Technical Report

Investigations and Monitoring Group

A review of nitrate toxicity to freshwater aquatic species

Report No. R09/57
ISBN 978-1-86937-997-1

Environment Canterbury
Your regional council

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Prepared for Environment Canterbury by

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Prepared for

Environment Canterbury

NIWA Client Report: HAM2009-099
June 2009

NIWA Project: ENC09201

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Executive summary

Environment Canterbury (ECan) is preparing an amendment to the proposed Natural Resources Regional Plan (NRRP) to better manage the cumulative effects of non-point source discharges of nutrients, sediment and pathogens on rivers, lakes, wetlands and groundwater. As part of this work numerical limits are to be set for key contaminants to ensure that management objectives for the region's surface and ground water bodies will be achieved.

Environment Canterbury commissioned a review of the ANZECC (2000) and the 2002 revised guideline value freshwater quality guidelines for chronic nitrate (NO_3-N) concentrations in surface waters and groundwaters, together with advice on application of guidelines to seasonally varying concentrations (i.e., in groundwaters, rivers and lowland streams). Specific consideration was also requested regarding the availability of data for indigenous and representative species, together with introduced species resident in Canterbury's aquatic ecosystems.

A review of the international literature and toxicological databases, including the US EPA AQUIRE database and Environment Canada data was undertaken to compile a database of acute (short-term) and chronic (long-term) toxicity data. The ANZECC and Environment Canada decision-making criteria were applied to this database to select appropriate species for guideline derivation. Data were specifically excluded for potassium nitrate, as high potassium is not a normal component of contamination of surface waters, and its toxicity has been shown to be significantly higher than sodium nitrate to both fish and macroinvertebrates. Tropical species data was also excluded from the guideline derivation. Recently published data provided sufficient chronic data for use in guideline derivation, which had previously been based only on acute data for the ANZECC (2000) derivation.

Sufficient data was available for both acute and chronic guideline derivations. The acute guideline derivation followed the US EPA (2002) protocol and the chronic guideline the ANZECC (2000)/Environment Canada (2007) approach. A total of 20 species were used for the acute derivation. The acute data had only four species found in Canterbury's water bodies (rainbow trout, lake trout & Chinook salmon), including one indigenous species, the native snail, (*Potamopyrgus antipodarum*). However, there were also five representative species, including amphipods, caddisflies and a snail. The chronic dataset includes three species found in Canterbury's rivers and lakes (rainbow trout, lake trout and Chinook salmon). These three fish species are represented by tests which fell in the lower 30 percentile of the sensitivity distribution. While there were other invertebrate species in the chronic data that could be considered representative of lake habitats (i.e., *Daphnia* and *Ceriodaphnia*), their sensitivity is markedly less than the most sensitive fish species (i.e., >9.8x). Overall, the acute nitrate data showed macroinvertebrates were the more sensitive organisms, while the chronic data showed fish to be more sensitive to long-term exposures.

The datasets are particularly lacking in species which are known to be of high sensitivity to other common toxic contaminants, and that dominate the fauna in river environments. Studies have shown that amphipods, mayflies and some native fish species are more sensitive to some chemical contaminants than standard test species, such as cladocerans and rainbow trout. No information is available on the sensitivity of native fish species to nitrate.

The recommended freshwater guidelines suitable for application to freshwaters of Canterbury are:

Guideline type	**Application to:**	**Guideline value (mg NO_3-N/L)[a]**
Acute	Very localised point source discharge.	20 mg NO_3-N/L
Chronic – high conservation value systems (99% protection)	Pristine environments with high biodiversity and conservation values.	1.0 mg NO_3-N/L
Chronic – slightly to moderately disturbed systems (95% protection)	Environments which are subjected to a range of disturbances from human activity.	1.7 mg NO_3-N/L
Chronic – highly disturbed systems (80 to 90% protection)	Specific environments which: (i) either have measurable degradation; or (ii) which receive seasonally high elevated background concentrations for significant periods of the year (1-3 months).	2.4 – 3.6 mg NO_3-N/L
Chronic – site-specific (species-specific protection)	Collection of specific data for representative species and life-stages with calculation of site-specific guideline values.	No data

[a] Multiply by conversion factor of 4.43x to convert to NO_3

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1 Background

Environment Canterbury (ECan) is preparing an amendment to the proposed Natural Resources Regional Plan (NRRP) to better manage the cumulative effects of point and non-point source discharges of nutrients, sediment and pathogens on rivers, lakes, wetlands and groundwater. As part of this work numerical limits are to be set for key contaminants to ensure that management objectives for the region's surface and ground water bodies will be achieved.

Environment Canterbury staff are reviewing the appropriateness of available standards and guideline values for a range of contaminants to achieve specific management objectives (e.g., protection of Canterbury's surface water ecosystems, drinking water). Questions have also been raised by consultants working for resource consent applicants over the appropriateness of the guideline values for nitrate (set out in ANZECC 2000) to protect New Zealand aquatic ecosystems. Environment Canterbury wants these values reviewed to establish confidence that they are relevant and applicable to New Zealand's freshwater ecology.

In addition to reviewing the guideline values for nitrate, Environment Canterbury is seeking advice on the following:

(i) the relative importance of the effects of short term exceedances of any toxicity threshold compared to longer-term exposure to concentrations below the guideline values. Many of Canterbury's lowland streams have very strong seasonal peaks in nitrate-N concentrations which may exceed current toxicity thresholds (of 7.2 mg N/L) for 1-3 months per year (usually late winter/spring) but concentrations may reduce considerably for the remainder of the year;

(ii) whether lower nitrate concentrations to provide protection for particularly sensitive species in specific areas are appropriate (as recommended by Carmago *et al.* 2005). In particular, is there justification for different thresholds in relatively undeveloped areas with very high natural water quality, such as the Mackenzie Basin.

Brief

A study brief (dated 17 October 2008) was supplied by ECan for this project.

The objective was: "To advise on the appropriateness of current nitrate guideline standards to protect New Zealand aquatic ecosystem values."

The specified tasks were:

1 Carry out a literature review to identify any new research (post 1998) on nitrate toxicity limits for surface water ecosystems. Review the relevance of nitrate toxicity literature and data to New Zealand aquatic fauna. The review will cover the published literature, water quality guidelines publications and international databases for toxicity testing (e.g., US EPA AQUIRE database).

2 Freshwater nitrate toxicity information will be summarised (Excel spreadsheet database) and reviewed and if appropriate used to calculate a revised water quality guideline for nitrate following the ANZECC (2000) guideline calculation procedures.

3 The relative sensitivity of species in the database will be compared with published data on the sensitivity of New Zealand aquatic species to nitrate contaminants (e.g., Hickey 2000) to provide a basis for addressing the specific issues below.

4 The adequacy of the species represented in the nitrate toxicity database will be assessed relative to known macroinvertebrate and fish distributions in Canterbury's rivers to provide a site-specific guideline assessment.

Comment on the following matters:

(i) Are there any reasons why international studies on nitrate toxicity will not be relevant to NZ aquatic fauna?

(ii) What is the importance of managing short term exceedances of any toxicity threshold as well as long-term exposure (i.e., 1 – 3 month period)?

(iii) Comment on the recommendations made by Carmago *et al*. (2005) regarding appropriate protection guideline values. Are there likely to be any sensitive aquatic communities in Canterbury that require a lower nitrate threshold?

(iv) Advise whether the ANZECC 2000 and the 2002 revised guideline value toxicity limits are appropriate for Canterbury water bodies, and if not provide, and justify the revised toxicity limits (see 2) and why these have been revised.

2 Methods

The original ANZECC (2000) guideline for nitrate was found to have an error in derivation, with a correction issued in 2002[1]. The 2002 revised guideline value is 7.2 mg NO_3-N/L for 95% species protection. The ANZECC (2000) guidelines are currently in an early stage of revision.

We undertook a search of the peer-reviewed scientific literature and toxicity databases to find nitrate information published since 1998. The primary sources were the US EPA AQUIRE (http://cfpub.epa.gov/ecotox/) database, the major published reviews of Camargo *et al*. (2005) and the Environment Canada (2003) nitrate ion guideline derivation. The Environment Canada (2003) procedure derived an "interim" water quality guideline of 13 mg NO_3/L (i.e., equivalent to 2.9 mg NO_3-N/L).

The AQUIRE database was searched for information on the nitrate ion (CAS 147-55-8), sodium nitrate (CAS 7631-99-4) and potassium nitrate (CAS 7757-79-1), with specific exclusion of ammonium nitrate (CAS 6484-52-2), because of the potential for ammoniacal-N toxicity with this compound. Data were loaded, or entered into, a summary Excel database and converted to a nitrogen (N) basis using the factors given in Table 2.1. Notably, a number of early publications were reported as the total salt weight (e.g., $NaNO_3$), with some of these data incorrectly reported in the ANZECC (2000) derivation.

Table 2.1 Conversion factors for various nitrate units to mg NO_3-N/L

Base unit	**Multiply by:**
mg NO_3/L	0.23
mg $NaNO_3$/L	0.16
mg KNO_3/L	0.14
mg NH_4NO_3/L	excluded
mg NO_3-N/L (ppm)	1
µg NO_3-N/L (ppb)	1000

We selected data suitable for acute and chronic endpoints following the ANZECC (2000) and Environment Canada (CCME 2007) selection criteria as detailed in Table 2.2. The selection procedures for categorising acute (short-term) and chronic (long-term) test durations, defining acceptable effects measures and endpoints generally follows documentation provided in ANZECC (2000), though we have adopted the more recent, and more specific, Environment Canada (CCME 2007) classification for durations and some of their document classifications. The generic derivation procedures were followed by selection of "site-specific" as required in the brief for this project. The selection criteria were:

1. **Effects:** The major effect classes included mortality (MOR), immobilisation (IMM), growth (GRO), reproduction (REP), population growth rate (PGR), and hatching (HAT) following the US EPA AQUIRE classifications as used in the ANZECC (2000) guidelines. A summary of effects codes is provided in Appendix 1. Biochemical effects (e.g., enzyme activity, serum protein concentrations) were not included as acceptable endpoints and while behavioural effects were included, special

[1] http://www.mfe.govt.nz/publications/water/anzecc-water-quality-guide-02/anzecc-nitrate-correction-sep02.html

consideration is made of these measured in the site-specific guideline derivation to assess whether the effects were relevant to survival.

2. **Duration:** The designation of acute and chronic durations generally followed the criteria used in the ANZECC (2000) guidelines, however because these are somewhat vague, the more specific Environment Canada (CCME 2007) criteria were used (Table 2.2). Generally, acute tests were up to 96 h exposure and with the predominant effect measured being survival, with an LC_{50} (lethal concentration causing a 50% effect) endpoint. The chronic tests had longer exposures, with the designation into "chronic" duration generally related to the life-span of the organism (shorter exposures are considered chronic for short-lived species), and favouring sub-lethal effects (e.g., growth, reproduction). Some best professional judgement was required for classification of some species/effects. Note that the ANZECC (2000) guidelines were only based on chronic guideline determinations.

3. **Endpoints:** Either an LC_{50} or EC_{50} (lethal or effective concentration causing a 50% effect, see Glossary (Section 6)) for the various effect measures were selected as suitable acute endpoints. According to the ANZECC (2000) procedures the "No Observed Effect Concentration" (NOEC) is the preferred endpoint for chronic exposures. Other reported chronic endpoints (e.g., "Lowest Observed Effect Concentration", LOEC; "Maximum Acceptable Toxicant Concentration", MATC) were converted to an estimated NOEC using the conversion factors used in ANZECC (2000) (Table 2.2).

In the updated Environment Canada procedures for guideline derivation (CCME 2007) the toxicity endpoint preference is for regression-based statistical evaluation (i.e., EC_x, values identifying no- or low-effects thresholds) over endpoints obtained through hypothesis-based statistical evaluation (i.e., NOEC and LOEC values). In hypothesis-based evaluations, the arbitrary nature of the selection of exposure concentrations, and dilution factors between consecutive dilutions, can result in a highly variable NOEC value relative to the onset of statistically significant effects detected at the LOEC concentration. While the use of the NOEC may provide a precautionary approach, it may also provide excessively conservative values depending on the design of the particular studies. Thus the Environment Canada preference ranking order is: EC_{10}>EC_{11-25}>MATC>NOEC>EC_{26-49}>non-lethal EC_{50}. In practice, threshold EC_{10} values are commonly not reported, especially in older literature, and hypothesis-based values must be used by default if data from those studies are to be included.

4. **Reference quality:** Publications with the ANZECC (2000) classification of "I" (insufficient data) or "Unacceptable data" from the Environment Canada (CCME 2007) classification system (see Appendix 2), were not included without specific justification. These classification scores are provided in the database where a publication has been previously considered. Note that the Environment Canada (2003) nitrate guideline derivation uses primary, secondary and "Ancillary source (A)" data classifications, and we have taken the latter as being equivalent to the "Unacceptable data" classification. We have used the ANZECC (2000) scoring procedure (Appendix 2) to classify any publication-derived data on highly sensitive species (in the lower 25%ile of the sensitivity distribution curve) which significantly affect the guideline derivation procedure.

5. **Calculation procedure:** The generic calculation procedure for guideline derivation involves selection of the longest duration exposure/effect/endpoint combination for each species/publication, and calculating a geometric mean for multiple independent studies where the same combination occurs (e.g., same species, same endpoint).

Notably, some of the ANZECC (2000) derivations calculate a geometric mean of a range of reported exposure durations (e.g., 24 h, 48, 96 h) from the same study, which does not follow their reported methodology. Providing there is adequate data (8 species for the ANZECC approach), the guideline derivation procedure involved modelling the cumulative species sensitivity distribution (SSD) estimating the 5%ile effect level (i.e., 95% protection level) of the SSD which provided the primary guideline derivation. This SSD approach is also the recommended primary calculation procedure for the recently revised Environment Canada procedure (CCME 2007).

For the acute guideline calculation, we have used the SSD approach to calculate a community 5%ile effect threshold based on LC_{50}/EC_{50} effects data. This acute 5%ile effects value then has an application factor (AF) of 2 applied to generate a final acute guideline following the US EPA standard procedure (Stephan *et al*. 1985; US EPA 2002). Though the recent Environment Canada protocol (CCME 2007) includes short-term exposure guidelines, which are: "meant to estimate severe effects and to protect most species against lethality during intermittent and transient events (e.g., spill events to aquatic-receiving environments, infrequent releases of short-lived/non-persistent substances)", their acceptance of a 50% effect for some species at the guideline level is probably inconsistent with the New Zealand Resource Management Act legislation (RMA 1991), as this would potentially constitute a significant adverse effect on aquatic life. Because of the large number of acute guideline derivations following the US EPA procedure, we consider that this is the preferred approach for use in this study to benchmark the acute nitrate toxicity relative to the available data.

The SSD model used for all guideline derivations was the BUR III model referred to in the ANZECC (2000) procedures.

6. **Key species:** Guideline derivation procedures generally include special consideration of data adequacy for rare and endangered species, and commercially or recreationally important species. This provision was explicitly included in the ANZECC (2000) guidelines and in the Environment Canada (CCME 2007) procedures, as defined by the "protection clause". This component of the derivation requires that effects and endpoint values for key species which fall below the 5%ile effect guideline be specifically considered on a case-by-case basis. If this endpoint is a moderate- or severe-effect level endpoint for a species at risk, then this value becomes the default guideline value (CCME 2007). Specific consideration of key species data was undertaken as a component of the procedure used here. An additional generic consideration in regard to key species, is the adequacy of the database in providing representative species/genus data for the diversity of species present in a given environment. Native and introduced species are also identified as part of this toxicity review.

7. **Site-specific selections:** A general site-specific selection was applied to exclude tropical species data. This was based on the contention that tropical species would not inhabit the temperate New Zealand freshwater aquatic environments. Tests for tropical species at ≥28ºC was the criteria for exclusion from the generic derivation.

Site-specific derivations were considered for species inhabiting particular environments (e.g., rivers, lakes, groundwaters) in the Canterbury region. This component of the study was assessed following the generic guideline derivation. The elimination of generic (i.e., non-local) species reduces the number of species and results in a dataset which is unsuitable for guideline calculation following the SSD model procedures.

Table 2.2 Decision criteria summary for data inclusion for site-specific nitrate guideline calculations

Number	Selection criteria	Comments	Reference
1	Database search	US EPA AQUIRE, Environment Canada, bibliographic references	
2	Effects: MOR, IMM, GRO, REP, PGR, PSR, HAT, DEV	Footnote a	ANZECC (2000)
3	Durations: acute (short-term): fish & amphibians, 96h; aquatic invertebrates, 48-96h; aquatic plants, case-by-case basis; algae, 24h; chronic (long-term): fish & amphibians, >21d, or >7d for eggs & larvae; aquatic invertebrates, >96h for short-lived, >7d for long-lived and non-lethal endpoints, >21d for long-lived and lethal endpoints; plants, case-by-case; algae, >72-96h	Classifications required for acute and chronic guideline derivations. Some best professional judgement required for classification of durations that fall outside these classes.	CCME (2007)
4	Selected endpoints: NOEC, LOEC, MATC & ECx for chosen EFFECTS	Footnote b & Glossary for terms	
5	Specific exclusions: toxicant	Only sodium nitrate (CAS: 7631994); exclusion of potassium nitrate, ammonium nitrate. Conversion of all data to standard measure: nitrate-nitrogen (NO_3-N)	
6	Specific exclusions: water quality	no marine or salt (>5 ppt) for freshwaters; or "NR" (not recorded)	
7	Specific exclusions: reference quality	Remove low reliability data. Based on "I" score for ANZECC & US EPA scoring system; "Unacceptable data" classification from Environment Canada. Specific justification for exclusion.	ANZECC (2000) & CCME (2007)
8	Effect selection	Multiple effects endpoints per species considered for species sensitivity distribution: most sensitive of traditional effects (e.g., growth, reproduction, survival) - with selection of most sensitive life-stage and effect; as well as endpoints for other effects (e.g., behavioural, physiological).	CCME (2007)
9	Duration selection	Longest duration within acute or chronic datasets for each author.	ANZECC (2000)
10	Endpoint selection and conversion	Conversion of chronic endpoints to NOEC values using following criteria: LOEC, MATC, LC/EC_{50} values divided by assessment factors of 2.5, 2 and 5 respectively. Toxicity data where insufficient concentration at the higher range (i.e., "toxicity greater than") included - on the basis that this will not result in an under-protective guideline. Note: NOEC is the preferred endpoint for ANZECC (2000); Environment Canada selection priority is for EC_{10} or LOEC.	ANZECC (2000)
11	Averaging	Geometric mean for each species having multiple authors/studies with common endpoints.	ANZECC (2000) & CCME (2007)
11	Key species selection	Specific consideration for inclusion of high economic, recereational or ecologically important species (i.e., exclusion from geometric mean averaging).	ANZECC (2000) & CCME (2007)
12	Site-specific: temperature	Tropical species and exposures at high temperatures (≥28°C) were excluded.	Site-specific criteria
13	Site-specific: environments	Separation of data into speciesinhabiting specific environments (e.g., rivers, lakes, groundwaters).	Site-specific criteria
14	Site-specific: species	Exclusion of exotic species not present in any local envionment. Best professional judgement with justification for exclusion.	Site-specific criteria

[a] Effects codes in Appendix 1

[b] NOEC, no observed effect concentration; LOEC, lowest observed effect concentration, MATC, geometric mean of NOEC + LOEC; LCx, lethal concentration causing x% effect; ECx, effective concentration causing x% effect

3 Results

3.1 Review of the ANZECC (2000) nitrate guideline derivation

A marked-up review of the ANZECC (2000) guidelines derivation for freshwater nitrate toxicity is provided in Appendix 3. The two chronic species and endpoints which were originally included in the ANZECC (2000) derivation have recently been identified as being potassium salts (R. van Dam, Environmental Research Institute of the Supervising Scientist (ERISS), Australia, pers. comm.) and should not have been included in the data set. The revision of the guideline undertaken in 2002[2] was based on a modification of the calculation approach to consider these two chronic data separately from the acute data in the guideline derivation process. Additional errors in original data and averaging have also been identified.

The publication of more recent chronic data since the ANZECC (2000) derivation means that these derivations are based on this new data rather than relying on the original acute database.

3.2 Update of nitrate guideline derivation

The data for all species are summarised in the accompanying Excel spreadsheet (NIWA_nitrate_2009.xls) [3]. This database includes annotations for analysis of nitrate in the tests, ranking of the source and the publication references. References are flagged to indicate those included in either the ANZECC (2000) or Environment Canada (2003) derivations. Details of the final acute and chronic datasets, together with the statistical model plots are provided in Appendix 4.

The derivation does not include potassium nitrate data, as both acute and chronic toxicity tests with the potassium salt have shown that it is markedly more toxic than the sodium salt for a range of fish and invertebrate species (Table 3.1). Notably, chronic data for two species which were included in the ANZECC (2000) derivation have recently been identified as being potassium salts. These data have not been included in this derivation and were identified in the marked-up review of the ANZECC (2000) derivation (Section 3.1).

[2] http://www.mfe.govt.nz/publications/water/anzecc-water-quality-guide-02/anzecc-nitrate-correction-sep02.html

[3] A copy of the data can be obtained upon request to Environment Canterbury

Table 3.1 Relative toxicity of sodium and potassium nitrate to freshwater organisms (from Environment Canada 2003)

Organism	Duration (h)	Endpoint	$[NO_3^-]$ (mg $NO_3^-\cdot L^{-1}$)		Reference
			K^+ Salt	Na^+ Salt	
Lepomis macrochirus (bluegill)	96	LC_{50}	1840	8753	Trama (1954)
	24	LC_{50}	3373	9338	Dowden and Bennett (1965)
Daphnia magna (water flea)	96	TL_m	552	3069	Dowden and Bennett (1965)
Polycelis nigra (planaria)	48	survival	555	2696	Jones (1940)
Gasterosteus aculeatus (stickleback)	240	lethal concen-tration limit	79	1348	Jones (1939)
Hydra attenuata (hydra)	288	NOEL	150 - 250	< 50	Tesh et al. (1990)

3.2.1 Acute data

A summary of the 20 acute results are provided in Table 3.2 and shown in Figure 3-1. These include: 9 fish; 9 invertebrate; and 2 amphibian species. The dataset spans a 37-fold range in sensitivity with the most sensitive species being an amphipod (*Echinogammarus echinosetosus*) with an LC_{50} of 56.2 mg NO_3-N/L. In general, the invertebrates appear to be more acutely sensitive to nitrate than fish (Figure 3-1), with rainbow trout 19x less sensitive than the most sensitive species, and the most sensitive fish (Siberian sturgeon) 7x less sensitive than the most sensitive species in the dataset.

Seven of the fourteen publications which contributed to the selected acute toxicity data were included in the ANZECC (2000) guideline derivation. All of the selected acute publications constituting the lower quartile of the sensitivity distribution were of reliable quality earning either a "primary" classification from Environment Canada or an "M" (moderate) classification from the ANZECC (2000) procedure (Appendix 2), with reference codes and classifications shown in Appendix 4.

Acute data for seven tropical species from six studies (Colt and Tchobanoglous 1976; Meade and Watts 1995; Tilak *et al*. 2002; Tilak *et al*. 2006a; Tilak *et al*. 2006b; Tilak *et al*. 2006c) were excluded from the guideline derivation procedure.

Table 3.2 Summary of acute toxicity data for sodium nitrate exposure selected for the 2009 derivation. Highlighted (white on black) indicate species which are resident in Canterbury's rivers and lakes bold indicates representative species with closely related families in rivers

Group	Common name	Latin Name	Life Stage	Duration (h, d)	End-point	Effect	Temp (°C)	EC_{50}/LC_{50} (mg NO_3-N/L)[a]	Rank	Cumulative %	Author
Invertebrate	**Amphipod**	***Echinogammarus echinosetosus***	**Adults**	**120h**	**LC50**	**MOR**	**17.9**	**56.2**	**1**	**0**	**Camargo *et al*. (2005)**
Invertebrate	**Amphipod**	***Eulimnogammarus toletanus***	**Adults**	**120h**	**LC50**	**MOR**	**17.9**	**73.1**	**2**	**5.2**	**Camargo *et al*. (2005)**
Invertebrate	**Caddisfly**	***Hydropsyche accidentalis***	**Last instar larvae**	**120h**	**EC50**	**MOR**	**18**	**77.2**	**3**	**10.5**	**Camargo & Ward (1992)**
Invertebrate	Caddisfly	*Cheumatopsyche pettiti*	Early instar larvae	120h	LC50	MOR	18	107	4	15.7	Camargo & Ward (1992)
Invertebrate	**Caddisfly**	***Hydropsyche exocellata***	**Last instar larvae**	**120h**	**LC50**	**MOR**	**17.9**	**230**	**5**	**21**	**Camargo *et al*. (2005)**
Amphibian	Pacific Treefrog	*Pseudacris regilla*	tadpoles	10d	LC50	MOR	22	266	6	26.3	Schuytema & Nebeker (1999c)
Invertebrate	Water flea	*Ceriodaphnia dubia*	Neonates	48h	LC50	MOR	25	374	7	31.5	Scott & Crunkilton (2000)
Fish	Siberian sturgeon	*Acipenser baeri*	Adults	96h	LC50	MOR	22.5	397	8	36.8	Hamlin (2006)
Invertebrate	Water flea	*Daphnia magna*	Neonates	48h	EC50	MOR		479	9	42.1	**Geometric mean**
Invertebrate	**Snail**	***Lymnaea sp***	**eggs**	**96h**	**LC50**	**HAT**	**NR**	**535**	**10**	**47.3**	**Dowden & Bennett (1965)**
Invertebrate	**Snail**	***Potamopyrgus antipodarum***	**Adults**	**96h**	**LC50**	**MOR**	**20.4**	**1042**	**12**	**52.6**	**Alonso & Camargo (2003)**
Fish	**Rainbow trout**	***Oncorhynchus mykiss***	**fingerlings**	**7d**	**LC50**	**MOR**	**13-14**	**1061**	**13**	**57.8**	**Westin (1974)**
Fish	**Chinook salmon**	***Oncorhynchus tshawytscha***	**fingerlings**	**7d**	**LC50**	**MOR**	**13-14**	**1084**	**14**	**63.1**	**Westin (1974)**
Fish	Eastern mosquitofish	*Gambusia holbrooki*		96h	LC50	MOR		1095	11	68.4	Wallen *et al*. (1957)
Fish	**Lake Trout**	***Salvelinus namaycush***	**fry**	**96h**	**LC50**	**MOR**	**7.5**	**1121**	**15**	**73.6**	**McGurk *et al*. (2006)**
Amphibian	African clawed frog	*Xenopus laevis*	tadpoles	10d	LC50	MOR	22	1236	16	78.9	Schuytema & Nebeker (1999c)
Fish	Fathead minnows	*Pimephales promelas*	Larvae	96h	LC50	MOR		1317	17	84.2	**Geometric mean**
Fish	Catfish	*Ictalurus punctatus*	Fingerlings	96h	LC50	MOR	22	1355	18	89.4	Colt & Tchobanoglous (1976)
Fish	Lake Whitefish	*Coregonus clupeaformis*	fry	96h	LC50	MOR	7.5	1903	19	94.7	McGurk *et al*. (2006)
Fish	Bluegill	*Lepomis macrochirus*	fingerlings	96h	LC50	MOR		2094	20	100	**Geometric mean**

[a] Multiply by conversion factor of 4.43x to convert to NO_3

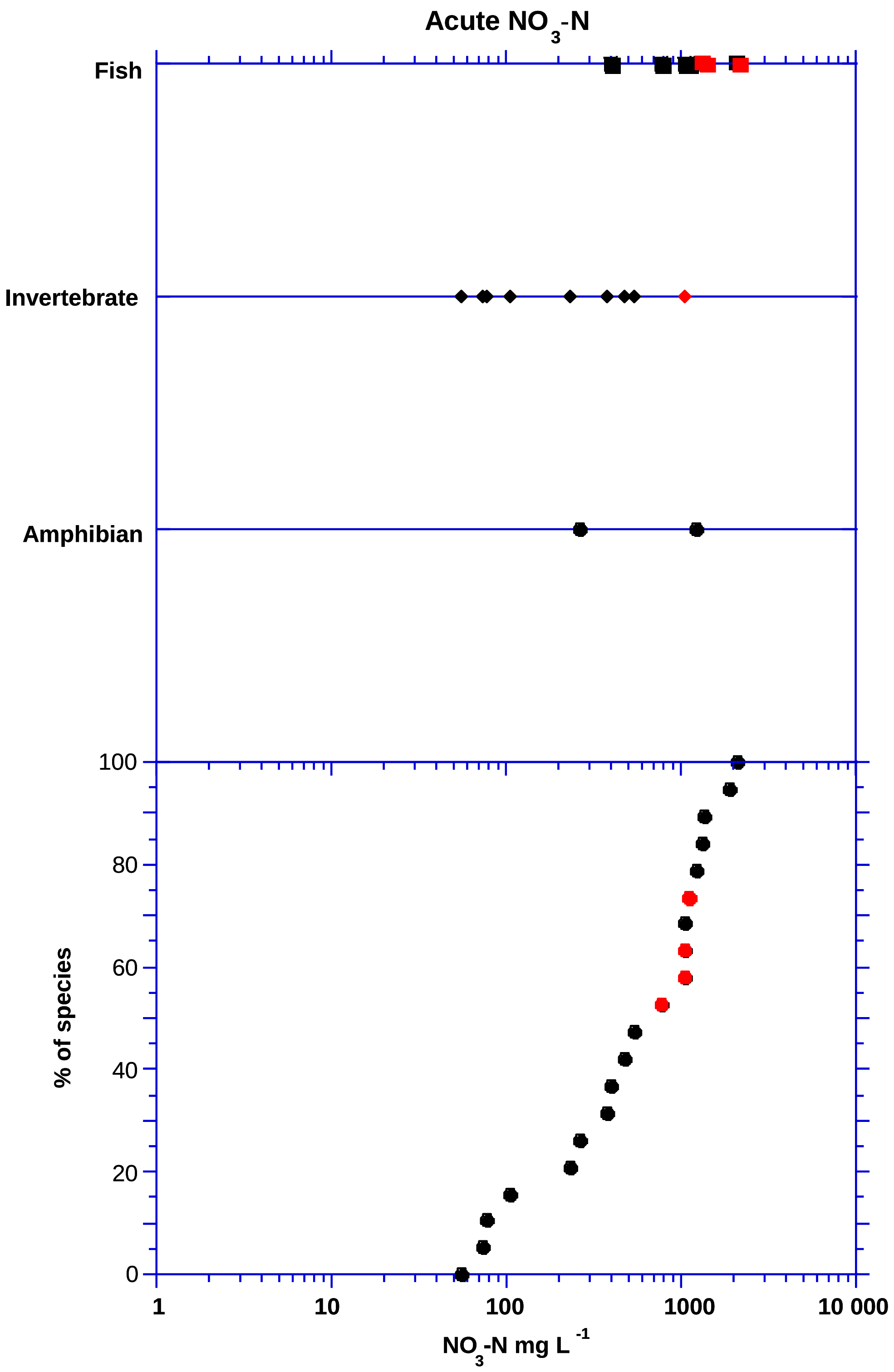

Figure 3-1 Cumulative species sensitivity distribution for <u>acute</u> toxicity dataset. Species resident in Canterbury indicated in red

3.2.2 Chronic data

A summary of the 16 chronic NOEC results are provided in Table 3.3 and shown in Figure 3-2. The details are contained in Appendix 4. These include: 7 fish, 4 invertebrate; and 3 amphibian species. The dataset spans a 224-fold range in sensitivity, with lake trout (*Salvelinus namaycush*) the most sensitive, with a NOEC of 1.6 mg NO_3-N/L for both growth and development endpoints measured after a 146 day exposure. In general, the chronic fish data indicates higher exposure sensitivity, though both fish and invertebrates show wide ranges in sensitivity (Figure 3-2). The most sensitive invertebrate NOEC (a freshwater crayfish) was 8.8x less sensitive than the most sensitive fish NOEC.

None of the eight publications included in the selected chronic studies were used in the ANZECC (2000) derivation. Most of these included studies scored a "C" classification ("complete") based on the ANZECC (2000) system, and either a "primary" or "secondary" under the Environment Canada classification (Appendix 2), with the exception of the "A" classification ("ancillary") for Kinchloe *et al*. (1979), which is addressed below. The reference codes and classifications are shown in Appendix 4.

The key primary data are the recent long-term (126-146 day) chronic studies of fish sensitivity by McGurk *et al*. (2006), who measured acute and chronic sensitivity of embryos, alevins, and swim-up fry of lake trout (*Salvelinus namaycush*) and lake whitefish (*Coregonus clupeaformis*) under laboratory conditions. The lake trout were the most sensitive species with a NOEC of 1.6 mg NO_3-N/L, and LOEC values of 6.25 mg NO_3-N/L for both growth (GRO) and development (DEV) endpoints (Table 3.3). Growth showed a progressive concentration-response with a 12% reduction in wet-weight at the LOEC value and a 22% reduction at 25 mg NO_3-N/L. The delayed development endpoint (>90% fry) is included for comparison but was not included in the guideline derivation calculation because growth was considered a more ecologically relevant measure.

The rainbow trout data was limited to two concurrent tests undertaken for fry of resident and anadromous ("Steelhead") rainbow trout by Kinchloe *et al*. (1979). This study measured mortality effects on eggs and fry after a 30 day exposure period. The egg sensitivity data in this study was compromised by the mortalities associated with *Saprolegnia* fungal infestations and the data was not included for consideration. There is no indication that the fry were adversely affected by fungal infestation, with good control survival (>95%) and a partial concentration-response for the "non-anadromous" rainbow trout. The NOEC values for the two trout types were 1.1 mg NO_3-N/L and >4.5 mg NO_3-N/L. We have included a geometric mean value for the reported nominal NOEC concentrations for use in the guideline derivation. Neither the stock solution nor the exposure solution concentrations were analytically confirmed in this study. Environment Canada (2003) did not include the results of this study in their nitrate guideline derivation because of fungal concerns about the fungal infestations.

The only tropical data excluded from the guideline derivation was for the freshwater prawn (*Macrobrachium rosenbergii*) (Wickins 1976) that is cultivated in New Zealand only in heated aquaculture facilities.

Environment Canada (2003) provide additional review comments on nitrate publications, together with reasons for exclusion of some studies from their derivation process.

Table 3.3: Summary of chronic toxicity data for sodium nitrate exposure selected for the 2009 derivation. Highlighted (white on black) indicate species that are resident in Canterbury's rivers and lakes.

Group	Common name	Scientific name	Life Stage	Duration (h/d)	End-point	Effect	Temp (ºC)	NOEC (mg/L NO_3-N)[a]	LOEC (mg/L NO_3-N)[a]	Author
Fish	**Lake Trout**	***Salvelinus namaycush***	**Fry**	**146d**	**NOEC**	**DVP**	**7.5**	**1.6**	**6.25**	**McGurk *et al*. (2006)**
Fish	**Lake Trout**	***Salvelinus namaycush***	**Fry**	**146d**	**NOEC**	**GRO**	**7.5**	**1.6**	**6.25**	**McGurk *et al*. (2006)**
Fish	**Rainbow trout**	***Oncorhynchus mykiss***	**Fry**	**30d**	**NOEC**	**MOR**	**10**	**2.2**	**2.3, >4.5**	**Kinchloe *et al*. (1979) (Geo mean)**
Fish	**Chinook salmon**	***Oncorhynchus tshawytscha***	**Fry**	**30d**	**NOEC**	**MOR**	**10**	**2.3**	**4.5**	**Kinchloe *et al*. (1979)**
Fish	Lahontan cutthroat trout	*Salmo clarki*	Fry	30d	NOEC	MOR	13	4.5	7.6	Kinchloe *et al*. (1979)
Fish	Coho salmon	*Oncorhynchus kisutch*	Fry	30d	NOEC	MOR	10	>4.5	>4.5	Kinchloe *et al*. (1979)
Fish	Lake Whitefish	*Coregonus clupeaformis*	Fry	126d	NOEC	DVP	7.5	6.25	25	McGurk *et al*. (2006)
Amphibian	American Toad	*Bufo americanus*	Egg	23d	NOEC	HAT	5-10	>9.26		Laposata & Dunson (1998)
Amphibian	Pacific Treefrog	*Pseudacris regilla*	tadpoles	10d	NOEC	GRO	22	12.0		Schuytema & Nebeker (1999c)
Invertebrate	Freshwater crayfish	*Astacus astacus*		7d	NOAEL	MOR	15	>14.0		Jensen (1996)
Invertebrate	Water flea	*Ceriodaphnia dubia*	neonates					15.6		Scott & Crunkilton (2000) (Geo mean)
Amphibian	African clawed frog	*Xenopus laevis*	Embryo	120h	NOEC	GRO	22	24.8		Schuytema & Nebeker (1999a)
Fish	Lake Whitefish	*Coregonus clupeaformis*	Fry	126d	NOEC	MOR	7.5	25.0	100	McGurk *et al*. (2006)
Invertebrate	Florida apple snail	*Pomacea paludosa*						25.3		Corrao *et al*. (2006) (Geo mean)
Fish	Fathead minnows	*Pimephales promelas*	Embryos and larvae	11d	NOEC	MOR	25	358		Scott & Crunkilton (2000)
Invertebrate	Water flea	*Daphnia magna*	neonates	7d	NOEC	REP	25	358		Scott & Crunkilton (2000)

[a] Multiply by conversion factor of 4.43x to convert to NO_3

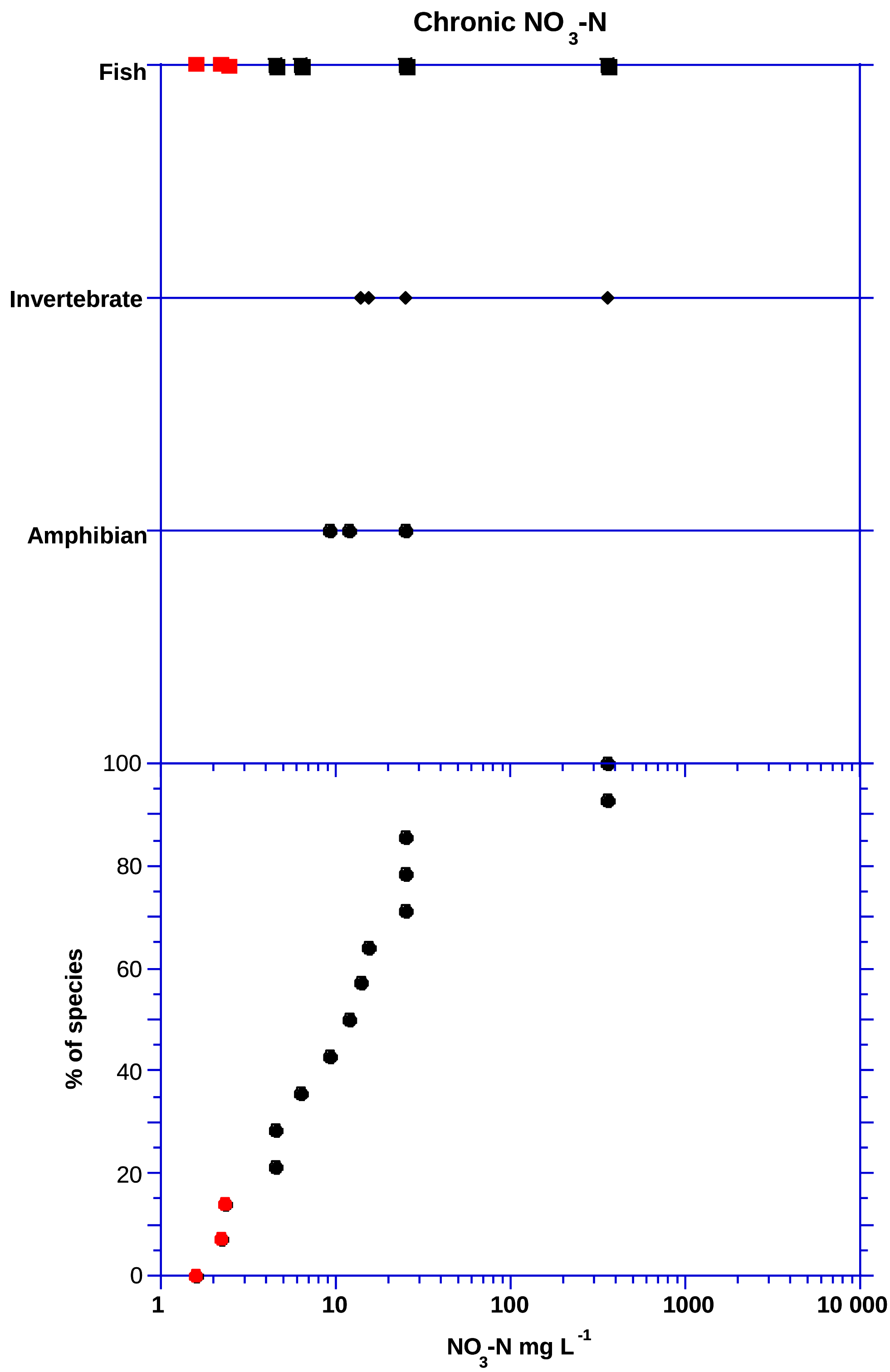

Figure 3-2 **Cumulative species sensitivity distribution for chronic toxicity dataset. Species resident in Canterbury indicated in red**

3.2.3 Generic acute and chronic guideline derivation

An **acute guideline** may be estimated from the species sensitivity distribution (Figure 3-1, Appendix 4 for model fit). The BurrIII model gave an acute 95%ile protection value of 39.7 mg NO_3-N/L. Following the US EPA procedure this value would be divided by a factor of 2 to provide an acute guideline of **20 mg NO_3-N/L**. This could be applicable to either short-term (<96 h) exposures or for application within mixing zones.

The **chronic guideline** trigger values were derived from the whole chronic dataset (Figure 3-3). Figure 3-3 shows a good fit of the BurrIII model to the dataset, with other alternative models (log-logistic, log-normal) shown. The trigger values were: 1.0 mg NO_3-N/L for 99% protection; **1.7 mg NO_3-N/L for 95% protection**; 2.4 mg NO_3-N/L for 90% protection; and 3.6 mg NO_3-N/L for 80% protection.

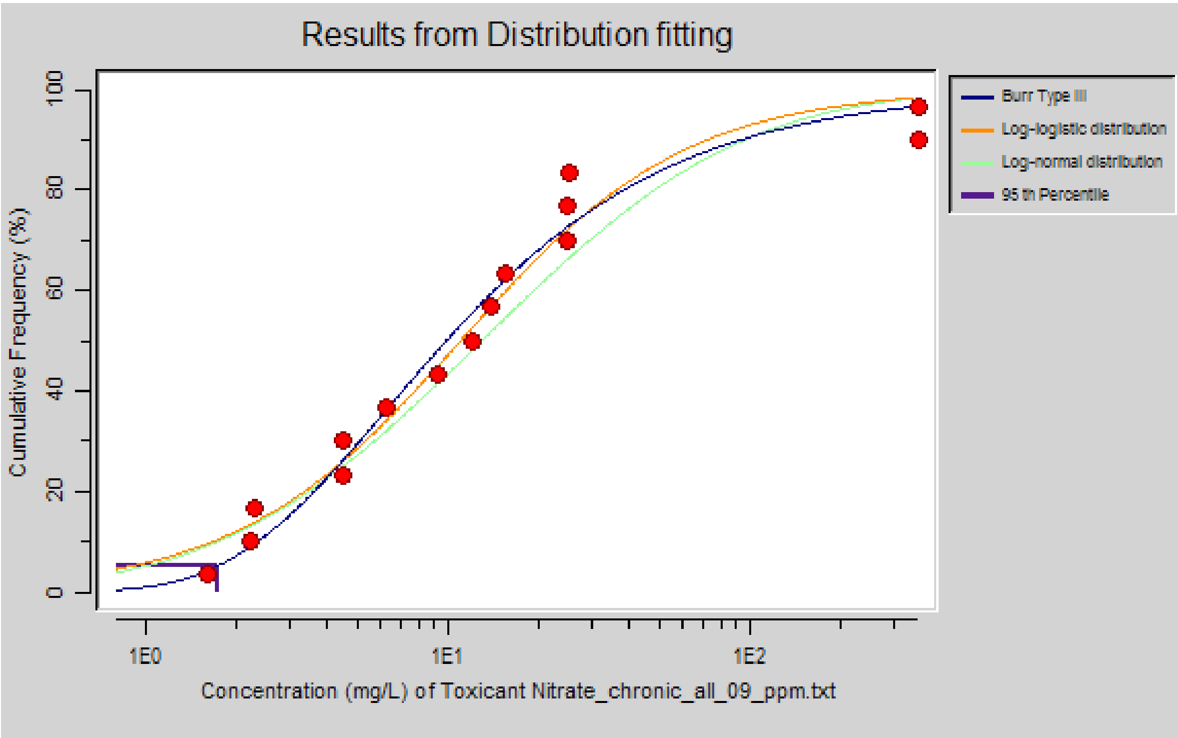

Figure 3-3 Cumulative frequency distribution plot with BurrIII model fit for chronic data. The 95th percentile guideline (1.7 mg NO_3-N/L) is shown

3.3 Site-specific guideline derivation

A site-specific guideline can be calculated by three alternative processes: (i) selection of local resident species from the acute or chronic datasets; (ii) recalculation using specific endpoints (e.g., recalculated from original data in publication); or (ii) selection of the most sensitive acute species and application of an acute-to-chronic ratio (ACR) to provide an estimated NOEC value.

The acute and chronic datasets have been highlighted for locally resident riverine and lake species and “representative” riverine species (Tables 3.2 & 3.3). The basis of this selection is discussed in the Section 4. Acute data is available for four resident species (a snail, rainbow trout, lake trout[4] & Chinook salmon), and eight representative/resident riverine species. However, the chronic dataset was more limited, with only three resident species (rainbow trout, lake trout & Chinook salmon), and no invertebrate species considered representative of riverine environments. These three fish species are represented by tests which fell in the lower 30 percentile of the sensitivity distribution. While there were other invertebrate species in the chronic data that could be considered representative of lake habitats (i.e., *Daphnia* and *Ceriodaphnia*), their sensitivity is markedly less than the most sensitive fish

[4] Lake trout or mackinaw (*Salvelinus namaycush*) are only present in Lake Pearson and are “often mistaken for poor-conditioned brown trout” McDowall (2000)

species (i.e., >9.8x). Notably, the rainbow trout data (Kincheloe *et al*. 1979) is among the most sensitive species (NOEC 2.2 mg NO_3-N/L), and may therefore be required to be retained as a “key” species. However, this publication is graded as “low-reliability” and therefore does not provide suitable assurance as the basis of a guideline. This very limited chronic dataset generally provides too few data to selectively modify to provide a site-specific, or species-specific derivation.

Some consideration can be given to recalculation of the guideline using alternative endpoints. The RMA (1991) is an effects-based legislation and thus consideration of the threshold for ecologically significant adverse effects should be considered. Examination of the nitrate chronic dataset shows the statistically significant effect threshold (LOEC) at 6.25 mg NO_3-N/L, with the threshold defined as the threshold effect concentration (TEC) (geometric mean of the NOEC + LOEC) of 3.2 mg NO_3-N/L.

An ACR of 9.9 was calculated for five species (3 fish, 2 invertebrate) based on acute (LC_{50}) and chronic (NOEC) data from two studies (Scott and Crunkilton 2000; McGurk *et al*. 2006) (Appendix 4, Table A4.3). The ACR values range widely (1.2 to 76), indicating the marked species-specific differences that may be expected for nitrate toxicity. Application of the average ACR to the most sensitive acute data (56.2 mg NO_3-N/L Table 3.2) gives an estimated NOEC of 5.7 mg NO_3-N/L, which is similar to the more sensitive chronic NOEC and LOEC values (Table 3.3). The ACR value could be applied to acute tests with site-specific native species to provide estimated NOECs for use in guideline derivation.

4 Discussion

Adequacy of the datasets

The derivation of water quality guidelines generally requires data of an international suite of species to be compiled in order to provide an adequately representative diversity of fish, invertebrates and other aquatic species. If there are substantive datasets, site-specific guidelines may be derived using a selection of species which are resident in the specific region or type of water-body (e.g., lakes or rivers). Additionally, tests with specific life-stages (e.g., eggs or embryo-larvae) may be omitted if they do not occur in the specific habitat.

We have identified the species known to be resident in Canterbury, together with representatives of those habitats from closely related families for the acute and chronic datasets (Tables 3.2 & 3.3). The acute data had only four species found in Canterbury's water bodies (rainbow trout, lake trout & Chinook salmon), including one indigenous species, the native snail, (*Potamopyrgus antipodarum*). However, there were also five representative species, including amphipods, caddisflies and a snail.

The two amphipods tested were the most sensitive acute species tested and would be expected to be representative of surface water and groundwater environments. Crustaceans have been found to be the predominant invertebrate group inhabiting Canterbury's and other New Zealand aquifers (Scarsbrook and Fenwick 2003; Gray *et al*. 2006). The most sensitive fish species was the rainbow trout, which was 19-fold less sensitive than the most sensitive species.

An acute exposure guideline of 20 mg NO_3-N/L has been calculated from this dataset. This could be applicable to either short-term (<96 h) exposures at specific sites or for application within mixing zones. An acute-to-chronic ratio (ACR) value may be used to estimate chronic exposure tolerance from measured acute values. New acute data for species representative of specific environments (e.g., groundwaters, lakes, trout spawning streams, lowland streams) could be used to provide the basis of site-specific "chronic" guidelines based on application of the ACR. Normally, five to eight species would be required to apply the SSD guideline derivation approach.

The chronic dataset has only three species that are found in Canterbury's rivers and lakes (rainbow trout, lake trout and Chinook salmon). These species are represented by three chronic fry exposures with endpoints in the lower 30 percentile of the sensitivity distribution (Table 3.3). While there are data on the sensitivity of invertebrate species to chronic nitrate exposure, the species are more commonly found in lentic (i.e., pond, lake) habitats (e.g., *Daphnia* & *Ceriodaphnia*), so riverine invertebrates are under-represented.

Are there any reasons why international studies will not be relevant to NZ aquatic fauna?

Some international aquatic studies contain species which are not present in the site-specific New Zealand environment. We have excluded tropical species data from the "generic" guideline derivations.

Amphibians and salamanders could be excluded from the dataset. The sensitivity of these species is generally poorly known compared to the more common fish and macroinvertebrate assemblages. As amphibians would be expected to be present in ponds and lakes, it would therefore be prudent to retain the existing amphibian data. These groups are not overly represented in the nitrate toxicity datasets, and excluding these groups would bias the guideline derivation.

The lack of chronic amphipod data raises concerns that this chronic guideline fails to protect species in groundwater environments. For example, the most sensitive invertebrates (freshwater crayfish, *Astacus astacus*; and the crustacean, *Ceriodaphnia dubia*) have chronic NOECs of >14.0 & 15.6 mg NO_3-N/L, which is 8.2-fold higher than the 95^{th} percentile guideline value. An estimated chronic NOEC for the most sensitive acute amphipod species is 5.7 mg NO_3-N/L (using the ACR conversion), which would be adequately protected by the 95^{th} percentile guideline value. Without benchmarking sensitivity data for relevant (preferably local) species the adequacy, or otherwise, cannot be determined.

What is the importance of managing short-term exceedences of any toxicity threshold as well as long-term exposure (i.e., 1 – 3 month period)?

Exposures of 1–3 months would be considered chronic exposure periods. Shorter duration exposures may occur in point source mixing zones or irrigation bywash flows where intermittent exposure could occur. We have calculated an acute guideline which could be used for such short-term exposures.

A chronic guideline with a lower protection threshold could be used for these seasonal periods of high background nitrate. An 80% percentile chronic guideline would be 3.6 mg NO_3-N/L. Use of this guideline for seasonal maxima would not be expected to result in marked ecological effects on broad ecological communities, given that the remainder of the year had lower concentrations of nitrate.

However, for communities making important seasonal use of these environments for critical life-stages at these times this might not hold true. For example, trout and salmon spawning in lowland spring creeks could be disproportionately affected by 1–3 month periods of high nitrate concentration exposure to eggs and fry in these high risk periods. Therefore, care should be exercised in applying the chronic guidelines that offer lower degrees of protection.

Generally, a conservative application of the 95%ile chronic guideline would be applied to discharge or managed inflow (e.g., groundwater intrusions) situations. Under the RMA, a chronic guideline would be applied after consideration of “reasonable mixing” with the receiving water.

Comment on the recommendations made by Carmago et al. (2005). Are there likely to be any sensitive aquatic communities in Canterbury that require a lower nitrate threshold?

Camargo *et al*. (2005) recommend (p1264) “… a maximum level of 2.0 mg NO_3-N/L would be appropriate for protection the most sensitive freshwater species”. This value is similar to that which we have derived using the ANZECC (2000) and the Environment Canada (CCME 2007) methodology with the updated chronic dataset. Notably, the Environment Canada (2003) derives a “interim” water quality guideline of 2.9 mg NO_3-N/L [5].

Canterbury’s groundwaters would be considered in many countries to be pristine and to contain potentially highly sensitive species, however, others are more modified and reflect the cumulative effects of the last 150 years of farming. The ANZECC (2000) guidelines would classify the pristine aquifers as (p3.1-10):

High conservation/ecological value systems *— effectively unmodified or other highly-valued ecosystems, typically (but not always) occurring in national parks, conservation reserves or in remote and/or inaccessible locations. While there are no aquatic ecosystems in Australia and New Zealand that are entirely without some human influence, the ecological integrity of high conservation/ecological value systems is regarded as intact.*

Such environments would be afforded a 99^{th} percentile protection level, which is 1.0 mg NO_3-N/L. However, based on consideration of the species sensitivity distribution in the chronic dataset, we would not recommend application of this data for use in sensitive groundwater environments without first benchmarking the sensitivity of representative key species (e.g., toxicity testing of native amphipods).

The majority of groundwaters (and surface waters) would be classified as:

Slightly to moderately disturbed systems — *ecosystems in which aquatic biological diversity may have been adversely affected to a relatively small but measurable degree by human activity. The biological communities remain in a healthy condition and ecosystem integrity is largely retained. Typically, freshwater systems would have slightly to moderately cleared catchments and/or reasonably intact riparian vegetation; marine systems would have largely intact habitats and associated biological communities. Slightly–moderately disturbed systems could include rural streams receiving runoff from land disturbed to varying degrees*

[5] Note: (i) that the Canadian guideline value is 13 mg NO_3/L, which is multiplied by 0.23 to convert to mg NO_3-N/L; (ii) The Canadian guideline is derived from the measured effect threshold on the most sensitive species with the application of a 0.1x “safety factor”

by grazing or pastoralism, or marine ecosystems lying immediately adjacent to metropolitan areas.

Such environments would be afforded a 95th percentile protection level, which is 1.7 mg NO_3-N/L.

The more modified groundwaters (or surface waters) could be classified as:

Highly disturbed systems. *These are measurably degraded ecosystems of lower ecological value. Examples of highly disturbed systems would be some shipping ports and sections of harbours serving coastal cities, urban streams receiving road and stormwater runoff, or rural streams receiving runoff from intensive agriculture or horticulture.*

Such environments would be afforded a 90th or 80th percentile protection level, which is 2.4 – 3.6 mg NO_3-N/L. Alternatively, a site-specific guideline could be calculated for these environments based on sensitivity measurements for representative local or valued species. Such measurements could be based on either chronic data or acute data with application of the ACR to estimate suitable chronic guidelines.

Advise whether the ANZECC 2000 toxicity limits are appropriate for Canterbury's water bodies, and if not comment on the revised toxicity limits (see 2) and why these have been revised.

As noted earlier, the ANZECC (2000) guidelines for nitrate contains errors in the derivation procedure, although Environment Canterbury has been using the corrected guideline value of 7.2 mg NO_3-N/L[2]. However, our present more detailed review has identified further transcription/calculation errors from the original papers which were not cited in the 2002 review, including the use of potassium nitrate data (see Appendix 3). We have corrected those errors in this review and incorporated the corrected data in this derivation. The original ANZECC (2000) derivation was based on 12 nominal "acute" results with the use of an ACR of 10 to derive the guideline. This review includes updated and expanded data with chronic results for 15 species.

We would recommend that this revised nitrate guideline value for 95% protection of 1.7 mg NO_3-N/L be used for Canterbury's rivers and lakes. Site-specific consideration for seasonally varying background levels (1–3 months duration), could use the lower protection threshold of 3.6 mg NO_3-N/L (80% protection value), if the seasonal period did not specifically serve sensitive species or life-stages, recognising that the remainder of the year would provide higher levels of protection.

Discontinuous point source discharges should not exceed the acute guideline, 20 mg NO_3-N/L, after "reasonable mixing". The acute guideline value could be applicable to either short-term (<96 h) exposures or for application within mixing zones.

5 Recommendations

Some information gaps were identified in undertaking this review. Normally, the requirements for guideline derivation would include representative photo-trophic species (i.e., planktonic algae and/or macrophytes). However, as nitrate is a beneficial nutrient for plant growth, the assessment of sensitivity to plants would not be required for normal environmental exposures.

The datasets are particularly lacking in species which are known to be of high sensitivity to contaminants and dominate the fauna in river environments. Studies have shown that amphipods, mayflies and some native fish species are more sensitive to some chemical contaminants than the standard international test species such as cladocerans and rainbow trout (Hickey 2000). No information is currently available on the sensitivity of native fish species to nitrate.

Validation of the nitrate guideline value could use a combination of laboratory testing with selected species and field assessment validation of effects on invertebrate communities. River sites with high nitrate groundwater inflows could provide suitable sites with gradients of mixed concentrations suitable for these studies. Environment Canada (CCME 2007) does not accept field studies for guideline derivation because the sites usually have a range of unmeasured variables (stressors) operating between sites, but they serve useful validation for laboratory-based tests.

However, for nitrate, we consider that a range of sites may be found which would provide a suitable basis for a field-based validation of guidelines. We have previously used this approach for investigating thresholds for effects on stream macroinvertebrates of: oxidation ponds (Quinn and Hickey 1993); inorganic suspended solids (Quinn *et al*. 1992); heavy metals (Hickey and Clements 1998); substrate particle size (Quinn and Hickey 1990b); and land-use development (Quinn and Hickey 1990a). We have also successfully used river mesocosms for establishing macroinvertebrate thresholds for ammoniacal-N (Hickey *et al*. 1999) and heavy metals (Hickey and Golding 2002).

A summary of the 2009 revision of the freshwater nitrate guidelines suitable for application to freshwaters of Canterbury is provided in Table 5.1.

Table 5.1 Summary of site-specific guidelines for nitrate (NO_3-N) for application to freshwater environments in Canterbury

Guideline type	**Application to:**	**Guideline value (mg NO_3-N/L)[a]**
Acute	Very localised point source discharges.	20 mg NO_3-N/L
Chronic – high conservation value systems (99% protection)	Pristine environments with high biodiversity and conservation values.	1.0 mg NO_3-N/L
Chronic – slightly to moderately disturbed systems (95% protection)	Environments which are subjected to a range of disturbances from human activity.	1.7 mg NO_3-N/L
Chronic – highly disturbed systems (80 to 90% protection)	Specific environments which: (i) either have measurable degradation; or (ii) which receive seasonally high elevated background concentrations for significant periods of the year (1-3 months).	2.4 – 3.6 mg NO_3-N/L
Chronic – site-specific (species-specific protection)	Collection of specific data for representative species and life-stages with calculation of site-specific guideline values.	No data

[a] Multiply by conversion factor of 4.43x to convert to NO_3

6 Acknowledgements

This work was supported by Environment Canterbury, with a contribution from the Ministry for the Environment. The authors wish to thank the Dr Rick van Dam and Dr Andrew Harford, Environmental Research Institute of the Supervising Scientist, Australia for undertaking a comprehensive review of an earlier version the report, and staff at Environment Canterbury for their comments.

7 Glossary

Acute toxicity Is a discernible adverse effect (lethal or sublethal) induced in the test organisms within a short period (relative to the duration of the species life cycle) of exposure to a test material.

Chronic toxicity Implies long-term effects that are related to changes in metabolism, development, growth, reproduction, or ability to survive. In this test, chronic toxicity is a discernible adverse effect (lethal or sublethal) induced in the test organism during a significant and sensitive part of the life-cycle.

EC_{50} Is the median effective concentration (i.e., the concentration of material in water that is estimated to produce a specifically quantified effect to 50% of the test organisms) after a specified exposure time. The EC_{50} and its 95% confidence limits are usually derived by statistical analysis of a quantal, "all or nothing", response (such as death, fertilization, germination, or development) in several test concentrations, after a fixed period of exposure.

Endpoint The adverse biological response in question that is measured. May vary with the level of biological organisation examined, but may include biochemical markers, mortality or reproduction. End points are used in toxicity tests as criteria for effects.

IC_{50} Is the median inhibition concentration, i.e., the concentration estimated to cause a 50% reduction in growth compared to a control. The exposure time must be specified, e.g., "IC_{50} (72 h)", for a growth rate derived IC_{50} and a test duration of 72 h.

Indigenous Species that have evolved in or spread naturally into this habitat. Sometimes termed **native** or **endemic** species.

Introduced Species that have become able to survive and reproduce outside the habitats where they evolved or spread naturally. Sometimes termed **exotic** or **non-indigenous** species.

Lethal Means causing death by direct action. Death of fish is defined as the cessation of all visible signs of movement or other activity.

LC_{50}, LC_{20} The lethal toxicant concentration resulting in a 50% or 20% mortality (respectively) at a specific time of exposure, (e.g. 48 hr LC_{50}).

LOEC Lowest observed effect concentration. The lowest concentration tested causing a statistically measurable effect to the test system. Derivation of this value is strongly influenced by the selected test concentrations.

MATC Maximum Acceptable Toxicant Concentration = geometric mean of NOEC + LOEC.

NOEC	No observed effect concentration. The highest concentration tested causing no statistically measurable effect to the test system. Derivation of this value is strongly influenced by the selected test concentrations.
Representative	Species that have the same genus in this habitat.
TEC	Threshold Effect Concentration = geometric mean of NOEC + LOEC.
Toxicity test	Is a method to determine the effect of a material on a group of selected organisms under defined conditions. An aquatic toxicity test usually measures either (a) the proportions of organisms affected (quantal) (e.g., by measuring EC_{50}), or (b) the degree of effect shown (graded or quantitative) after exposure to specific concentrations of whole effluents or receiving water as measured by an IC_{50}.
Toxicity	Is the inherent potential or capacity of a material to cause adverse effects on living organisms.

8 List of Acronyms

ANZECC	Australian and New Zealand Environment and Conservation Council
CAS	Chemical Abstracts Service
CCME	Canadian Council of Ministers of the Environment
CCREM	Canadian Council of Resource and Environment Ministers
CV	coefficient of variation
[C]WQG	[Canadian] Water Quality Guidelines
DIN	dissolved inorganic nitrogen
DO	dissolved oxygen
DOC	dissolved organic carbon
DOM	dissolved organic matter
DON	dissolved organic nitrogen
EC	effects concentration
ECan	Environment Canterbury
EC_{50}	median effects concentration
KNO_3	potassium nitrate
LC_{50}	median lethal concentration
LO[A]EL	lowest observed [adverse] effects level
LOEC	lowest observed effects concentration
MATC	Maximum Acceptable Toxicant Concentration
N_2	molecular nitrogen
$NaNO_3$	sodium nitrate
NH_3	un-ionized ammonia
NH_4^+	ammonium ion
NH_4NO_3	ammonium nitrate
NO_2	nitrite
NO_3	nitrate
NO_3-N	nitrate-nitrogen
NO[A]EL	no observed [adverse] effects level
NOEC	no observed effect concentration
SSD	species sensitivity distribution

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Appendix 1 Effect codes

Table A1.1 Effects codes used (from ANZECC 2000)

Effect Code	Effect
ABD	Abundance
ABN	Abnormality
BIOLUM	Bioluminescent
BIOMASS	Biomass
DVP	Development
EMR	Emergence
FRT	Fertilisation
GRO	Growth (length or weight)
HAT	Hatchability
IMM	Immobilisation
LUM	Luminescent
MOR	Mortality
NR	Not Recorded
PGR	Population growth rate
POP	Population
PRP	Predator-prey dynamics
PSE	Photosynthesis
PSR	Photosynthetic rate
REP	Reproduction

Appendix 2 Data quality assessment

The ANZECC (2000) water quality guidelines used a data documentation system based on the US EPA AQUIRE classification system operating at that time. The fields and score allocations are summarised below (Table 8.3.1 from ANZECC 2000). The scores were categorised as: complete ("C" = 86-100); moderate ("M" = 51-85) or incomplete ("I" = <51). Data with an "I" classification were not included in the guideline derivation procedure without special consideration. ANECC (2000) also provides guidance on dealing with outlying data (section 8.3.4.2).

Table 8.3.1 AQUIRE (1994) fields and scores

AQUIRE Field	Score Points
Exposure duration	20
Control type	5
Organism characteristics	5
Chemical analysis method	5
Exposure type	5
Test location	4
Chemical grade	4
Test media	4
Hardness (freshwater exposures) Salinity (saltwater) 4 total	2
Alkalinity (freshwater exposures) Salinity (saltwater)	2
Dissolved oxygen	2
Temperature	2
pH	2
End-point	20
Trend of effect	5
Effect percent	5
Statistical significance	4
Significance level	4

Environment Canada (CCME 2007) provides specific recommendations for assessing data quality based on and a three level classification system. These are:

(i) "Primary data", with requirements including: toxicity tests must employ currently acceptable laboratory or field practices of exposure and environmental controls; as a minimum requirement for primary data, substance concentrations must be measured at the beginning and end of the exposure period; generally, static laboratory tests are not classified as primary data unless it can be shown that substance concentrations did not change during the test; preferred test endpoints from a partial or full life-cycle test include a determination of effects on embryonic development, hatching, or germination success, survival of juvenile stages, growth, reproduction, and survival of adults. Additional test endpoints, such as behavioural or endocrine-disrupting effects, can be included if it can be shown that these effects are a result of exposure to the parameter in question, lead to an ecologically relevant negative impact, and are scientifically sound; a clear dose-response relationship should be demonstrated in the study; controlled microcosm and mesocosm studies are acceptable and are ranked according to the applicable categorization criteria.

(ii) “Secondary data”, with requirements including: Secondary data are those that originate from studies where primary data cannot be generated, but are still of acceptable quality and documentation. Toxicity tests may employ a wider array of methodologies (e.g., measuring toxicity while test species are exposed to additional stresses such as low temperatures, lack of food, or high salinity). All relevant environmental variables that modify toxicity should be measured and reported. The survival of controls must be measured and reported; static tests, calculated substance concentrations, and measurements taken in stock solutions are generally acceptable; appropriate test replication is necessary; Preferred test endpoints include those listed for primary data as well as pathological, behavioural (if their ecological relevance can be shown, but not as clearly as for primary data), and physiological effects.

(iii) “Unacceptable data”, with requirements including: Toxicity data that do not meet the criteria of primary or secondary data are unacceptable for guideline derivation purposes. Unacceptable data cannot be used to fulfil minimum data set requirements for any derivation procedure; these data should be discussed and the reasons for their rejection clearly stated.

Appendix 3: June 2009 review of the ANZECC (2000) nitrate guideline derivation

Table A3.1 Original document from ANZECC (2000) document: "TOX-TVderivation.pdf" (p26), showing error in initial derivation. Note: this does not include new acute or chronic data.

Nitrate (NO_3)
Freshwater
Fails HR
MR Calculations

The data used to derive the TV were:

~~340406.4~~ 4854500 (a)	~~896847.9~~ (b)	~~7630126~~ 6391817 (c)	~~5582000~~ 5584429 (d)
~~14000~~ (e)	5998000	5799000	~~720085.7~~ 1252086 (f)
733058.5	576645.9	~~723490.7~~ 2198980 (g)	~~9000~~ (e)

HC1 50% = ~~167.4~~ 139330
HC5 50% = ~~6855~~ 482018
HC10 50% = ~~33909~~ 823270
HC20 50% = ~~167737~~ 1411644

NOTE: Because this TV was derived using acute toxicity data it is a MR TV and must be divided by either a default AF of 10 or an ACR. There was no ACR for this chemical.

~~MR TV = 6855/10 = 685.5 = 685 µg/L~~

MR TV = 482018/10 = 48201.8 = 48201 µg/L

The other levels of protection are:

99%	95%	90%	80%
~~16.74~~	~~685.5~~	~~3390.9~~	~~16773.7~~
13933	48201	82327	141164

These were rounded off to

~~17 700 3400 17 000 µg/L~~

2009 conversion to mg NO_3-N (x0.00023)

99%	**95%**	**90%**	**80%**
3.2	**11.1**	**18.9**	**32.5**

Footnotes for 2009 mark-up revision:

(a) corrected value based on highest time exposure only (Eastern mosquito fish)
(b) potassium salt (Guppy))
(c) 10x transcription error (Bluegills)
(d) 10x transcription error (Guadalupe bass)
(e) potassium salts and chronic tests (Purple Spotted Gudgeon & Hydra)
(f) errors in database (Daphnia)
(g) database errors (Pond snail – *Lymnaea*)

Table A3.2 Marked-up download from ANZECC (2000) database (downloaded 2/8/2002) showing corrected nitrate (NO_3) concentrations and identifying potassium nitrate values

Water Quality Search Results **Date:** **2/08/2002 download from ANZECC 2000 database**

Toxicant **nitrate (NO_3)**

Latin Name / Common	Test Media	Test Type	Duration (h)	Endpoint	Effect	Concent-ration Used	Units	Ref ID	Ref	Notes/Comments	Corrected NO_3 Conc	Use Y/N
Fish												
U											ug/L NO_3	
Gambusia holbrooki Eastern mosquitofish	Freshwater	Acute	48	LC50	MORT	1.00E+07	ug/L	200508	Wallen et al. (1957)	NaNO3 value. Correct value from paper	7300000	N
U												
Gambusia holbrooki Eastern mosquitofish	Freshwater	Acute	48	LC50	MORT	137000	ug/L	200508	Wallen et al. (1957)	Potassium nitrate value	no value	N
U												
Gambusia holbrooki Eastern mosquitofish	Freshwater	Acute	96	LC50	MORT	99000	ug/L	200508	Wallen et al. (1957)	NaNO3 value. Correct value from paper	4854500	Y
U												
Gambusia holbrooki Eastern mosquitofish	Freshwater	Acute	96	LC50	MORT	99000	ug/L	200508	Wallen et al. (1957)	Potassium nitrate value	no value	N
Geometric	340406.42									all 4 above geomean	5952969.8	
U												
Lebistes reticulatus Guppy	Freshwater	Acute	48	LC50	MORT	969000	ug/L	207635	Rubin & Elmargahy (1997)	Potassium salt	no value	N
U												
Lebistes reticulatus Guppy	Freshwater	Acute	72	LC50	MORT	881000	ug/L	207635	Rubin & Elmargahy (1997)	Potassium salt	no value	N
U												
Lebistes reticulatus Guppy	Freshwater	Acute	96	LC50	MORT	845000	ug/L	207635	Rubin & Elmargahy (1997)	Potassium salt	no value	N
Geometric	896847.91											
U												
Lepomis macrochirus Bluegill	Freshwater	Acute	96	LC50	MORT	1.42E+07	ug/L	208037	Trama (1954)	Not sodium nitrate - could be calcium value (?).	no value	N
U												
Lepomis macrochirus Bluegill	Freshwater	Acute	96	LC50	MORT	885300	ug/L	208037	Trama (1954)	Wrong value, possibly incorrectly calculated, this value used in Camargo (2005)	1973000	

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Toxicant **nitrate**

Latin Name Common	Test Media	Test Type	Duration (h)	Endpoint	Effect	Concent-ration Used	Units	Ref ID	Ref	Notes	Corrected Conc	Use Y/N
U *Lepomis macrochirus* Bluegill	Freshwater	Acute	96	LC50	MORT	900000	ug/L	200930	Cairns & Scheier (1959)	10x error, value from Aquire	9000000	Y
U *Lepomis macrochirus* Bluegill	Freshwater	Acute	96	LC50	MORT	940000	ug/L	200930	Cairns & Scheier (1959)	10x error, value from Aquire	9400000	Y
U *Lepomis macrochirus* Bluegill	Freshwater	Acute	96	LC50	MORT	186000	ug/L	208037	Trama (1954)	Potassium nitrate value	no value	N
U *Lepomis macrochirus* Bluegill	Freshwater	Acute	96	LC50	MORT	1.00E+07	ug/L	200930	Cairns & Scheier (1959)	Wrongly transcribed	10000000	Y
Geometric	7630126.3											
U *Micropterus treculi* Guadalupe bass	Freshwater	Acute	96	LC50	MORT	558200	ug/L	211794	Tomasso & Carmichael (1986)	10x error, value confirmed from paper	5584428.6	Y
Geometric	5582000											
U *Oncorhynchus mykiss* Rainbow trout	Freshwater	Acute	96	LC50	MORT	599800	ug/L	205115	Westin (1974)	10x error, confirmed from paper	6000000	Y
Geometric	5998000									correct value		
U *Oncorhynchus* Chinook salmon	Freshwater	Acute	96	LC50	MORT	579900	ug/L	205115	Westin (1974)	10x error, confrimed from paper	5800000	Y
Geometric	5799000									correct value		
crustaceans												
U *Daphnia magna* Water flea	Freshwater	Acute	48	LC50	MORT	358100	ug/L	200915	Dowden & Bennett (1965)	10x error, NaNO3 value	2612330	N
U *Daphnia magna* Water flea	Freshwater	Acute	48	LC50	MORT	358100	ug/L	202465	Dowden (1961)	10x error, NaNO3 value	2610200	Y
U *Daphnia magna* Water flea	Freshwater	Acute	48	LC50	MORT	301000	ug/L	200915	Dowden & Bennett (1965)	Potassium nitrate value	no value	N

nitrate

Latin Name **Common**	**Test Media**	**Test Type**	**Duration (h)**	**Endpoint**	**Effect**	**Concent-ration Used**	**Units**	**Ref ID**	**Ref**	**Notes**	**Corrected Conc**	Use Y/N
U *Daphnia magna* Water flea	Freshwater	Acute	72	LC50	MORT	212500	ug/L	200915	Dowden & Bennett (1965)	calculated from paper	1550182.4	Y
U *Daphnia magna* Water flea	Freshwater	Acute	72	LC50	MORT	137000	ug/L	200915	Dowden & Bennett (1965)	Potassium nitrate value	no value	N
U *Daphnia magna* Water flea	Freshwater	Acute	96	LC50	MORT	420600	ug/L	200915	Dowden & Bennett (1965)	calculated from paper	485115.9	Y
U *Daphnia magna* Water flea	Freshwater	Acute	96	LC50	MORT	665000	ug/L	200915	Dowden & Bennett (1965)	Potassium nitrate value	no value	N
U *Daphnia magna* Water flea	Freshwater	Acute	96	LC50	MORT	23000	ug/L	200915	Dowden & Bennett (1965)	Potassium nitrate value	no value	N
Geometric	720085.68											
Insects												
U *Cheumatopsyche pettiti* Caddisfly	Freshwater	Acute	72	EC50	MORT	845000	ug/L	203879	Camargo & Ward (1992)	Calculated from paper, rounded	845000	Y
U *Cheumatopsyche pettiti* Caddisfly	Freshwater	Acute	72	EC50	MORT	930000	ug/L	203879	Camargo & Ward (1992)	Calculated from paper, rounded	930000	Y
U *Cheumatopsyche pettiti* Caddisfly	Freshwater	Acute	96	EC50	MORT	732000	ug/L	203879	Camargo & Ward (1992)	Calculated from paper, rounded	732000	Y
U *Cheumatopsyche pettiti* Caddisfly	Freshwater	Acute	96	EC50	MORT	502000	ug/L	203879	Camargo & Ward (1992)	Calculated from paper, rounded	502000	Y
Geometric	733058.47											
U *Hydropsyche* Caddisfly	Freshwater	Acute	72	LC50	MORT	657000	ug/L	203879	Camargo & Ward (1992)	Calculated from paper, rounded	657000	Y
U *Hydropsyche* Caddisfly	Freshwater	Acute	72	LC50	MORT	812000	ug/L	203879	Camargo & Ward (1992)	Calculated from paper, rounded	812000	Y

nitrate

Latin Name Common	Test Media	Test Type	Duration (h)	Endpoint	Effect	Concent-ration Used	Units	Ref ID	Ref	Notes	Corrected Conc	Use Y/N
U												
Hydropsyche Caddisfly	Freshwater	Acute	96	LC50	MORT	430000	ug/L	203879	Camargo & Ward (1992)	Calculated from paper, rounded	430000	Y
U												
Hydropsyche Caddisfly	Freshwater	Acute	96	LC50	MORT	482000	ug/L	203879	Camargo & Ward (1992)	Calculated from paper, rounded	482000	Y
Geometric	576645.92											
Molluscs												
U												
Lymnaea sp Pond snail	Freshwater	Acute	48	EC50	HAT	914000	ug/L	200508	Wallen (1957)	Potassium nitrate value. Not in Wallen (1957) based on AQUIRE; may be from Dowden & Bennet (1965) (?) --> corrected conc.	4712554.4	Y
U												
Lymnaea sp Pond snail	Freshwater	Acute	72	EC50	HAT	624000	ug/L	200508	Wallen (1957)	See above comment.	4340510.6	Y
U												
Lymnaea sp Pond snail	Freshwater	Acute	96	EC50	HAT	664000	ug/L	200915	Dowden & Bennett (1965)	See above comment.	2371596.7	Y
Geometric	723490.7											
Fish												
U												N
Mogurnda mogurnda Purple SpottedGudgeon	Freshwater	Chronic	216	NOEC	MORT	14000	ug/L	300119	Rippon & McBride	Potassium salt	no value	
Geometric	14000											
Coelentrates												
U												
Hydra viridissima Hydra	Freshwater	Chronic	144	NOEC	PGR	9000	ug/L	300119	Rippon & McBride	Potassium salt	no value	N
Geometric	9000											
Fish												
U												
Centropristis striata Black sea bass	Marine	Acute	96	LC50	MORT	1.0624E	ug/L	209424		Marine	no value	N
Geometric	10624000											

nitrate

U												
Monacanthus hispidus	Marine	Acute	96	LC50	MORT	253600	ug/L	209424		Marine	no value	N
Plane headFilefish												
Geometric	2536000											
U												
Oncorhynchus mykiss	Marine	Acute	96	LC50	MORT	465000	ug/L	205115	Westin (1974)	Marine 7d value	no value	N
Rainbow trout												
Geometric	4650000											
U												
Oncorhynchus	Marine	Acute	96	LC50	MORT	440200	ug/L	205115	Westin (1974)	Marine 7d value	no value	N
Chinook salmon												
Geometric	4402000											
U												
Pomacentrus	Marine	Acute	96	LC50	MORT	1.328E+	ug/L	209424				
Beaugregory												
Geometric	13280000											
U												
Trachinotus carolinus	Marine	Acute	96	LC50	MORT	442600	ug/L	209424		Marine		N
Florida pompano												
Geometric	4426000											
Molluscs												
U												
Crassostrea virginica	Marine	Acute	96	EC50	MORT	1.6821E	ug/L	205098		Marine		N
American or virginia												
U												
Crassostrea virginica	Marine	Acute	96	EC50	MORT	1.1509E	ug/L	205098		Marine		N
American or virginia												
U												
Crassostrea virginica	Marine	Acute	96	EC50	MORT	1.8946E	ug/L	205098		Marine		N
American or virginia												
U												
Crassostrea virginica	Marine	Acute	96	EC50	MORT	2.7578E	ug/L	205098		Marine		N
American or virginia												
Geometric	17833740											

nitrate

U - Unmodified	HC - Hardness	T - Unmodified Total
C - Converted NOEC	UI - Unmodifie	Tp -Total at pH8.0
H - Hardness Corrected	UD - Unmodifi	TpC -Total at pH8.0, Converted NOEC

Appendix 4: Revised nitrate guideline derivation

Table A4.1 A,B Acute data from database showing reference codes and classifications

A

Group	Common	Latin Name	Life Stage	Duration(h	Endpoint	Effect	Temp	EC_{50}/LC_{50} (mg NO_3-N/L)	Analysis	Author
Invertebrate	Amphipod	*Echinogammarus echinosetosus*	Adults	120h	LC50	MOR	17.9	56.2	U	Camargo et al (2005)
Invertebrate	Amphipod	*Eulimnogammarus toletanus*	Adults	120h	LC50	MOR	17.9	73.1	U	Camargo et al (2005)
Invertebrate	Caddisfly	*Hydropsyche accidentalis*	Last instar larvae	120h	EC50	MOR	18	77.2	M	Camargo & Ward (1992)
Invertebrate	Caddisfly	*Cheumatopsyche pettiti*	Early instar larvae	120h	LC50	MOR	18	106.5	M	Camargo & Ward (1992)
Invertebrate	Caddisfly	*Hydropsyche exocellata*	Last instar larvae	120h	LC50	MOR	17.9	230.2	U	Camargo et al (2005)
Amphibian	Pacific Treefrog	*Pseudacris regilla*	tadpoles	10d	LC50	MOR	22	266.2	M	Schuytema & Nebeker (1999c)
Invertebrate	Water flea	*Ceriodaphnia dubia*	Neonates	48h	LC50	MOR	25	374.0	M	Scott & Crunkilton (2000)
Fish	Siberian sturgeon	*Acipenser baeri*	Adults	96h	LC50	MOR	22.5	397.0	M	Hamlin (2006)
Invertebrate	Water flea	*Daphnia magna*		48h	EC50	MOR		479.1		**Geometric mean**
Invertebrate	Snail	*Lymnaea sp*	eggs	96h	LC50	HAT	NR	535.5	NR	Dowden & Bennett (1965)
Invertebrate	Snail	*Potamopyrgus antipodarum*	Adults	96h	LC50	MOR	20.4	1042.0	U	Alonso & Camargo (2003)
Fish	Rainbow trout	*Oncorhynchus mykiss*	fingerlings	7d	LC50	MOR	13-14	1061.0	M	Westin (1974)
Fish	Chinook salmon	*Oncorhynchus tshawytscha*	fingerlings	7d	LC50	MOR	13-14	1083.9	M	Westin (1974)
Fish	Eastern mosquitofish	*Gambusia holbrooki*		96h	LC50	MOR		1095.4		Wallen et al.(1957)
Fish	Lake Trout	*Salvelinus namaycush*	fry	96h	LC50	MOR	7.5	1121.4	M	McGurk et al (2006)
Amphibian	African clawed frog	*Xenopus laevis*	tadpoles	10d	LC50	MOR	22	1236.2	M	Schuytema & Nebeker (1999c)
Fish	Fathead minnows	*Pimephales promelas*		96h	LC50	MOR		1316.6		**Geometric mean**
Fish	Catfish	*Ictalurus punctatus*	Fingerlings	96h	LC50	MOR	22	1355.0	unknown	Colt & Tchobanoglous (1976)
Fish	Lake Whitefish	*Coregonus clupeaformis*	fry	96h	LC50	MOR	7.5	1902.7	M	McGurk et al (2006)
Fish	Bluegill	*Lepomis macrochirus*	fingerlings	96h	LC50	MOR		2094.0		**Geometric mean**

B

Author	Env Canada classification	AQUIRE ref ID	ANZECC ref ID	NIWA ref ID	ANZECC ref classification	Group	Common	Latin Name
Camargo et al (2005)				500006	M	Invertebrate	Amphipod	*Echinogammarus echinosetosus*
Camargo et al (2005)				500006	M	Invertebrate	Amphipod	*Eulimnogammarus toletanus*
Camargo & Ward (1992)	1	3879	203879	203879	M	Invertebrate	Caddisfly	*Hydropsyche accidentalis*
Camargo & Ward (1992)	1	3879	203879	203879	M	Invertebrate	Caddisfly	*Cheumatopsyche pettiti*
Camargo et al (2005)				500006	M	Invertebrate	Caddisfly	*Hydropsyche exocellata*
Schuytema & Nebeker (1999c)	1	20488		6020488		Amphibian	Pacific Treefrog	*Pseudacris regilla*
Scott & Crunkilton (2000)	1			400001	C	Invertebrate	Water flea	*Ceriodaphnia dubia*
Hamlin (2006)				500005		Fish	Siberian sturgeon	*Acipenser baeri*
Geometric mean						Invertebrate	Water flea	*Daphnia magna*
Dowden & Bennett (1965)	A, b, c	915	200915	200915		Invertebrate	Snail	*Lymnaea sp*
Alonso & Camargo (2003)				500008	M	Invertebrate	Snail	*Potamopyrgus antipodarum*
Westin (1974)	2	5115	205115	205115	M	Fish	Rainbow trout	*Oncorhynchus mykiss*
Westin (1974)	2	5115	205115	205115	M	Fish	Chinook salmon	*Oncorhynchus tshawytscha*
Wallen et al.(1957)		508	200508	200508		Fish	Eastern mosquitofish	*Gambusia holbrooki*
McGurk et al (2006)		95870		6095870	C	Fish	Lake Trout	*Salvelinus namaycush*
Schuytema & Nebeker (1999c)	1	20488		6020488		Amphibian	African clawed frog	*Xenopus laevis*
Geometric mean						Fish	Fathead minnows	*Pimephales promelas*
Colt & Tchobanoglous (1976)	2			400002		Fish	Catfish	*Ictalurus punctatus*
McGurk et al (2006)		95870		6095870	C	Fish	Lake Whitefish	*Coregonus clupeaformis*
Geometric mean						Fish	Bluegill	*Lepomis macrochirus*

Note: see Environment Canada footnotes under Chronic data

Figure A4.1 Acute data for 2009 revision fitted to BurIII model (ANZECC 2000).

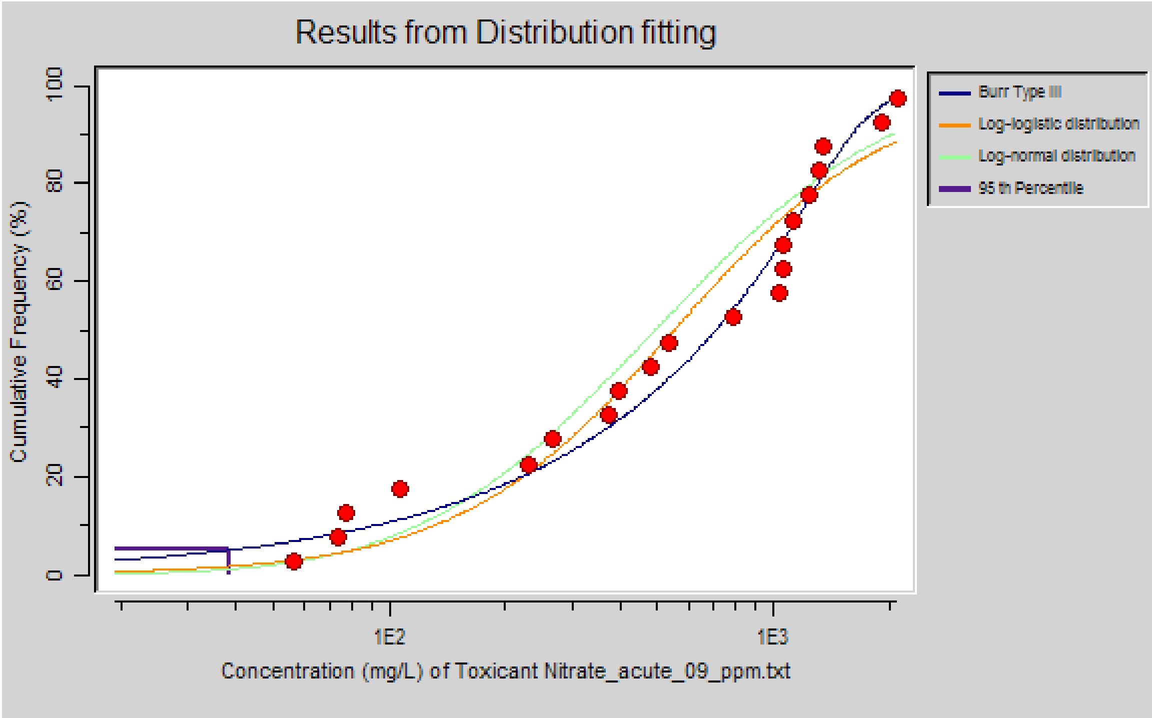

Table A4.2 A,B Chronic data from database showing reference codes and classifications

A

Group	Common name	Scientific name	Converted	Life Stage	Exposure conditions	Test Type	Duration (h/d)	Endpoint	Effect	Temp	NOEC (mg/L NO_3-N)	LOEC (mg/L NO_3-N)	Analysis	Author
Fish	Lake Trout	*Salvelinus namaycush*	U	Fry	R	Chronic	146d	NOEC	DVP	7.5	1.6	6.25	M	McGurk et al (2006)
Fish	Lake Trout	*Salvelinus namaycush*	U	Fry	R	Chronic	146d	NOEC	GRO	7.5	1.6	6.25	M	McGurk et al (2006)
Fish	Rainbow trout	*Oncorhynchus mykiss*	U	Fry	F	Chronic	30d	NOEC	MOR	10	2.2	2.3, >4.5	NR	Kinchloe et al (1979) (Geo mean)
Fish	Chinook salmon	*tshawytscha*	U	Fry	F	Chronic	30d	NOEC	MOR	10	2.3	4.5	NR	Kinchloe et al (1979)
Fish	Lahontan cutthroat trout	*Salmo clarki*	U	Fry	F	Chronic	30d	NOEC	MOR	13	4.5	7.6	NR	Kinchloe et al (1979)
Fish	Coho salmon	*Oncorhynchus kisutch*	U	Fry	F	Chronic	30d	NOEC	MOR	10	>4.5	>4.5	NR	Kinchloe et al (1979)
Fish	Lake Whitefish	*Coregonus clupeaformis*	U	Fry	R	Chronic	126d	NOEC	DVP	7.5	6.25	25	M	McGurk et al (2006)
Amphibian	American Toad	*Bufo americanus*	C	Egg	R	Chronic	23d	NOEC	HAT	5-10	>9.26		M	Laposata & Dunson (1998)
Amphibian	Pacific Treefrog	*Pseudacris regilla*	U	tadpoles	R	Chronic	10d	NOEC	GRO	22	12.0		M	Schuytema & Nebeker (1999c)
Invertebrate	Freshwater crayfish	*Astacus astacus*	U			Acute	7d	NOAEL	MOR	15	>14.0		U	Jensen (1996)
Invertebrate	Water flea	*Ceriodaphnia dubia*		neonates							15.6			Scott & Crunkilton (2000) (Geo mean)
Amphibian	African clawed frog	*Xenopus laevis*	C	Embryo	R	Chronic	120h	NOEC	GRO	22	24.8		M	Schuytema & Nebeker (1999a)
Fish	Lake Whitefish	*Coregonus clupeaformis*	U	Fry	R	Chronic	126d	NOEC	MOR	7.5	25.0	100	M	McGurk et al (2006)
Invertebrate	Florida apple snail	*Pomacea paludosa*									25.3			Corrao et al (2006) (Geo mean)
Fish	Fathead minnows	*Pimephales promelas*	U	Embryos and larvae	F	Chronic	11d	NOEC	MOR	25	358		U	Scott & Crunkilton (2000)
Invertebrate	Water flea	*Daphnia magna*	U	neonates	S, R	Chronic	7d	NOEC	REP	25	358		U	Scott & Crunkilton (2000)

B

Author	Env Canada classification	AQUIRE ref ID	ANZECC ref ID	NIWA ref iD	Classification	Selector	Group	Common name	Scientific name
McGurk et al (2006)		95870		6095870	C	N	Fish	Lake Trout	*Salvelinus namaycush*
McGurk et al (2006)		95870		6095870	C	Y	Fish	Lake Trout	*Salvelinus namaycush*
Kinchloe et al (1979) (Geo mean)	A	95870		6095870	C	Y	Fish	Rainbow trout	*Oncorhynchus mykiss*
Kinchloe et al (1979)	A			400003	C	Y	Fish	Chinook salmon	*Oncorhynchus tshawytscha*
Kinchloe et al (1979)	A			400003	C	Y	Fish	Lahontan cutthroat trout	*Salmo clarki*
Kinchloe et al (1979)	A			400003	C	Y	Fish	Coho salmon	*Oncorhynchus kisutch*
McGurk et al (2006)		95870		6095870	C	Y	Fish	Lake Whitefish	*Coregonus clupeaformis*
Laposata & Dunson (1998)	2, I	19803		6019803		Y	Amphibian	American Toad	*Bufo americanus*
Schuytema & Nebeker (1999c)	1	20488		6020488		Y	Amphibian	Pacific Treefrog	*Pseudacris regilla*
Jensen (1996)				500009		Y	Invertebrate	Freshwater crayfish	*Astacus astacus*
Scott & Crunkilton (2000) (Geo mean)				400001	C	Y	Invertebrate	Water flea	*Ceriodaphnia dubia*
Schuytema & Nebeker (1999a)	1			500010		Y	Amphibian	African clawed frog	*Xenopus laevis*
McGurk et al (2006)		95870		6095870	C	Y	Fish	Lake Whitefish	*Coregonus clupeaformis*
Corrao et al (2006) (Geo mean)				500007		Y	Invertebrate	Florida apple snail	*Pomacea paludosa*
Scott & Crunkilton (2000)	1			400001	C	Y	Fish	Fathead minnows	*Pimephales promelas*
Scott & Crunkilton (2000)	1			400001	C	Y	Invertebrate	Water flea	*Daphnia magna*

Notes: ND = no data provided; NR = not recorded
Test Types: R = renewal, S = static, F = flow-through
Environment Canada footnotes: Ranking Scheme: 1 = primary source, 2 = secondary source, A = ancillary source
a $LC_{0.01}$ extrapolated from Camargo and Ward (1992) LC_{50} data, therefore not used in guideline development
b tests run with filtered local lake water
c insufficient test details / water quality information provided
d lack of statistical support
e non-resident, or tropical species
f distilled water used as test medium
g lack of clear dose-response relationship
h potassium salts not suitable for guideline derivation
i inadequate test design or conditions
j control mortality > 10%
k organisms only exposed to one test concentration
l lowest observable effect level beyond nitrate concentration range tested
m >10% change in nitrate concentration in test containers
n the ecological significance of this endpoint is uncertain

Acute-to-chronic ratio (ACR)

Table A4.3: Acute and chronic toxicity data used for acute-to-chronic ratio (ACR) calculation. Highlight (white on black) indicates species which are present in Canterbury's rivers and lakes.

			Acute		Chronic		ACR	Reference
Group	**Common Name**	**Species**		**LC_{50} mg/L NO_3-N [a]**		**NOEC mg/L NO_3-N [a]**		
Fish	**Lake Trout**	***Salvelinus namaycush***	**96 h swim up fry survival**	**1121**	**Embryo to swim up fry survival**	**100**	**11.2**	**McGurk *et al.* (2006)**
Fish	Lake Whitefish	*Coregonus clupeaformis*	96 h swim up fry survival	1903	Embryo to swim up fry survival	25	76.1	McGurk *et al.* (2006)
Fish	Fathead minnow	*Pimephales promelas*	96 h Survival	1317	Larvae 7 d post hatch growth	358	3.7	Scott & Crunkilton (2000)
Crustacea	Water flea	*Daphnia magna*	48h Survival	447	7d reproduction	358	1.2	Scott & Crunkilton (2000)
Crustacea	Water flea	*Ceriodaphnia dubia*	48h Survival	374	7d reproduction (geometric mean of 5)	15.6	24.0	Scott & Crunkilton (2000)
							9.9	**Geometric mean**

[a] Multiply by conversion factor of 4.43x to convert to NO_3

Table A4.4: Species list for all species in the nitrate database.

Species No.	Scientific name	Group	Commoni name
1	*Gambusia holbrooki (G. Affinis)*	Fish	Eastern mosquitofish
2	*Lebistes reticulatus*	Fish	Guppy
3	*Lepomis macrochirus*	Fish	Bluegill
4	*Micropterus treculi*	Fish	Guadalupe bass
5	*Oncorhynchus mykiss*	Fish	Rainbow trout (nonanadromous)
6	*Oncorhynchus tshawytscha*	Fish	Chinook salmon
7	*Coregonus clupeaformis*	Fish	Lake whitefish
8	*Salvelinus namaycush*	Fish	Lake trout
9	*Catla catla*	Fish	Indian major carp
10	*Labeo rohita*	Fish	Carp (Roha)
11	*Cirrhinus mrigala*	Fish	Mrigal Carp
12	*Cyprinus carpio*	Fish	Common carp
13	*Ctenopharyngodon idella*	Fish	Grass Carp
14	*Acipenser baeri*	Fish	Siberian sturgeon
15	*Pimephales promelas*	Fish	Fathead minnow
16	*Ictalurus punctatus*	Fish	Catfish
17	*Carassius carassius*	Fish	Crucian carp
18	*Oncorhynchus mykiss*	Fish	Rainbow trout (Steelhead anadromous)
19	*Salmo clarki*	Fish	Lahontan cutthrpoat trout
20	*Daphnia magna*	Invertebrate	Waterflea
21	*Mogurnda mogurnda*	Fish	Purple spotted gudgeon
22	*Salvelinus namaycush*	Fish	Lake trout
23	*Ceriodaphnia dubia*	Invertebrate	Waterflea
24	*Cheumatopsyche pettiti*	Invertebrate	Caddisfly
25	*Hydropsyche accidentalis*	Invertebrate	Caddisfly
26	*Eulimnogammarus toletanus*	Invertebrate	Amphipod
27	*Echinogammarus echinosetosus*	Invertebrate	Amphipod
28	*Hydropsyche exocellata*	Invertebrate	Caddisfly
29	*Oncorhynchus kisutch*	Fish	Coho salmon
30	*Bufi bufo*	Amphibian	Common toad
31	*Bufo boreas*	Amphibian	Western toad
32	*Acnthocyclops vernalis*	Invertebrate	Stygobite copepod
33	*Lymnaea sp*	Invertebrate	Snail
34	*Potamopyrgus antipodarum*	Invertebrate	Snail
35	*Macrobrachium rosenbergii*	Invertebrate	Freshwater prawn
36	*Pomacea paludosa*	Invertebrate	Florida apple snail
37	*Hydra viridissima*	Invertebrate	Hydra
38	*Cherax quadricarinatus*	Invertebrate	Australian crayfish
39	*Pseudacris regilla*	Amphibian	Pacific treefrog
40	*Xenopus laevis*	Amphibian	African clawed frog
41	*Hydra attenuatta*	Invertebrate	Hydra
42	*Rana catesbeiana*	Amphibian	Bullfrog
43	*Rana temporaria*	Amphibian	European common frog
44	*Polycelis niagra*	Invertebrate	Planaria
45	*Bufo americanus*	Amphibian	American toad
46	*Proasellus slavus vindobonensis*	Invertebrate	Stygobite isopod
47	*Paracyclops fimbriatus*	Invertebrate	Epigean copepod
48	*Diacyclops bicuspidatus*	Invertebrate	Stygobite copepod
49	*Rana clamitans*	Amphibian	Green frog
50	*Astacus astacus*	Invertebrate	Freshwater crayfish

Table A4.5: References used for acute and chronic guideline derivation.

	References - NIWA 2009 IDs			
	Envt Canada	**USEPA**	**ANZECC**	**NIWA2009**
Alonso & Camargo (2003)				500008
Baker & Waights (1993)	A, e, f			400006
Buhl & Hamilton (2000)		47875		6047875
Cairns & Scheier (1959)		930	200930	200930
Camargo & Ward (1992)	1	3879	203879	203879
Camargo *et al*. (2005)				500006
Colt & Tchobanoglous (1976)	2			400002
Corrao *et al*. (2006)				500007
Dowden & Bennett (1965)	A, b, c	915	200915	200915
Dowden (1961)		2465	202465	202465
Hamlin (2006)				500005
Jensen (1996)				500009
Johansson *et al*. (2001)	A, e,g			400005
Jones (1940)	A, c,f			400007
Jones (1941)	A, c,f			400008
Kinchloe *et al*. (1979)	A			400003
Laposata & Dunson (1998)	2, l	19803		6019803
McGurk *et al*. (2006)		95870		6095870
Meade & Watts (1995)		19529		6019529
Mosslacher (2000)		100653		60100653
Rippon & McBride (1994)			300119	300119
Rubin & Elmargahy (1997)	1, e, h	7635	207635	207635
Schuytema & Nebeker (1999a)	1			500010
Schuytema & Nebeker (1999b)	1			500011
Schuytema & Nebeker (1999c)	1	20488		6020488
Scott & Crunkilton (2000)	1			400001
Sullivan & Spence (2003)				500012
Tesh *et al*. (1990)	A, c,d			400004
Tilak, Lakshmi & Susan (2002)				500001
Tilak, Vardhan & Kumar (2006)				500004
Tilak, Veeraiah & Lakshmi (2006)				500002
Tilak, Veeraiah & Raju (2006)				500003
Tomasso & Carmichael (1986)	A, c	11794	211794	211794
Trama (1954)	2	8037	208037	208037
Wallen *et al*. (1957)		508	200508	200508
Westin (1974)	2	5115	205115	205115
Wickins (1976)	2	2320		602320

Envt Canada reference footnotes – see Table A4.2B

Appendix 5: References in nitrate dataset

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