)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{-1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{-1}$)	Hardness ($mg \cdot L^{-1}$)	Alkalinity ($mg \cdot L^{-1}$)	pH	Reference	Ranking (rationale)
aquatic plants and algae	*Scenedesmus obliquus*	algae		<1% ethanol including solvent controls	72 hour EC50 (growth)	89,120	Static			artificial medium (HB4)			Zhang *et al.* 2012	U (99% purity, 3 = Klimisch score from RIVM 2014, highes test conc was 10 mg/L so extrapolated effect conc.).
aquatic plants and algae	*Scenedesmus obliquus*	algae		<1% ethanol including solvent controls	96 hour EC50 (growth)	70,100	Static			artificial medium (HB4)			Zhang *et al.* 2012	U (99% purity, 3 = Klimisch score from RIVM 2014, highes test conc was 10 mg/L so extrapolated effect conc.).
aquatic plants and algae	*Synechococcus leopolensis*	Blue-green algae		No solvent added	96 hour EC50 (growth)	33,600	Static	28±1		artificial medium			Ferrari *et al.* 2004	2 (>99% purity; Klimisch classification by RIVM 2014)
aquatic plants and algae	*Synechococcus leopolensis*	Blue-green algae		No solvent added	96 hour NOEC (growth)	17,500		28±1					Ferrari *et al.* 2004	2 (>99% purity; Klimisch classification by RIVM 2014)
aquatic plants and algae	*Synechococcus leopolensis*	Blue-green algae		No solvent added	48 hour LOEC (growth)	7,070		23±1				7.6	Andreozzi *et al.* 2004	U (six pharmaceutical mixture)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{1}$)	Hardness ($mg \cdot L^{1}$)	Alkalinity ($mg \cdot L^{1}$)	pH	Reference	Ranking (rationale)
aquatic plants and algae	River biofilm	algae, blue-green algae, bacteria		No solvent added	56 day LOEC (decrease in biomass of cyanobacteria)	10				freshwater			Lawrence *et al.* 2005	U (only one concentration tested = 10 ug/L)
aquatic plants and algae	River biofilm	algae, blue-green algae, bacteria		No solvent added	56 day NOEC (no effect on thickness of biofilm)	10				freshwater			Lawrence *et al.* 2005	U (only one concentration tested = 10 ug/L)
aquatic plants and algae	River biofilm	algae, blue-green algae, bacteria		No solvent added	56 day LOEC (decrease in biomass of cyanobacteria)	10				freshwater			Lawrence *et al.* 2005	U (only one concentration tested = 10 ug/L)
aquatic plants and algae	River biofilm	algae, blue-green algae, bacteria		No solvent added	56 day LOEC (decrease in total extracellular polymeric substance)	10				freshwater			Lawrence *et al.* 2005	U (only one concentration tested = 10 ug/L)
aquatic plants and algae	River biofilm	algae, blue-green algae, bacteria		No solvent added	56 day LOEC (significant changes in the composition of the biofilm community)	10				freshwater			Lawrence *et al.* 2005	U (only one concentration tested = 10 ug/L)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{1}$)	Hardness ($mg \cdot L^{1}$)	Alkalinity ($mg \cdot L^{1}$)	pH	Reference	Ranking (rationale)
aquatic plants and algae	River biofilm	algae, blue-green algae, bacteria		No solvent added	56 day NOEC (increase in proportion of live cells; increase in bacterial counts)	10				freshwater			Lawrence *et al.* 2005	U (only one concentration tested = 10 ug/L)

Acute Saltwater Toxicity Data – Carbamazepine

No data available

Chronic Saltwater Toxicity Data – Carbamazepine

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{-1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{-1}$)	Hardness ($mg \cdot L^{-1}$)	Alkalinity ($mg \cdot L^{-1}$)	pH	Reference	Ranking (rationale)
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day NOEC - effect of CBZ on mussel haemocyte lysosomal membrane stability (destabilization time, i.e. the time at which more than 50% of the lysosomes appeared discoloured)	<0.1	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - assessed using neutral red retention assay; lysosomal membrane stability significantly reduced to about 35 min (−60%) and 18 min (−80%) after a 7-day exposure to 0.1 and 10 g/L CBZ, respectively.
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day LOEC - effect of CBZ on mussel haemocyte lysosomal membrane stability (destabilization time, i.e. the time at which more than 50% of the lysosomes appeared discoloured)	0.1	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - assessed using neutral red retention assay; lysosomal membrane stability significantly reduced to about 35 min (−60%) and 18 min (−80%) after a 7-day exposure to 0.1 and 10 g/L CBZ, respectively.
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day NOEC - accumulation of neutral lipids and lipofuscins	<0.1	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - neutral lipids were slightly but significantly increased with respect to the control animals.

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration (µg·L^{1})	Test Type	Temp (°C)	DO (mg·L^{1})	Hardness (mg·L^{1})	Alkalinity (mg·L^{1})	pH	Reference	Ranking (rationale)
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day LOEC - accumulation of neutral lipids and lipofuscins	0.1	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - neutral lipids were slightly but significantly increased with respect to the control animals.
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day NOEC - Lipid peroxidation assessed as MDA (malondialdehyde) content in digestive glands	>10	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - no effect of CBZ in digestive gland
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day NOEC - Lipid peroxidation assessed as MDA (malondialdehyde) content in gills	<0.1	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - a significant increase in MDA content was observed in gills of mussels exposed to 0.1 and 10 ug/L
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day LOEC - Lipid peroxidation assessed as MDA (malondialdehyde) content in gills	0.1	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - a significant increase in MDA content was observed in gills of mussels exposed to 0.1 and 10 ug/L
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day NOEC - Lipid peroxidation assessed as MDA (malondialdehyde) content in mantle/gonads	0.1	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - a significant increase in MDA content was observed in mantle/gonads of mussels exposed to 10 ug/L

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration (µg·L^{1})	Test Type	Temp (°C)	DO (mg·L^{1})	Hardness (mg·L^{1})	Alkalinity (mg·L^{1})	pH	Reference	Ranking (rationale)
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day LOEC - Lipid peroxidation assessed as MDA (malondialdehyde) content in mantle/gonads	10	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - a significant increase in MDA content was observed in mantle/gonads of mussels exposed to 10 ug/L
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day LOEC - Glutathione S-transferase activity in digestive glands	0.1*	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - *significant increase compared to control at 0.1 ug/L CBZ, but no significant difference from control at 10 ug/L CBZ
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day NOEC - Glutathione S-transferase activity in gills	>10	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - No effect was observed in gills
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day NOEC - Glutathione S-transferase activity in mantle/gonads	<0.1	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - significant increase at both 0.1 and 10 ug/L CBZ
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day LOEC - Glutathione S-transferase activity in mantle/gonads	0.1	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - significant increase at both 0.1 and 10 ug/L CBZ

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration (µg·L^{1})	Test Type	Temp (°C)	DO (mg·L^{1})	Hardness (mg·L^{1})	Alkalinity (mg·L^{1})	pH	Reference	Ranking (rationale)
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day NOEC - Catalase activity in digestive glands	<0.1	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - CAT activity was significantly increased in digestive gland after exposure to 0.1 and 10 g/L CBZ.
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day LOEC - Catalase activity in digestive glands	0.1	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - CAT activity was significantly increased in digestive gland after exposure to 0.1 and 10 g/L CBZ.
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day NOEC - Catalase activity in gills	>10	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - No effectwas observed in gills.
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day NOEC - Catalase activity in mantle/gonads	0.1	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - CAT activity was significantly increased in mantle/gonads but only at 10 ug/L CBZ.
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day LOEC - Catalase activity in mantle/gonads	10	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - CAT activity was significantly increased in mantle/gonads but only at 10 ug/L CBZ.

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration (µg·L^{1})	Test Type	Temp (°C)	DO (mg·L^{1})	Hardness (mg·L^{1})	Alkalinity (mg·L^{1})	pH	Reference	Ranking (rationale)
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day NOEC - Levels of tail DNA damage in haemocytes	>10	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - No differences in levels of primary DNA damage were noted in haemocytes from mussels exposed for 7 days to CBZ as evaluated by the alkaline Comet assay and expressed as percentage of DNA in the tail
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day NOEC - Content of cAMP in digestive glands	0.1	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - Significant reductions were observed in cAMP content in digestive gland at 10 g/L CBZ.
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day LOEC - Content of cAMP in digestive glands	10	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - Significant reductions were observed in cAMP content in digestive gland at 10 g/L CBZ.
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day NOEC - Content of cAMP in gills	0.1	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - Significant reductions were observed in cAMP content in gills at 10 g/L CBZ.

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration (µg·L^{1})	Test Type	Temp (°C)	DO (mg·L^{1})	Hardness (mg·L^{1})	Alkalinity (mg·L^{1})	pH	Reference	Ranking (rationale)
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day LOEC - Content of cAMP in gills	10	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - Significant reductions were observed in cAMP content in gills at 10 g/L CBZ.
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day NOEC - Content of cAMP in mantle/gonads	<0.1	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - Significant reductions were observed in cAMP content in mantle/gonads at 0.1 and 10 ug/L CBZ.
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day LOEC - Content of cAMP in mantle/gonads	0.1	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - Significant reductions were observed in cAMP content in mantle/gonads at 0.1 and 10 ug/L CBZ.
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day NOEC - Activity of PKA in digestive glands	0.1	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - Significant activity reductions were observed in digestive gland at 10 g/L CBZ.
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day LOEC - Activity of PKA in digestive glands	10	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - Significant activity reductions were observed in digestive gland at 10 g/L CBZ.

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration (µg·L^{1})	Test Type	Temp (°C)	DO (mg·L^{1})	Hardness (mg·L^{1})	Alkalinity (mg·L^{1})	pH	Reference	Ranking (rationale)
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day NOEC - Activity of PKA in gills	0.1	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - Significant activity reductions were observed in gills at 10 g/L CBZ.
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day LOEC - Activity of PKA in gills	10	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - Significant activity reductions were observed in gills at 10 g/L CBZ.
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day NOEC - Activity of PKA in mantle/gonads	<0.1	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - Significant activity reductions were observed in mantle/gonads at 0.1 and 10 ug/L CBZ.
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day LOEC - Activity of PKA in mantle/gonads	0.1	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - Significant activity reductions were observed in mantle/gonads at 0.1 and 10 ug/L CBZ.
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day NOEC - Transcript levels of MgPgp in digestive glands	0.1	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - CBZ at 10 ug/L induced a significant reduction in MgPgp transcript levels in digestive gland

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration (µg·L^{1})	Test Type	Temp (°C)	DO (mg·L^{1})	Hardness (mg·L^{1})	Alkalinity (mg·L^{1})	pH	Reference	Ranking (rationale)
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day LOEC - Transcript levels of MgPgp in digestive glands	10	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - CBZ at 10 ug/L induced a significant reduction in MgPgp transcript levels in digestive gland
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day NOEC - Transcript levels of MgPgp in gills	0.1	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - CBZ at 10 ug/L induced a significant reduction in MgPgp transcript levels in gills
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day LOEC - Transcript levels of MgPgp in gills	10	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - CBZ at 10 ug/L induced a significant reduction in MgPgp transcript levels in gills
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day NOEC - Transcript levels of MgPgp in mantle/gonads	<0.1	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - significant reduction in MgPgp transcript levels in mantle/gonads at 0.1 and 10 ug/L CBZ
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day LOEC - Transcript levels of MgPgp in mantle/gonads	0.1	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - significant reduction in MgPgp transcript levels in mantle/gonads at 0.1 and 10 ug/L CBZ

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration (µg·L^{1})	Test Type	Temp (°C)	DO (mg·L^{1})	Hardness (mg·L^{1})	Alkalinity (mg·L^{1})	pH	Reference	Ranking (rationale)
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day NOEC - Transcript levels of MgMrp2 in digestive glands	<0.1	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - CBZ significantly reduced the mRNA expression of MgMrp2 at both 0.1 and 10 ug/L
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day LOEC - Transcript levels of MgMrp2 in digestive glands	0.1	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - CBZ significantly reduced the mRNA expression of MgMrp2 at both 0.1 and 10 ug/L
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day NOEC - Transcript levels of MgMrp2 in gills	0.1	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - only the highest CBZ concentration significantly reduced the mRNA expression of MgMrp2 in gills
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day LOEC - Transcript levels of MgMrp2 in gills	10	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - only the highest CBZ concentration significantly reduced the mRNA expression of MgMrp2 in gills
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day NOEC - Transcript levels of MgMrp2 in mantle/gonads	<0.1	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - CBZ significantly reduced the mRNA expression of MgMrp2 at both 0.1 and 10 ug/L

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration ($\mu g \cdot L^{1}$)	Test Type	Temp (°C)	DO ($mg \cdot L^{1}$)	Hardness ($mg \cdot L^{1}$)	Alkalinity ($mg \cdot L^{1}$)	pH	Reference	Ranking (rationale)
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day LOEC - Transcript levels of MgMrp2 in mantle/gonads	0.1	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - CBZ significantly reduced the mRNA expression of MgMrp2 at both 0.1 and 10 ug/L
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day NOEC - Transcript levels of MgMvp in digestive glands	0.1	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - only the highest CBZ concentration significantly reduced the mRNA expression of MgMvp in digestive glands
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day LOEC - Transcript levels of MgMvp in digestive glands	10	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - only the highest CBZ concentration significantly reduced the mRNA expression of MgMvp in digestive glands
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day NOEC - Transcript levels of MgMvp in gills	0.1	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - only the highest CBZ concentration significantly reduced the mRNA expression of MgMvp in gills
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day LOEC - Transcript levels of MgMvp in gills	10	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - only the highest CBZ concentration significantly reduced the mRNA expression of MgMvp in gills

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration (µg·L^{1})	Test Type	Temp (°C)	DO (mg·L^{1})	Hardness (mg·L^{1})	Alkalinity (mg·L^{1})	pH	Reference	Ranking (rationale)
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day NOEC - Transcript levels of MgMvp in mantle/gonads	<0.1	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - CBZ significantly reduced the mRNA expression of MgMvp at both 0.1 and 10 ug/L
Invertebrate	*Mytilus galloprovincialis*	Mediterranean mussel	Adults (collected from the northwestern Adriatic Sea coast)	Final DMSO concentration was 0.001% (v/v)	7 day LOEC - Transcript levels of MgMvp in mantle/gonads	0.1	Daily static renewal	16		artificial 35 psu seawater			Martin-Diaz *et al.* 2009	U (endpoint not ecologically relevant) - CBZ significantly reduced the mRNA expression of MgMvp at both 0.1 and 10 ug/L
bacteria	*Vibrio fischeri*	bioluminescent bacteria		No solvent added	30 minute EC50 (inhibition of bioluminescence)	>81,000	Static	15				7.0-8.5	Ferrari *et al.* 2003; Ferrari *et al.* 2004	2
bacteria	*Vibrio fischeri*	bioluminescent bacteria		≤0.5% DMSO in final exposure	5 minute EC50 (inhibition of bioluminescence)	52,500 (49,200-56,100)	Static						Kim *et al.* 2007	2
bacteria	*Vibrio fischeri*	bioluminescent bacteria		≤0.5% DMSO in final exposure	15 minute EC50 (inhibition of bioluminescence)	52,200 (45,800-59,500)	Static						Kim *et al.* 2007	2
bacteria	*Vibrio fischeri*	bioluminescent bacteria		1% DMSO in stock solution	5 minute EC50 (inhibition of bioluminescence)	64,265 (272 uM)	Static	15					Jos *et al.* 2003	2

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration (µg·L^{1})	Test Type	Temp (°C)	DO (mg·L^{1})	Hardness (mg·L^{1})	Alkalinity (mg·L^{1})	pH	Reference	Ranking (rationale)
bacteria	*Vibrio fischeri*	bioluminescent bacteria		1% DMSO in stock solution	15 minute EC50 (inhibition of bioluminescence)	78,442 (332 uM)	Static	15					Jos *et al.* 2003	2
bacteria	*Vibrio fischeri*	bioluminescent bacteria		1% DMSO in stock solution	60 minute EC50 (inhibition of bioluminescence)	87,420 (370 uM)	Static	15					Jos *et al.* 2003	2
bacteria	*Vibrio fischeri*	bioluminescent bacteria		DMSO in stock solution (conc. not specified)	15 minute EC50 (inhibition of bioluminescence)	28,300	Static						Harada *et al.* 2008	2
bacteria	*Vibrio fischeri*	bioluminescent bacteria		0.1 M ethanol in stock may have been used	30 minute EC50 (inhibition of bioluminescence)	53,200	Static						Escher *et al.* 2005	2
bacteria	*Vibrio fischeri*	bioluminescent bacteria		0.1 M ethanol in stock may have been used	30 minute EC50 (inhibition of bioluminescence)	489,700	Static						Escher *et al.* 2005	U (exceeds solubility limit)

Group	Organism	Common Name	Age, Size or Life Stage	Solvent Used	Endpoint	Effect concentration (µg·L^{1})	Test Type	Temp (°C)	DO (mg·L^{1})	Hardness (mg·L^{1})	Alkalinity (mg·L^{1})	pH	Reference	Ranking (rationale)
aquatic plants and algae	*Dunaliella tertiolecta*	marine phytoplankton		final acetone concentration of 0.4% in each treatment	96 hour EC50 (cell density)	>80,000	Static	25					DeLorenzo and Fleming 2008	2 (a statistical comparison between 0.4% acetone controls and no-solvent controls revealed no significant difference in algal growth. Concentrations greater than 80,000 lg/L precipitated out of solution).