State Water Resources Control Board
Ex Parte Communication Disclosure Regarding Pending Order

Pending Order
Draft Order WQ re: Waste Discharge Requirements General Order R5-2012-0116

Name, title, contact information of person completing form
Steve Shimek, The Otter Project, 831/663-9460, exec@otterproject.org

Date, time, location of meeting
November 30, 2017, 2:00 p.m. – 3:00 p.m., Cal-EPA Building

Type of Communication
Oral communication occurred.
Handouts.

Participants
Steven Moore
Phil Wyels
Steve Shimek
Sean Bothwell

Name of person(s) who initiated the communication
Steve Shimek

Describe Communication
Discussed some of the technical aspects of the Eastern San Joaquin order, including:

- Applied and Removed v Nitrogen Uptake
- Toxicity Testing using appropriate organisms
- Biostimulatory substance narrative standard

The attached documents were discussed and made available:

1) Nitrogen Requirements and N Status Determination of Lettuce
2) 2015 SWAMP Update of Toxicity Testing
3) 2015 Morrissey et al review of neonicitinoid impacts
4) 2010 SWAMP Interpreting Narrative Objectives for Biostimulatory Substances

HortScience 47(12):1768–1774. 2012.

Nitrogen Requirements and N Status Determination of Lettuce

Thomas G. Bottoms
Department of Plant Sciences, University of California, 1 Shields Avenue, Davis, CA 95616

Richard F. Smith and Michael D. Cahn
University of California Cooperative Extension, Salinas, CA

Timothy K. Hartz[1]
Department of Plant Sciences, University of California, 1 Shields Avenue, Davis, CA 95616

Additional index words. fertilizer efficiency, soil and tissue testing, *Lactuca sativa*

***Abstract*. As concern over NO_3-N pollution of groundwater increases, California lettuce growers are under pressure to improve nitrogen (N) fertilizer efficiency. Crop growth, N uptake, and the value of soil and plant N diagnostic measures were evaluated in 24 iceberg and romaine lettuce (*Lactuca sativa* L. var. *capitata* L., and *longifolia* Lam., respectively) field trials from 2007 to 2010. The reliability of presidedressing soil nitrate testing (PSNT) to identify fields in which N application could be reduced or eliminated was evaluated in 16 non-replicated strip trials and five replicated trials on commercial farms. All commercial field sites had greater than 20 mg·kg^{-1} residual soil NO_3-N at the time of the first in-season N application. In the strip trials, plots in which the cooperating growers' initial sidedress N application was eliminated or reduced were compared with the growers' standard N fertilization program. In the replicated trials, the growers' N regime was compared with treatments in which one or more N fertigation through drip irrigation was eliminated. Additionally, seasonal N rates from 11 to 336 kg·ha^{-1} were compared in three replicated drip-irrigated research farm trials. Seasonal N application in the strip trials was reduced by an average of 77 kg·ha^{-1} (73 kg·ha^{-1} vs. 150 kg·ha^{-1} for the grower N regime) with no reduction in fresh biomass produced and only a slight reduction in crop N uptake (151 kg·ha^{-1} vs. 156 kg·ha^{-1} for the grower N regime). Similarly, an average seasonal N rate reduction of 88 kg·ha^{-1} (96 kg·ha^{-1} vs. 184 kg·ha^{-1}) was achieved in the replicated commercial trials with no biomass reduction. Seasonal N rates between 111 and 192 kg·ha^{-1} maximized fresh biomass in the research farm trials, which were conducted in fields with lower residual soil NO_3-N than the commercial trials. Across fields, lettuce N uptake was slow in the first 4 weeks after planting, averaging less than 0.5 kg·ha^{-1}·d^{-1}. N uptake then increased linearly until harvest (≈9 weeks after planting), averaging ≈4 kg·ha^{-1}·d^{-1} over that period. Whole plant critical N concentration (N_c, the minimum whole plant N concentration required to maximize growth) was estimated by the equation N_c (g·kg^{-1}) = 42 − 2.8 dry mass (DM, Mg·ha^{-1}); on that basis, critical N uptake (crop N uptake required to maintain whole plant N above N_c) in the commercial fields averaged 116 kg·ha^{-1} compared with the mean uptake of 145 kg·ha^{-1} with the grower N regime. Soil NO_3-N greater than 20 mg·kg^{-1} was a reliable indicator that N application could be reduced or delayed. Neither leaf N nor midrib NO_3-N was correlated with concurrently measured soil NO_3-N and therefore of limited value in directing in-season N fertilization.**

The coastal valleys of central California produce nearly 60,000 ha of lettuce annually, more than half of the nation's supply. In this region, lettuce is typically produced in rotation with other leafy vegetables. Production systems are characterized by two to three crops per year with frequent irrigation and heavy N fertilization. Water quality monitoring in the agricultural watersheds in this region has shown that both surface water and groundwater often exceed the federal drinking water standard of 10 mg·L^{-1} NO_3-N. Vegetable growers are under increasing regulatory pressure to improve both their fertilization and irrigation practices to protect environmental water quality. Recently proposed regulations would require growers to report N fertilization rates and to bring N loading from fertilizer and irrigation water into approximate balance with crop N uptake. In this region, lettuce N uptake has been reported to average 130 kg·ha^{-1} for iceberg and 107 kg·ha^{-1} for romaine (Breschini and Hartz, 2002). However, a recent field survey found that lettuce received an average seasonal N fertilization rate of 184 kg N/ha (Hartz et al., 2007), suggesting that significant N rate reduction would be required to meet these new regulations.

Received for publication 8 Aug. 2012. Accepted for publication 18 Oct. 2012.
[1]To whom reprint requests should be addressed; e-mail tkhartz@ucdavis.edu.

Studies on lettuce response to N fertilization have reported widely varying results. Seasonal N rates required to maximize crop yield have ranged from 100 to 150 kg·ha^{-1} (Gardner and Pew, 1972, 1974, 1979; Tei et al., 2003) to greater than 220 kg·ha^{-1} (Hoque et al., 2010; Welch et al., 1979). Much of this variability may be attributed to field-specific factors affecting crop yield potential and N fertilizer efficiency; these factors include plant population, precipitation, irrigation efficiency, residual soil NO_3-N, and soil N mineralization potential. Given the high crop value and strict market standards for lettuce, growers commonly use standard fertilization programs with little field-specific modification; they are reluctant to modify current N fertilizer practices without a sound understanding of the interaction of these factors and reliable diagnostic techniques to guide field-specific N fertilization.

Adding to the uncertainty regarding efficient N management of lettuce, California growers continue to modify production practices to increase yield. Average lettuce yield rose ≈11% between 2000 and 2010 (Monterey County Agricultural Commissioner, 2000, 2010); factors potentially responsible included modified planting configurations that increased plant population and widespread adoption of drip irrigation. We undertook this study to develop detailed information on lettuce N requirements under current production practices used in California's central coast region and to critically evaluate the value of soil and plant diagnostic techniques to guide in-season N fertilizer management.

Materials and Methods

Lettuce N uptake and response to N fertilization were evaluated in 24 field trials in the Salinas Valley of California from 2007 through 2010. Sixteen of these were non-replicated strip trials in commercial fields comparing a reduced N fertilization regime with the growers' standard N fertilization program. Replicated comparisons of reduced N management strategies and growers' N management were conducted in five additional commercial fields. All commercial fields had been in long-term rotations of cool-season vegetables. The remaining three trials, conducted at a research facility, were replicated N rate comparisons.

Strip trials. Sixteen commercial lettuce fields were selected in 2009 and 2010 to evaluate the reliability of PSNT in identifying fields in which N fertilization could be reduced or delayed with no loss of marketable yield. The fields, which were seeded between 21 Mar. and 1 Aug., were selected based on the presence of at least 20 mg·kg^{-1} NO_3-N in the top 30 cm of soil after crop thinning (typically 14 to 21 d after planting); this soil NO_3-N threshold was suggested by prior research on lettuce (Breschini and Hartz, 2002; Hartz et al., 2000). Twelve fields were planted with iceberg cultivars and four fields with romaine. The Salinas Valley is

Table 1. Effect of sidedress N reduction on aboveground lettuce fresh biomass, and biomass nitrogen (N), in the commercial strip trials.

Trial	Lettuce type	Germination water date	Soil texture	Soil NO_3-N ($mg \cdot kg^{-1}$)[z]	Seasonal N ($kg \cdot ha^{-1}$)		Fresh biomass ($Mg \cdot ha^{-1}$)		Biomass N ($kg \cdot ha^{-1}$)	
					Grower N	Reduced N	Grower N	Reduced N	Grower N	Reduced N
1	Iceberg	21 Mar.	Clay	36	144	25	80	78	140	136
2	Iceberg	1 Apr.	Silty clay	20	138	40	109	101	190	177
3	Iceberg	11 Apr.	Clay loam	48	132	29	82	85	152	151
4	Iceberg	30 May	Clay	55	143	48	85	86	158	160
5	Iceberg	22 June	Silty loam	33	112	50	101	99	157	171
6	Iceberg	1 July	Sandy clay loam	20	203	115	107	107	174	168
7	Iceberg	1 July	Silty clay loam	24	89	36	85	85	145	146
8	Iceberg	15 July	Sandy clay loam	48	190	119	86	85	147	148
9	Iceberg	16 July	Clay	32	85	36	84	84	136	134
10	Iceberg	16 July	Silty clay	71	190	119	119	113	200	197
11	Iceberg	1 Aug.	Clay	46	144	25	126	128	189	188
12	Iceberg	18 May	Clay loam	36	216	151	71	71	95	91
13	Romaine	6 June	Clay	29	148	114	74	78	158	169
14	Romaine	27 June	Sandy clay loam	20	142	47	79	78	164	124
15	Romaine	1 Aug.	Sandy clay loam	23	148	98	77	76	136	120
16	Romaine	1 Aug.	Clay	68	179	108	74	75	152	139
Avg					150	73	90	89	156	151

[z]Post-thinning, before treatment initiation.

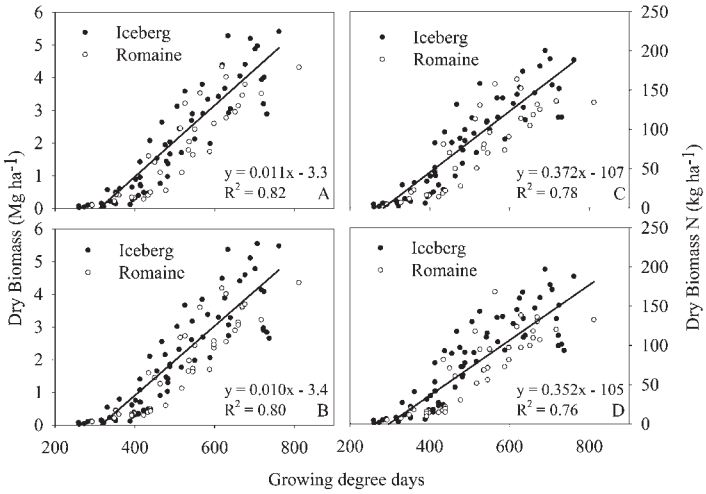

Fig. 1. Lettuce aboveground dry biomass, and dry biomass nitrogen (N), as a function of cumulative growing degree-days in the strip trial fields; grower N treatment (**A** and **C**) and reduced N treatment (**B** and **D**). Growing degree-days were calculated using 5 and 30 °C threshold temperatures.

essentially rain-free during the lettuce production period, and growers use a variety of irrigation systems and irrigation schedules. Most fields are irrigated with well water. Wells vary widely in NO_3-N concentration with most wells between 2 and 20 $mg \cdot L^{-1}$. All fields were sprinkler-irrigated for stand establishment with two fields switched to drip irrigation and one field switched to furrow irrigation after establishment. Soil texture ranged from sandy clay loam to clay. The planting configuration was either two plant rows per 1-m raised bed or five to six plant rows per 2-m raised bed; plant population varied from 72,000 to 112,000 ha. Preplant N fertilization was banded in the beds at rates ranging from 0 to 40 $kg \cdot ha^{-1}$.

Before the first sidedress N application, a strip plot in the center of each field was identified to receive a reduced N fertilization regime. These strip plots were the length of the field × 12 to 24 beds wide and averaged 0.4 ha. The width of the strip plot was set to accommodate one pass of the commercial harvest crew and equipment, which varied by grower. In all fields, the grower applied an N sidedressing 20 to 28 d after planting. Sidedress applications were typically applied in bands 5 to 10 cm deep in the bed; a variety of N fertilizers were used. The strip plot received either no sidedressing (14 fields) or a half rate sidedressing (two fields) at the cooperating growers' discretion. After the first sidedressing, the reduced N plots received all subsequent N fertilization applied by the grower, whether by additional sidedressing or by fertigation.

Soil samples (0 to 30 cm depth in the plant row) were taken before the first N sidedressing and repeated on 7- to 10-d intervals until harvest. Samples were collected separately from the head and tail ends of the reduced N plot. Samples of the grower N regime from the head and tail ends of the field were collected from the areas adjacent to the reduced N plot; samples drawn from each side of the reduced N plot were blended so that for each sampling date, a total of four composite samples per field was collected; each comprised of eight to 10 cores. Matching samples of whole plants and recently mature leaves were also collected at each soil sampling date after the initial N sidedressing. Each of the four composite samples per field per collection date contained 12 whole plants and 20 leaves; the leaves were subsequently divided into blade and midrib samples. Plant, leaf, and midrib samples were oven-dried at 65 °C to a constant weight and ground to pass a 40-mesh screen. N concentration of whole plants and leaf blades was determined by a N gas analyzer (Model FP-528; LECO Corp., St. Joseph, MI). Midrib NO_3-N was measured by flow injection analysis (Lachat Instruments, Milwaukee, WI) after extraction with 2% acetic acid. Field-moist soil was extracted in 2 N KCl and analyzed for NO_3-N by the flow injection method. Plant population was determined based on post-thinning plant counts in four representative 4 m wide × 30-m long strips within the trial area of each field.

Just before commercial harvest, aboveground biomass was determined by the collection of 32 randomly selected whole plants in both the head and tail ends of the reduced N plot and in the adjacent grower N plots, as previously described. Subsamples were oven-dried, weighed, and analyzed for total N concentration. During the commercial harvest, the harvest crews recorded marketable yield separately in the reduced N strip and in the adjacent areas receiving the full grower N regime.

Replicated trials. Five replicated field trials were conducted in drip-irrigated commercial lettuce fields between 2007 and 2009. Three fields were planted with iceberg and two fields with romaine cultivars. All of the fields were sprinkler-irrigated for stand establishment and then switched to drip irrigation. Soil texture ranged from loam to clay loam. Fields were planted between 3 Mar. and 2 Aug. N fertilization treatments differed among fields based on the grower practices. Within fields, up to four levels of seasonal N application were established by eliminating

Table 2. Effect of nitrogen (N) fertigation on lettuce fresh biomass, and biomass N, in the replicated commercial drip-irrigated trials.

Trial	Yr	Lettuce type	Germination water date	Soil texture	Soil NO_3-N ($mg \cdot kg^{-1}$)[z]	N treatment	Number of fertigations	Seasonal N ($kg \cdot ha^{-1}$)	Fresh biomass ($Mg \cdot ha^{-1}$)	Biomass N ($kg \cdot ha^{-1}$)
1	2007	Iceberg	5 June	Loam	20	Grower	3	189	96 a[y]	116 a
						Reduced 1	1	103	93 a	102 b
						Reduced 2	0	47	81 b	94 b
2	2007	Iceberg	15 June	Loam	27	Grower	4	192	87 a	115 a
						Reduced 1	2	72	91 a	113 a
						Reduced 2	0	20	83 a	100 a
3	2007	Romaine	15 Aug	Loam	21	Grower	2	129	77 a	114 a
						Reduced	1	75	77 a	97 b
4	2008	Iceberg	3 March	Clay loam	20	Grower	4	236	94 a	128 a
						Reduced 1	3	183	97 a	133 a
						Reduced 2	2	140	92 a	111 a
						Reduced 3	1	86	84 b	108 a
5	2009	Romaine	2 Aug	Loam	21	Grower	3	175	77 a	134 a
						Reduced	3	144	77 a	132 a

[z]Post-thinning, before treatment initiation.
[y]Means within columns and trials separated using the REGWQ multiple range test.

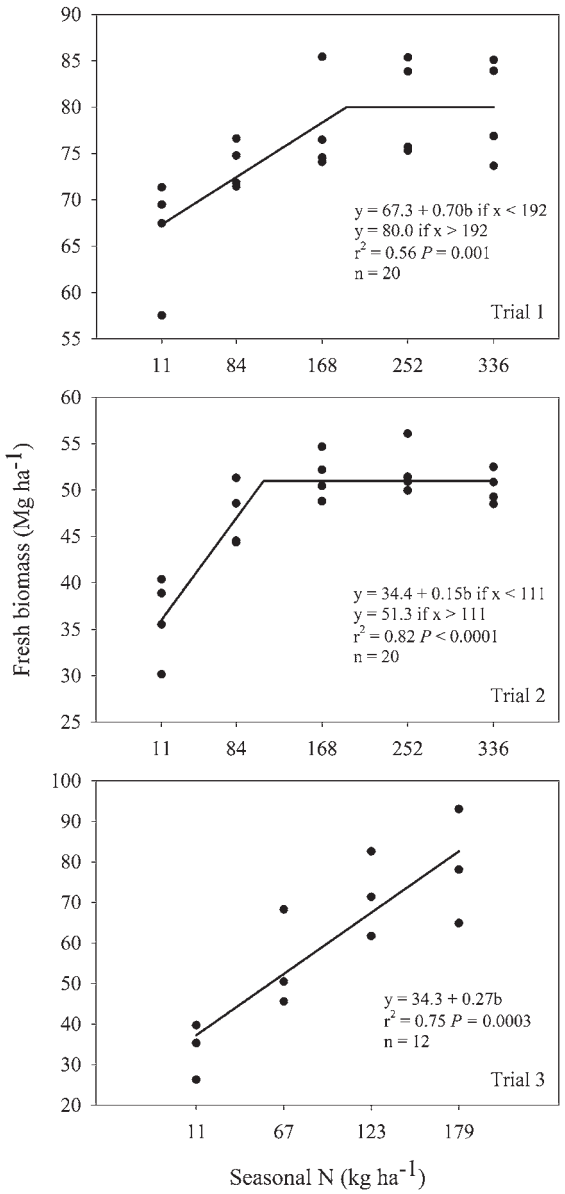

Fig. 2. Lettuce fresh biomass as affected by seasonal nitrogen (N) rate in research farm trials; linear-plateau models fit by the method of Waugh et al. (1973).

one or more of the grower N fertigations. All fields had soil NO_3-N greater than 20 $mg \cdot kg^{-1}$ (0 to 30 cm depth) at the time of the initial in-season N application. A randomized complete block experimental design was used in all fields with four replications per N treatment. Individual plots were four 1-m beds wide × 9 to 15 m long. Data were collected on the middle two beds of each plot. Soil, whole plant, leaf, and midrib sampling was done on 7- to 10-d intervals as previously described. The final plant sampling was conducted just before commercial harvest. Fresh and dry biomass of 24 randomly selected whole plants per plot was determined.

Three additional N rate trials were conducted between 2009 and 2010 at the Hartnell College research farm in Salinas, CA. All trials were seeded with romaine cultivars and grown using drip irrigation. Each trial was organized in a randomized complete block design with four replications (Trials 1 and 2) or three replications (Trial 3) per N rate. Each plot consisted of two 1 m wide beds 50 m long. Seasonal N rates ranged from 11 to 336 $kg \cdot ha^{-1}$ (Trials 1 and 2) and from 11 to 179 $kg \cdot ha^{-1}$ (Trial 3). N was applied preplant (11 $kg \cdot ha^{-1}$) and in three fertigations at ≈4, 5, and 6 weeks post-planting. Soil NO_3-N (0 to 30 cm depth) at the first N fertigation was 13, 9, and 7 $mg \cdot kg^{-1}$ in Fields 1, 2, and 3, respectively. At commercial maturity, aboveground biomass was determined on 80 randomly selected whole plants per plot.

Calculation of growing degree-days. To allow comparison of lettuce growth across fields and production seasons, growing degree-days (GDDs) were calculated from air temperature data provided by the California Irrigation Management Information System (Pruitt et al., 1987). GDDs were calculated using a single sine method (Allen, 1976) with upper and lower thresholds of 30 and 5 °C, respectively. GDD accumulation began on the day of the first irrigation rather than at seeding because seeding was typically done in dry soil.

Statistical analysis. Parallel line analysis was used to compare the regression slopes of romaine and iceberg lettuce dry biomass accumulation over time using SigmaPlot (Systat Software, Inc., San Jose, CA). All other statistical analyses were conducted using the SAS statistical package (SAS Institute, Cary, NC). Comparison of the crop biomass of the grower and reduced N management treatments in the strip trials was

done with the GLM procedure using fields as replications to evaluate the reliability of the 20 mg·kg^{-1} PSNT residual soil NO_3-N threshold as a diagnostic tool to improve N management. Comparison of lettuce biomass among N treatments in the replicated commercial trials was accomplished using the GLM procedure and the REGWQ multiple range test. Optimum N rates in the research farm trials were estimated by the linear-plateau model described by Waugh et al. (1973) using the NLIN procedure.

Results

Aboveground lettuce fresh biomass in the reduced N treatment was not different from the grower N management treatment in the strip trials (P = 0.92), confirming the reliability of PSNT in identifying fields in which the first sidedress N application could be reduced or delayed (Table 1). Across the 16 fields, total fresh biomass at harvest averaged 89.9 and 89.3 Mg·ha^{-1} in the grower N and reduced N treatments, respectively. Marketable yield was obtained from the commercial harvest crews in 12 of the fields, and the reduced N treatment averaged 41.0 Mg·ha^{-1} compared with 40.8 Mg·ha^{-1} in the grower N treatment (P = 0.97). Seasonal N application (including preplant fertilization) averaged 150 and 73 kg·ha^{-1} in the grower N and reduced N treatments, respectively. Aboveground biomass N in the reduced N treatment averaged 151 kg·ha^{-1} compared with 156 kg·ha^{-1} in the grower N treatment, suggesting inefficient use of the N applied at first sidedressing, which averaged 77 kg·ha^{-1}.

Lettuce showed a characteristic growth pattern across the strip trial fields (Fig. 1A–B). Aboveground dry biomass accumulation averaged less than 0.3 Mg·ha^{-1} over the first 300 GDD (≈3 to 4 weeks at Salinas Valley temperatures) and then increased in a linear fashion until harvest. There was no significant difference between iceberg and romaine lettuce in DM accumulation [regression slopes during the rapid growth phase were not significantly different (P = 0.51)]. There was a trend toward higher DM with increasing plant population [DM (Mg·ha^{-1}) = 0. 00003 (plants/ha) +1.44, r^2 = 0.14, P = 0.08]. Biomass N accumulation followed the same pattern as biomass accumulation (Fig. 1C–D). N uptake during the linear growth phase averaged 0.38 kg/GDD across N treatments and fields; at 10 to 12 GDD/d during the production season, daily aboveground N accumulation averaged ≈3.8 to 4.6 kg·ha^{-1}.

The replicated commercial trials also demonstrated that N fertigation could be reduced below current grower practice with no reduction in crop biomass (Table 2). Significant fresh biomass reduction was observed in only two of five fields and only in treatments in which multiple N fertigations were eliminated. In both cases of biomass reduction, the midseason soil NO_3-N had decreased to less than 10 mg·kg^{-1}. A significant response to N fertigation was observed in all research farm trials (Fig. 2). Seasonal N rates between 111 and 192 kg·ha^{-1} were sufficient to maximize fresh biomass, somewhat higher than observed in the other trials. The research farm trials began with lower residual soil NO_3-N (7 to 13 mg·kg^{-1}), and they followed a fallow period, whereas most of the commercial fields were planted after residue incorporation from a spring crop.

Collectively, these 24 trials provided extensive data on lettuce growth and plant N status on which to apply the "critical N concentration" concept (N_c, the minimum whole plant N concentration required to maximize growth; Greenwood et al., 1991; Fig. 3). Data points identified as N-deficient represented treatments in replicated trials in which DM was significantly (P < 0.05) below that of the highest N rate in that trial on a given sample date. Data points identified as "grower N" represented the grower N management in the strip trials and the replicated commercial trials plus the highest N rate in the research farm trials. Points identified as "reduced N" represented reduced N treatments from all strip trials plus reduced N treatments from replicated trials for which DM was not statistically different (P > 0.05) from the grower N treatment on a given sample date. The critical N equation [N_c = 45.6 DM (Mg·ha^{-1})$^{-0.357}$], developed in a 3-year study of lettuce in Italy by Tei et al. (2003), generally distinguished N deficiency from sufficiency. However, that equation had been validated only for DM values between 0.9 and 3.4 Mg·ha^{-1} and was clearly inappropriate for earlier growth stages. We empirically fit a linear function (N_c = 42.0 – 2.8 DM), which distinguished N-deficient from N-sufficient samples with reasonable accuracy across the entire season.

Based on the empirically derived N_c equation, the crop N uptake required to maintain whole plant N above the N_c (critical N uptake, N_{upt} = –2.8 DM^2 + 42 DM) was compared with actual crop N uptake of the grower N treatment in the commercial field trials (Fig. 4). Aboveground DM at harvest in the grower N treatment ranged from 2.4 to 5.4 Mg·ha^{-1}, and N uptake ranged from 94 to 200 kg·ha^{-1}, averaging 145 kg·ha^{-1}. The calculated N_{upt} ranged from 86 to 145 kg·ha^{-1}, averaging only 116 kg·ha^{-1}, indicating that a substantial amount of "luxury" uptake occurred

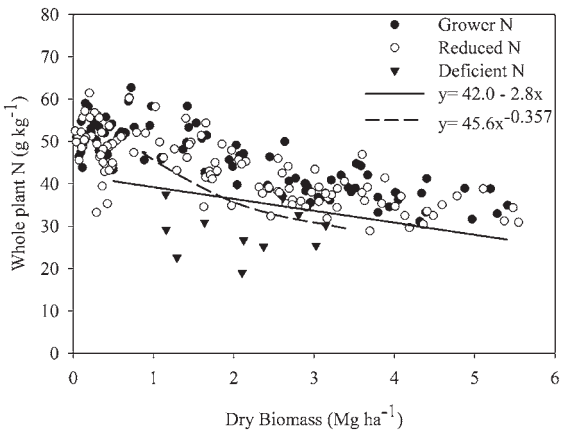

Fig. 3. The relationship between dry biomass (DM) and whole plant nitrogen (N) concentration. Dashed line represents plant critical N concentration (N_c = 45.6 DM$^{-0.357}$) from Tei et al. (2003). Solid line represents N_c as an empirically derived linear function (N_c = 42.0 – 2.8 DM).

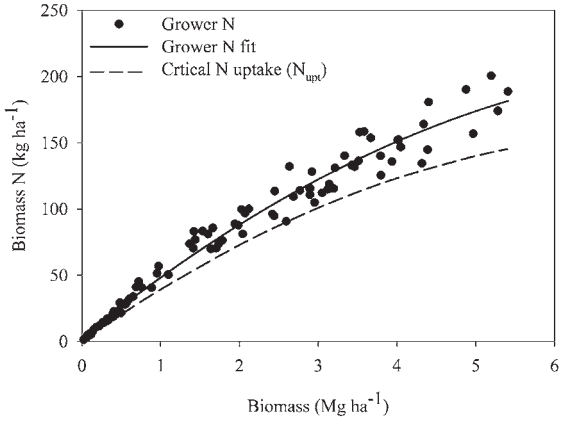

Fig. 4. Whole plant nitrogen (N) (all commercial field trials) as function of dry biomass (DM) for grower N treatment. Solid line represents grower N uptake (y = –2.8 DM^2 + 48 DM + 3); dashed line represents critical N uptake (N_{upt}, y = –2.8 DM^2 + 42 DM).

in these fields. N_{upt} during the rapid growth phase ranged between 3 and 4 kg·ha^{-1}·d^{-1} for Salinas Valley summer conditions.

Neither leaf N nor midrib NO_3-N was correlated with concurrently measured soil NO_3-N during either early growth (less than 1.5 Mg·ha^{-1} biomass) or the heading stage (greater than 1.5 Mg·ha^{-1}; Fig. 5). This insensitivity across a wide range of soil NO_3-N suggested that these tissue diagnostics provided no insight on current soil N availability. Leaf N was correlated with whole plant N (Fig. 6A). However, there was substantial variability in that relationship, indicating that leaf N was not a dependable surrogate for whole plant N. Midrib NO_3-N was not correlated with whole plant N (Fig. 6B). Based on the limited number of N-deficient leaf and midrib samples encountered in this study, empirically derived critical levels appeared to be ≈40 g·kg^{-1} leaf N and 6 g·kg^{-1} midrib NO_3-N throughout the season (Fig. 7). However, the separation between deficient and sufficient samples was not clear, and applying these critical levels would have resulted in unnecessary fertilization in some fields. Given the limitations just described, using either tissue N diagnostic to guide N fertilization, in the absence of soil NO_3-N data, would not be warranted.

The average soil NO_3-N concentration in the top 30 cm at harvest in the strip trials was 20 and 14 mg·kg^{-1} for the grower N and reduced N treatments, respectively (Fig. 8). This difference in soil NO_3-N of 6 mg·kg^{-1} represented 23 kg N/ha in the top 30 cm, assuming a typical bulk density of 1.4 g·cm^{-3}. Taking into account the slight increase in crop N uptake (≈5 kg·ha^{-1}) obtained in the grower N treatment in these fields, less than half of the extra 77 kg·ha^{-1} N applied in that treatment was accounted for at harvest, suggesting substantial in-season leaching below 30 cm. At harvest, soil NO_3-N was less than 10 mg·kg^{-1} in the reduced-N treatment in nine of the 14 fields in which data were collected and below that level in the grower N treatment in six fields. This documented that high-yield lettuce production can be managed to minimize residual soil NO_3-N at the end of the season.

Discussion

Lettuce growth was maximized by seasonal N fertilization rates substantially below current typical grower practices. The reduced N treatment in the strip plot trials received an average of only 73 kg N/ha and produced biomass equivalent to the more heavily fertilized grower N treatment. In the replicated commercial fertigation trials, the lowest seasonal N rate achieving maximum biomass averaged only 102 kg N/ha. The presence of high residual soil NO_3-N in these fields, which is common in this production system (especially after a spring crop), was a major factor limiting fertilizer N requirements. In the absence of substantial residual soil NO_3-N, fertilizer N requirements would undoubtedly

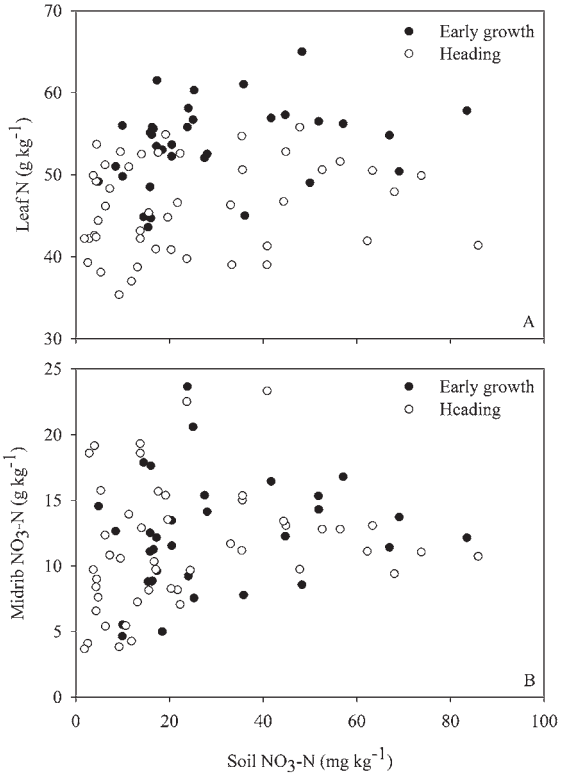

Fig. 5. Relationship between root zone soil NO_3-N and leaf nitrogen (N) (**A**) or midrib NO_3-N (**B**). Early growth and heading stages defined as dry biomass less than 1.5 Mg·ha^{-1} and greater than 1.5 Mg·ha^{-1}, respectively.

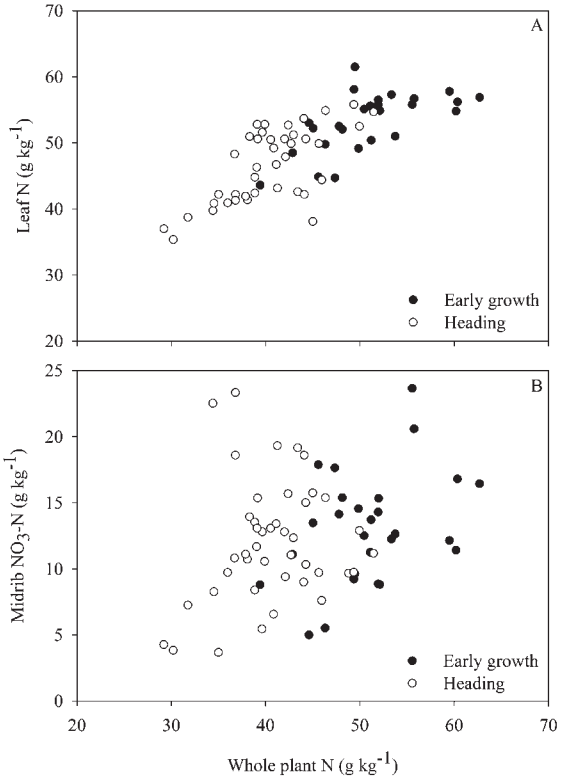

Fig. 6. Relationship between whole plant nitrogen (PN) concentration and leaf N (LN) concentration at the early growth (LN = 0.50 PN + 27.9, r^2 = 0.40) and heading stages (LN = 0.76 PN + 15.7, r^2 = 0.46, **A**). Relationship between PN concentration and midrib NO_3-N concentration at the early growth and heading stages (**B**). Early growth and heading stages defined as dry biomass less than 1.5 Mg·ha^{-1} and greater than 1.5 Mg·ha^{-1}, respectively.

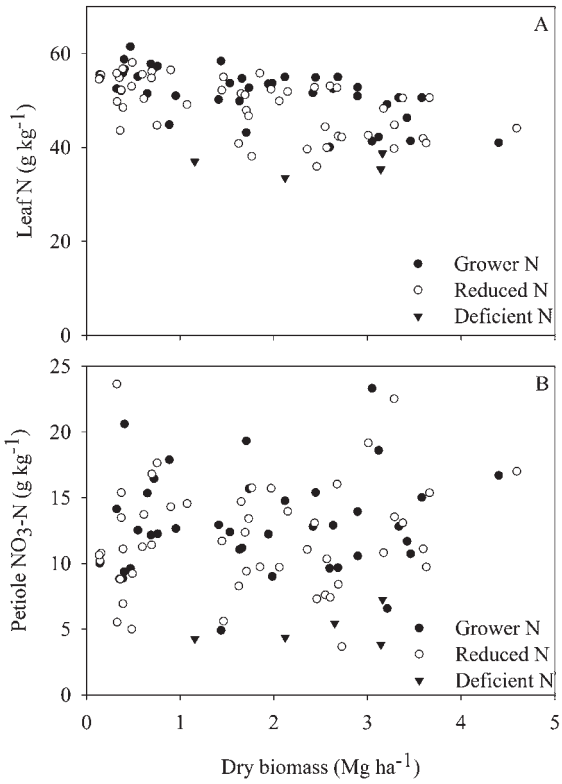

Fig. 7. Leaf nitrogen (N) (**A**) and midrib NO_3-N (**B**) as a function of dry biomass; data include all growth stages from all fields.

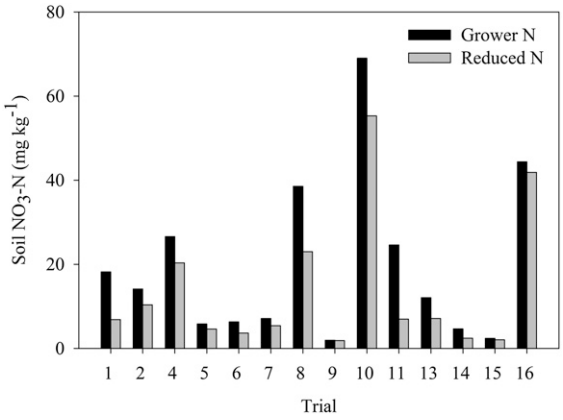

Fig. 8. Residual soil NO_3-N in the surface 30 cm at harvest in the strip trial fields.

be higher, as was the case in the research farm trials.

Crop uptake of the extra N applied in the grower N treatment was minimal. On average the apparent fertilizer recovery (AFR) of the N applied by growers at the first sidedressing was only 7% in the strip trials. In the replicated commercial fertigation trials, crop N uptake in the grower N treatment was on average only 13 kg·ha^{-1} higher than the lowest reduced N treatment that produced equivalent biomass, representing an AFR of 16% for the extra N applied by growers. Greenwood et al. (1989) reported that AFR in lettuce declined as N rate increased; at N rates greater than 100 kg·ha^{-1}, AFR was less than 15%. In this production system where multiple crops are produced annually, the overall AFR of N applied to a spring crop may be improved by subsequent recovery by a summer-planted crop. However, lettuce is shallowly rooted with most roots concentrated in the top 30 cm of soil (Jackson, 1995). The potential for NO_3-N leaching during the germination irrigation for the summer crop is substantial, and leaching losses with winter precipitation would be even more significant. Jackson et al. (1994) found that annual NO_3-N leaching loss in a double-cropped lettuce field in the Salinas Valley was ≈150 kg·ha^{-1}.

The reliability of PSNT in identifying lettuce fields in which N sidedressing can be reduced or delayed confirmed earlier California studies (Breschini and Hartz, 2002; Hartz et al., 2000). PSNT has been successfully applied to other crops, including cabbage (*Brassica oleracea* L. var. *capitata* L.; Heckman et al., 2002), celery (*Apium graveolens* L.; Hartz et al., 2000), and corn (*Zea mays* L.; Fox et al., 1989; Heckman et al., 1995); action thresholds have ranged from 20 to 30 mg·kg^{-1} soil NO_3-N. Most prior research on PSNT evaluated this approach as a once per season test to determine sidedress N requirements. However, for high-value vegetable crops on which multiple in-season N applications are common, repeated soil testing would allow growers more flexibility and confidence. Breschini and Hartz (2002) successfully demonstrated such a system in lettuce, testing soil NO_3-N up to three times per crop and on each occasion applying only enough N to bring the soil up to a 20 mg·kg^{-1} NO_3-N threshold.

Based on the observed lettuce N uptake requirements in the weeks before harvest (3 to 4 kg·ha^{-1}·d^{-1}), and the assumption that most N uptake occurs in the top 30 cm of soil, plant N uptake would be expected to reduce root zone soil NO_3-N by no more than 1 mg·kg^{-1}·d^{-1}. Soil testing for the final time 2 weeks before expected harvest, and limiting N application to no more than the amount required to return the soil to 20 mg·kg^{-1} NO_3-N, should provide sufficient mineral N for maximum crop productivity while finishing the season with a moderate level of residual soil NO_3-N. The observation that soil NO_3-N at harvest in the reduced N treatment was less than 10 mg·kg^{-1} in most fields confirmed that such low season-ending soil NO_3-N was not growth-limiting. Minimizing residual soil NO_3-N at harvest is a crucial element in a groundwater protection program.

In contrast to the documented use of soil NO_3-N monitoring to guide in-season N fertilization, plant-based diagnostics were less useful. The close agreement of our data with that of Tei et al. (2003) regarding N_c suggested that whole plant N was a robust measure of N sufficiency. Early-season whole plant N could be a practical monitoring technique, and our empirical N_c equation suggested a pre-heading critical threshold of ≈40 g·kg^{-1}. As plants get larger, whole plant sampling becomes impractical. The correlation between leaf N and whole plant N was unsatisfactory to make it a precise surrogate for whole plant N. Leaf N was not correlated with soil NO_3-N over a range of soil values from very high (greater than 40 mg·kg^{-1}) to potentially growth-limiting (less than 5 mg·kg^{-1}). Maier et al. (1990) and Westerveld et al. (2003) found that leaf N critical level varied by cultivar and location. Such confounding effects may explain the variability in published diagnostic guidelines. Lorenz and Tyler (1983) reported a leaf N sufficiency threshold for lettuce at harvest of 25 g·kg^{-1}, whereas Jones et al. (1991) suggested 38 g·kg^{-1}. Our data agreed with Jones et al.

The practical value of midrib NO_3-N monitoring was particularly questionable. Midrib NO_3-N was unrelated to either soil

NO_3-N or whole plant N. Midrib (petiole) NO_3-N has been shown to be affected by environmental conditions unrelated to soil N availability (Bates, 1971; Maynard et al., 1976) or to crop N uptake (MacKerron et al., 1995). The much higher degree of variability in midrib NO_3-N encountered in the present study (samples ranged from 4 to 24 g·kg^{-1}) compared with either whole plant N or leaf N suggested that the rate of nitrate reduction in the plant was influenced by factors unrelated to soil NO_3-N availability or plant N status.

All plant-based N monitoring techniques share a fundamental limitation as a water quality protection practice. They can provide an indication of current crop N status. However, given the insensitivity of plant diagnostics to soil NO_3-N availability, a sufficient tissue N value provides no indication of future N fertilization requirements and therefore cannot accurately identify fields where in-season N application can be reduced or delayed.

In summary, seasonal N uptake in commercial lettuce fields averaged 145 kg·ha^{-1} with uptake over the last half of the growing season averaging ≈4 kg N/ha/d. Current commercial N fertilization rates can be reduced substantially with no reduction of crop yield. PSNT was a reliable technique on which to base N fertilization. Leaf N and midrib NO_3-N monitoring were of limited value in guiding in-season N management.

Literature Cited

Allen, J.C. 1976. A modified sine wave method for calculating degree-days. Environ. Entomol. 5:388–396.

Bates, T.E. 1971. Factors affecting critical nutrient concentrations in plants and their evaluation: A review. Soil Sci. 112:116–130.

Breschini, S.J. and T.K. Hartz. 2002. Presidedress soil nitrate testing reduces nitrogen fertilizer use and nitrate leaching hazard in lettuce production. HortScience 37:1061–1064.

Fox, R.H., G.W. Roth, K.V. Iversen, and W.P. Piekielek. 1989. Soil and tissue nitrate tests compared for predicting soil nitrogen availability to corn. Agron. J. 81:971–974.

Gardner, B.R. and W.D. Pew. 1972. Response of fall grown head lettuce to nitrogen fertilization. Univ. of Arizona Agr. Expt. Sta. Tech. Bul. 199.

Gardner, B.R. and W.D. Pew. 1974. Response of spring grown head lettuce to nitrogen fertilization. Univ. of Arizona Agr. Expt. Sta. Tech. Bul. 210.

Gardner, B.R. and W.D. Pew. 1979. Comparison of various nitrogen sources for the fertilization of winter-grown head lettuce. J. Amer. Soc. Hort. Sci. 104:534–536.

Greenwood, D.J., F. Gastal, G. Lemaire, A. Draycott, P. Millard, and J.J. Neeteson. 1991. Growth rate and % N of field grown crops: Theory and experiments. Ann. Bot. (Lond.) 67:181–190.

Greenwood, D.J., K. Kubo, I.G. Burns, and A. Draycott. 1989. Apparent recovery of fertilizer N by vegetable crops. Soil Sci. Plant Nutr. 35:367–381.

Hartz, T.K., W.E. Bendixen, and L. Wierdsma. 2000. The value of pre-sidedress soil nitrate testing as a nitrogen management tool in irrigated vegetable production. HortScience 35: 651–656.

Hartz, T.K., P.R. Johnstone, E. Williams, and R.F. Smith. 2007. Establishing lettuce leaf nutrient optimum ranges through DRIS analysis. HortScience 42:143–146.

Heckman, J.R., W.T. Hlubik, D.J. Prostak, and J.W. Paterson. 1995. Pre-sidedress soil nitrate test for sweet corn. HortScience 30: 1033–1036.

Heckman, J.R., T. Morris, J.T. Sims, J.B. Sieczka, U. Krogmann, P. Nitzsche, and R. Ashley. 2002. Pre-sidedress soil nitrate test is effective for fall cabbage. HortScience 37: 113–117.

Hoque, M.M., H. Ajwa, and M. Othman. 2010. Yield and postharvest quality of lettuce in response to nitrogen, phosphorus and potassium fertilizers. HortScience 45:1539–1544.

Jackson, L.E. 1995. Root architecture in cultivated and wild lettuce (*Lactuca* spp.). Plant Cell Environ. 18:885–894.

Jackson, L.E., L.J. Stivers, B.T. Warden, and K.K. Tanji. 1994. Crop nitrogen utilization and soil nitrate loss in a lettuce field. Fert. Res. 37:93–105.

Jones, J.B., B. Wolf, and H.A. Mills. 1991. Plant analysis handbook. Macro-Micro Publishing, Athens, GA.

Lorenz, O.A. and K.B. Tyler. 1983. Plant tissue analysis of vegetable crops, p. 24–29. In: Reisenaur, H.M. (ed.). Soil and plant tissue testing in California. California Coop. Ext. Bul. 1879.

Maier, N.A., A.P. Dahlenberg, and T.K. Twigden. 1990. Assessment of the nitrogen status of onions (Allium cepa L.) cv. Cream Gold by plant analysis. Aust. J. Exp. Agr. 30:853–859.

MacKerron, D.K.L., M.W. Young, and H.V. Davies. 1995. A critical assessment of the value of petiole sap analysis in optimizing the nitrogen nutrition of the potato crop. Plant Soil 172:247–260.

Maynard, D.N., A.V. Barker, P.L. Minotti, and N.H. Peck. 1976. Nitrate accumulation in vegetables. Adv. Agron. 28:71–117.

Monterey County Agricultural Commissioner. 2000, 2010. Crop report. <http://www.ag.co.monterey.ca.us/resources/category/crop-reports>.

Pruitt, W.D., E. Fereres, and R.L. Snyder. 1987. Reference evapotranspiration (ET_o) for California. Univ. of Calif. Coop. Ext. Bul. 1922.

Tei, F., P. Benincasa, and M. Guiducci. 2003. Critical nitrogen in lettuce. Acta Hort. 627: 187–194.

Waugh, D.L., R.B. Cate, and L.A. Nelson. 1973. Discontinuous models for rapid correlation, interpretation and utilization of soil analysis and fertilizer response data. Tech. Bull. 7. Int. Soil Fertility Evaluation and Improvement Program. North Carolina State Univ., Raleigh, NC.

Welch, N.C., K.B. Tyler, and D. Ririe. 1979. Nitrogen stabilization in the Pajaro Valley in lettuce, celery and strawberries. Calif. Agr. 33:12–13.

Westerveld, S.M., A.W. McKeown, C.D. Scott-Dupree, and M.R. McDonald. 2003. How well do critical nitrogen concentrations work for cabbage, carrot and onion crops? HortScience 38:1122–1128.3.

Updated recommendations for monitoring current-use pesticide toxicity in water and sediment in the Surface Water Ambient Monitoring Program

Prepared by: Brian Anderson[1], Bryn Phillips[1], Marie Stillway and Linda Deanovic[2], Debra Denton[3], Michael Lyons[4], Mary Hamilton[5]

[1]*University of California, Davis–Granite Canyon*
[2]*University of California, Davis–Aquatic Toxicity Lab*
[3]*U.S. Environmental Protection Agency, Region 9*
[4]*California Regional Water Quality Control Board, Region 4*
[5]*California Regional Water Quality Control Board, Region 3*

SWAMP Technical Memorandum
SWAMP-TM-2015-0001
September 2015

TABLE OF CONTENTS

BACKGROUND – Changing Pesticides

A decade of evidence from the Surface Water Ambient Monitoring Program has indicated that toxicity to invertebrates is most often caused by pesticides (Anderson et al., 2011). As patterns of urban and agricultural pesticide use change in California, the species used to monitor water and sediment toxicity in SWAMP programs should be selected to properly evaluate these variations. While past data showed that much of the surface water toxicity was due to organophosphate pesticides such as diazinon and chlorpyrifos, these have largely been replaced by pyrethroids in most watersheds. In addition, recent data suggest new classes of pesticides are increasing in use, including phenylpyrazoles such as fipronil, and neonicotinoids such as imidacloprid. Decisions regarding toxicity monitoring for these pesticides should be based on their use patterns, and their relative toxicity to different test species and protocols. In addition, the decision to monitor in water and/or sediment depends on the solubility and stability of these pesticides, which dictates their environmental fate. The following discussion provides guidance for application of appropriate test species and protocols to

SWAMP monitoring coordinators interested in incorporating toxicity testing into their monitoring designs. Emphasis is placed on monitoring in freshwater habitats but two protocols are also recommended for marine receiving systems.

RELATIVE SPECIES SENSITIVITY

Four classes of pesticides that continue to be detected at toxicologically relevant concentrations in California streams are organophosphates (e.g., diazinon, chlorpyrifos, malathion), pyrethroids (e.g., bifenthrin, permethrin, cypermethrin), phenylpyrazoles (e.g., fipronil and its degradates), and neonicotinoids (e.g., imidacloprid, clothianidin, thiamethoxam). The relative acute toxicity of selected pesticides from these classes to standard test species is presented as 96-hour median lethal concentrations (LC50s) in Table 1. These data show that at 96 hours, the amphipod *Hyalella azteca* is the most sensitive to pyrethroids such as bifenthrin, the midge *Chironomus dilutus* is most sensitive to fipronil and its degradates, and the cladoceran *Ceriodaphnia dubia* is most sensitive to organophosphates such as chlorpyrifos. Both *C. dubia* (48-hour LC50) and *C. dilutus* have comparable acute sensitivities to imidacloprid, but evidence suggest that *C. dilutus* is more sensitive in chronic exposures. *Hyalella azteca* is also relatively sensitive to the organophosphate pesticide chlorpyrifos. Table 1 also lists a column of fathead minnow (*Pimephales promelas*) LC50 values to demonstrate the lower sensitivities of this vertebrate to current use pesticides. The other component of U.S. EPA three-species testing, the algae *Selenastrum*, does not respond to these pesticides, but could be used for monitoring involving potential toxicity caused by herbicides.

Because pesticides are usually detected in mixtures (U.S.G.S., 2006), the use of more than one toxicity test organism is recommended if multiple pesticides are present or suspected, and if the monitoring budget allows for it. Pesticide mixtures can be additive, synergistic, or antagonistic. Lydy et al. (2004) provides a review of challenges in regulating pesticide mixtures with differing modes of action and relative toxicities to aquatic organisms. Surface waters containing current use pesticides may include mixtures containing the parent compound and its toxic degradates. Phillips et al. (2014) demonstrated that monitoring the single active ingredient of the organophosphate mosquito control pesticide naled did not capture all of the potential impacts to receiving systems because the primary degradate, dichlorvos, was more toxic than the parent compound. This characteristic also applies to fipronil, where the degradates fipronil sulfone and fipronil sulfide are more toxic to *Chironomus dilutus* (Weston and Lydy 2014). Toxicity testing integrates the effects of mixture toxicity from different pesticides, as well as active ingredient and degradates.

Acute tests measure lethality, whereas chronic tests measure sub-lethal effects such as reduced reproduction, growth, or development. The differences between acute and chronic exposures in water column tests are typically defined by the protocol endpoint and test duration. Some pesticides demonstrate greater chronic toxicity to certain species so selection of chronic vs. acute toxicity test protocols should consider this characteristic. For example, there is little difference in 10 day and 28 day sediment exposures of *H. azteca* to the pyrethroid pesticide bifenthrin (Table 2; (Anderson et al., 2015)), but the difference in sensitivity between a 96 hour and 10 day water exposures of *H. azteca* to the neonicotinoid imidacloprid is much greater. The sensitivity of *C. dilutus* to imidacloprid in chronic water exposures is greater than that of *H. azteca*, and even *C. dubia*. Monitoring programs for pyrethroids will be adequately protective using the 96 hour water or 10 day sediment test protocols (note: water vs. sediment monitoring is discussed in the following section). Neonicotinoids, such as imidacloprid, demonstrate greater toxicity in longer term chronic toxicity tests (Table 2; see review (Morrissey et al., 2015)). Therefore, monitoring with longer-term tests using *C. dilutus* is recommended for receiving systems where imidacloprid is of concern (e.g., 10 day and 28 day water test protocols). Recent data by the California Department of Pesticide Regulation suggest that the highest concentrations of imidacloprid

have been measured in agricultural watersheds (Starner and Goh, 2012), so chronic testing in agriculture-dominated watersheds is a current priority. Although the imidacloprid 28 day LC50 for *C. dilutus* is 0.91 µg/L, Morrissey et al., (2015) suggest 0.1 µg/L for chronic sublethal effects. These authors also suggest a long-term chronic protective value based on a probabilistic risk assessment of 0.035 µg/L.

A source of acute and chronic benchmarks for standard test species used for the evaluation of pesticide registration is the U.S. EPA Office of Pesticide Programs (OPP) Aquatic Life Benchmarks Database. The database is maintained by OPP and provides acute and chronic endpoints for over 300 parent pesticide compounds and degradates in surface waters. These benchmarks are developed using data from ecological risk assessments for pesticide registration decisions. The results of toxicity tests using standard species are reported and these species typically include one or more species of fish, invertebrates, and both vascular and non-vascular plants.

Table 1. Acute water toxicity of representative pesticides to standard test species in water.

	96 hour water LC50 (µg/L)			
Pesticide	*Ceriodaphnia dubia*	*Hyalella azteca*	*Chironomus dilutus*	*Pimephales promelas*
Bifenthrin	0.142 [a]	0.0093 [e]	0.069 [i]	1.90 [k]
Fipronil	17.7 [b]	0.728 [f]	0.033 [f]	398 [k]
Imidacloprid	2.07 [c]	65.4 [g]	2.65 [j]	>1,000 [l]
Chlorpyrifos	0.053 [d]	0.086 [h]	0.29 [i]	203 [m]

[a] (Wheelock et al., 2004), [b] (Konwick et al., 2005), [c] 48-hour LC50 (Chen et al., 2010), [d] (Bailey et al., 1997), [e] (Anderson et al., 2006), [f] EC50 (Weston and Lydy, 2014), [g] (Stoughton et al., 2008), [h] (Phipps et al., 1995), [i] (Ding et al., 2012), [j] (LeBlanc et al., 2012), [k] (Beggel et al., 2010)(24-hour LC50), [l] (Lanteigne et al., 2015), [m] (Holcombe et al., 1982)

Table 2. Acute versus Chronic LC50s for bifenthrin and imidacloprid toxicity to *H. azteca* and *C. dilutus*. ND indicates not determined.

	Hyalella azteca			*Chironomus dilutus*	
Pesticide and Matrix	96 hour	10 day	28 day	96 hour	28 day
Bifenthrin in Sediment (ng/g)	ND	9.1 [a]	9.6 [a]	60.2 [c]	Uknown
Imidacloprid in Water (µg/L)	65.4 [b]	7.01 [b]	7.08 [b]	2.65 [d]	0.91 [b]

[a] (Anderson et al., 2015), [b] (Stoughton et al., 2008), [c] (Maul et al., 2008), [d] (LeBlanc et al., 2012)

*Morrissey et al., 2015 suggest 0.1 ug/L for chronic sublethal effects; these authors suggest a long term chronic protective value based on a probabilistic risk assessment of 0.035 ug/L.

WATER and SEDIMENT MATRICES and RECOMMENDATIONS

The environmental fate of current use pesticides largely depends on their relative stability and solubility in water. The octanol water partitioning coefficient (K_{ow}) is a laboratory derived parameter used as a surrogate measure for the potential of organic chemicals to accumulate in tissues; it is also used as an indicator of relative solubility. Pesticides with high log K_{ow} values are hydrophobic and pesticides with lower log K_{ow} values are more soluble. Pyrethroid pesticides like bifenthrin are highly hydrophobic and therefore readily partition to particles in water and accumulate in sediments. Urban stormwater and agriculture monitoring programs also routinely detect pyrethroids in water. Based on this, and the relative sensitivity of test species, the primary environmental compartment and matrix recommended for monitoring pyrethroids would be sediments using the 10-day *H. azteca* protocol (Table 3). Depending on resources, water toxicity testing for pyrethroids also provides useful information and the 96-hour water test with *H. azteca* is appropriate for this application. Fipronil and its degradates have moderate log K_{ow} values and therefore can be expected to accumulate in sediments and be detected in water. As with pyrethroids, they can be monitored in both matrices depending on resources. Toxicity testing should be conducted with the midge *C. dilutus* based on its greater sensitivity to this pesticide. For sediment, the 10-day test is applicable. For water, the 96 hour and 10 day tests are applicable, but the 10 day test is likely more sensitive (Table 3). Since fipronil is not registered for use in agriculture, monitoring for this pesticide should be restricted to urban watersheds. Neonicotinoids are highly soluble and are therefore not expected to accumulate in sediments. Because they are sufficiently stable to persist in receiving waters and exhibit greater potential for chronic toxicity to chironomids (testing at longer durations), water testing for this pesticide should use the 10-day test with *C. dilutus*.

Table 3. Log K_{ow} partitioning coefficients for selected current use pesticides, likely environmental compartments and recommended monitoring matrices.

Pesticide Class	Representative Compounds	Usage	Solubility (Log Kow)	Primary Recommended Test Species and Test	LC50 for species and exposure
Pyrethroids	Bifenthrin	Urban/Ag	6.4	*H. azteca* - 10-day Sediment	12.9 ng/g
	Cyhalothrin	Urban/Ag	7.1	*H. azteca* - 10-day Sediment	5.6 ng/g
	Cypermethrin	Urban/Ag	6.8	*H. azteca* - 10-day Sediment	14.9 ng/g
	Permethrin	Urban/Ag	6.3	*H. azteca* - 10-day Sediment	201 ng/g
Phenylpyrazoles	Fipronil	Urban	4.1	*C. dilutus* - 10-day Sediment	0.90 ng/g
	Fipronil Sulfide	Urban		*C. dilutus* - 10-day Sediment	1.11 ng/g
	Fipronil Sulfone	Urban		*C. dilutus* - 10-day Sediment	0.83 ng/g
Neonicotinoids	Imidacloprid	Ag/Urban	0.57	*C. dilutus* - 10-day Water	0.91-2.65 ug/L
Organophosphates	Chlorpyrifos	Ag	4.7	*C. dubia* - 96-hour Water	53 ng/L
	Diazinon	Ag	3.8	*C. dubia* - 96-hour Water	320 ng/L
	Malathion	Ag	2.4	*C. dubia* - 96-hour Water	2,120 ng/L

MARINE and ESTUARINE TESTING

The amphipod *H. azteca* is tolerant of a relatively wide range of salinities and can therefore be tested in estuarine systems up to 15‰. Standard U.S. EPA protocols using euryhaline species with high sensitivity to pesticides include the 10-day sediment test with the amphipod *Eohaustorius estuarius,* and the 96-hour acute and 7-day chronic water tests with the mysid *Americamysis bahia.*

STATUS of U.S. EPA PROTOCOLS

The U.S. EPA describes acute toxicity test methods for *C. dubia* in its freshwater acute toxicity test manual (U.S. EPA, 2002). This method allows a range of test durations from 24 to 96 hours. In addition, the manual includes a supplemental list of test species, including the amphipod *H. azteca* and the midge *C. dilutus*.

The U.S. EPA and United State Geological Survey describe 10-day, and 42-day sediment toxicity test protocols for *H. azteca* and *C. dilutus* (U.S. EPA, 2000). The 10-day sediment exposure procedure can be adapted for use as a 10-day water-only static renewal exposure with both *H. azteca* and *C. dilutus* (this is the procedure currently used at the UCD Granite Canyon Lab for water testing with these species).

Long term tests can also be adapted for shorter durations, such as the 28-day exposures with *H. azteca* (measuring growth and survival), and *C. dilutus* (measuring growth, survival and, potentially, emergence). U.S. EPA and USGS are currently in the process of updating the U.S. EPA 2000 sediment toxicity manual, which will include methods for testing both species in water and sediment using different exposure durations that range from 10 to 42 days for *H. azteca*, and 10 to ~50 days for *C. dilutus*. This revision is currently undergoing internal review within these agencies (personal communication, C. Ingersoll, USGS, Columbia, Missouri).

SUGGESTED CITATION

Anderson BS, Phillips BM, Denton D, Stillway M, Deanovic L, Lyons M, Hamilton M. 2015. Updated recommendations for monitoring current-use pesticide toxicity in water and sediment in the Surface Water Ambient Monitoring Program. SWAMP Technical Memorandum. SWAMP-TM-2015-0001.

REFERENCES

Anderson, B.S., Phillips, B.M., Hunt, J.W., Connor, V., Richard, N., Tjeerdema, R.S., 2006. Identifying primary stressors impacting macroinvertebrates in the Salinas River (California, USA): Relative effects of pesticides and suspended particles. Environ Poll 141, 402-408.

Anderson, B.S., Phillips, B.M., Voorhees, J.P., Peterson, M.A., Jennings, L.L., Fojut, T.L., Vasquez, M.E., Bucknell, P., Tjeerdema, R.S., 2015. Relative toxicity of bifenthrin to *Hyalella azteca* in 10-day vs. 28-day exposures. Integrated Environmental Assessment and Management 11, 319-328.

Bailey, H.C., Miller, J.L., Miller, M.J., Wiborg, L.C., Deanovic, L.A., Shed, T., 1997. Joint acute toxicity of diazinon and chlorpyrifos to *Ceriodaphnia dubia*. Environ Toxicol Chem 16, 2304-2308.

Beggel, S., Werner, I., Connon, R.E., Geist, J.P., 2010. Sublethal toxicity of commercial insecticide formulations and their active ingredients to larval fathead minnow (Pimephales promelas). Science of the Total Environment 408, 3169-3175.

Chen, X.D., Culbert, E., Hebert, V., Stark, J.D., 2010. Mixture effects of the nonylphenyl polyethoxylate, R-11 and the insecticide, imidacloprid on population growth rate and other parameters of the crustacean, Ceriodaphnia dubia. Ecotoxicology and Environmental Safety 73, 132-137.

Ding, Y., Landrum, P.F., You, J., Harwood, A.D., Lydy, M.J., 2012. Use of Solid Phase Microextraction to Estimate Toxicity: Relating Fiber Concentrations to Toxicity - Part I. Environ Toxicol Chem 31, 2159-2167.

Holcombe, G.W., Phipps, G.L., Tanner, D.K., 1982. The acute toxicity of kelthane, dursban, disulfoton, pydrin and permethrin to fathead minnows, *Pimephales promelas*, and rainbow trout, *Salmo gairdneri*. Environmental Pollution Series A Ecological and Biological 29, 167-178.

Konwick, G.J., Fisk, A.T., Garrison, A.W., Avants, J.K., Black, M.C., 2005. Acute enantioselective toxicity of fipronil and its deslfinyl photoproduct to *Ceriodaphnia dubia*. Environ Toxicol Chem 24, 2350-2355.

Lanteigne, M., Whiting, S.A., Lydy, M.J., 2015. Mixture Toxicity of Imidacloprid and Cyfluthrin to Two Non-target Species, the Fathead Minnow Pimephales promelas and the Amphipod Hyalella azteca. Archives of Environmental Contamination and Toxicology 68, 354-361.

LeBlanc, H.M.K., Culp, J.M., Baird, D.J., Alexander, A.C., Cessna, A.J., 2012. Single Versus Combined Lethal Effects of Three Agricultural Insecticides on Larvae of the Freshwater Insect Chironomus dilutus. Archives of Environmental Contamination and Toxicology 63, 378-390.

Maul, J.D., Brennan, A.A., Harwood, A., Lydy, M.J., 2008. Effect of sediment-associated pyrethroids, fipronil, and metabolites on *Chironomus tentans* growth rate, body mass, condition index, immobilization, and survival. Environ Toxicol Chem 27, 2582-2590.

Phipps, G.L., Mattson, V.R., Ankley, G.T., 1995. The relative sensitivity of three benthic test species to ten chemicals. Arch Environ Toxicol Chem 28, 281-286.

Stoughton, S.J., Liber, K., Culp, J., Cessna, A., 2008. Acute and Chronic Toxicity of Imidacloprid to the Aquatic Invertebrates *Chironomus tentans* and *Hyalella azteca* under Constant- and Pulse-Exposure Conditions. Arch Environ Contam Toxicol 54, 662-673.

Weston, D.P., Lydy, M.J., 2014. Toxicity of the Insecticide Fipronil and Its Degradates to Benthic Macroinvertebrates of Urban Streams. Environ Sci Tech 48, 1290-1297.

Wheelock, C.E., Miller, J.L., Miller, M.J., Gee, S.J., Shan, G., Hammock, B.D., 2004. Development of toxicity identification evaluation procedure for pyrethroid detection using esterase activity. Environ Toxicol Chem 23, 2699-2708.

Environment International 74 (2015) 291–303

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Review

Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: A review

Christy A. Morrissey [a,b,*], Pierre Mineau [c], James H. Devries [d], Francisco Sanchez-Bayo [e], Matthias Liess [f], Michael C. Cavallaro [b], Karsten Liber [b,g]

[a] Department of Biology, University of Saskatchewan, 112 Science Place, Saskatoon, Saskatchewan, Canada
[b] School of Environment and Sustainability, 117 Science Place, University of Saskatchewan, Saskatoon, Saskatchewan, Canada
[c] Pierre Mineau Consulting, 124 Creekside Dr., Salt Spring Island, BC V8K 2E4, Canada
[d] Institute for Wetland and Waterfowl Research, Ducks Unlimited Canada, P.O. Box 1160, Stonewall, Manitoba, Canada
[e] Faculty of Agriculture & Environment, University of Sydney, NSW 2015, Australia
[f] UFZ, Helmholtz Centre for Environmental Research, Department System-Ecotoxicology, Permoserstrasse 15, 04318 Leipzig, Germany
[g] Toxicology Centre, University of Saskatchewan, 44 Campus Drive, Saskatoon, Saskatchewan, Canada

ARTICLE INFO

Article history:
Received 29 July 2014
Received in revised form 27 October 2014
Accepted 28 October 2014
Available online xxxx

Keywords:
Water quality guidelines
Pesticides
Neonicotinoids
Risk assessment
Species sensitivity distribution
Aquatic invertebrates

ABSTRACT

Neonicotinoids, broad-spectrum systemic insecticides, are the fastest growing class of insecticides worldwide and are now registered for use on hundreds of field crops in over 120 different countries. The environmental profile of this class of pesticides indicate that they are persistent, have high leaching and runoff potential, and are highly toxic to a wide range of invertebrates. Therefore, neonicotinoids represent a significant risk to surface waters and the diverse aquatic and terrestrial fauna that these ecosystems support. This review synthesizes the current state of knowledge on the reported concentrations of neonicotinoids in surface waters from 29 studies in 9 countries world-wide in tandem with published data on their acute and chronic toxicity to 49 species of aquatic insects and crustaceans spanning 12 invertebrate orders. Strong evidence exists that water-borne neonicotinoid exposures are frequent, long-term and at levels (geometric means = 0.13 μg/L (averages) and 0.63 μg/L (maxima)) which commonly exceed several existing water quality guidelines. Imidacloprid is by far the most widely studied neonicotinoid (66% of the 214 toxicity tests reviewed) with differences in sensitivity among aquatic invertebrate species ranging several orders of magnitude; other neonicotinoids display analogous modes of action and similar toxicities, although comparative data are limited. Of the species evaluated, insects belonging to the orders Ephemeroptera, Trichoptera and Diptera appear to be the most sensitive, while those of Crustacea (although not universally so) are less sensitive. In particular, the standard test species *Daphnia magna* appears to be very tolerant, with 24–96 hour LC_{50} values exceeding 100,000 μg/L (geometric mean > 44,000 μg/L), which is at least 2–3 orders of magnitude higher than the geometric mean of all other invertebrate species tested. Overall, neonicotinoids can exert adverse effects on survival, growth, emergence, mobility, and behavior of many sensitive aquatic invertebrate taxa at concentrations at or below 1 μg/L under acute exposure and 0.1 μg/L for chronic exposure. Using probabilistic approaches (species sensitivity distributions), we recommend here that ecological thresholds for neonicotinoid water concentrations need to be below 0.2 μg/L (short-term acute) or 0.035 μg/L (long-term chronic) to avoid lasting effects on aquatic invertebrate communities. The application of safety factors may still be warranted considering potential issues of slow recovery, additive or synergistic effects and multiple stressors that can occur in the field. Our analysis revealed that 81% (22/27) and 74% (14/19) of global surface water studies reporting maximum and average individual neonicotinoid concentrations respectively, exceeded these thresholds of 0.2 and 0.035 μg/L. Therefore, it appears that environmentally relevant concentrations of neonicotinoids in surface waters worldwide are well within the range where both short- and long-term impacts on aquatic invertebrate species are possible over broad spatial scales.

* Corresponding author at: Dept. of Biology, University of Saskatchewan, 112 Science Place, Saskatoon, Saskatchewan S7N 5E2, Canada.
E-mail address: christy.morrissey@usask.ca (C.A. Morrissey).

http://dx.doi.org/10.1016/j.envint.2014.10.024
0160-4120/

Contents

1. Introduction

1.1. Background on neonicotinoids

Neonicotinoids belong to the group of nitroguanidine systemic insecticides frequently applied to crops as soil and seed treatments at planting to protect seedlings from early-season root and leaf-feeding pests, as well as via later season foliar treatments. Imidacloprid-containing products now dominate the insecticide market and are registered for use on more than 140 different crops in 120 countries (Jeschke and Nauen, 2008). The neonicotinoid class of insecticides was first developed and registered in the early 1990s, partly in response to ongoing pest resistance, concerns over cumulative exposure from organophosphorous and carbamate insecticides, and increasing evidence linking impaired neural development in children to cholinesterase-inhibiting insecticides (Eskenazi et al., 1999). Following on the industry success of imidacloprid, development and sale of other neonicotinoid insecticides with similar chemistries rapidly followed after 2000, specifically acetamiprid, clothianidin, dinotefuran, nitenpyram, thiacloprid and thiamethoxam among others, under various trade names. Neonicotinoids now represent the largest selling class of insecticide and seed treatments on the global market (Jeschke et al., 2010).

Due to their systemic activity, improved rain fastness, and convenience of use as a seed treatment, neonicotinoids are extremely popular for pest control on a broad range of crops (Elbert et al., 2008; Main et al., 2014; USGS, 2012). However, they exhibit chemical properties that enhance environmental persistence and susceptibility to transport into aquatic ecosystems through runoff and drainage of agricultural areas (Armbrust and Peeler, 2002). Recent reports suggest toxic residues of imidacloprid and other neonicotinoids have been detected in water bodies and researchers in the Netherlands have found correlative links to reduced aquatic insect populations (Van Dijk et al., 2013) and insectivorous farmland birds (Hallmann et al., 2014). However, in most countries there is a general lack of systematic environmental monitoring data for neonicotinoids in surface waters and until recently, analytical procedures were often insufficient to report the low concentrations known to cause harm to aquatic invertebrates.

Neonicotinoids are successful insecticides largely because the acute toxicity to mammals is lower than its replacements, they are extremely toxic to most insect pests and can be conveniently used as a systemic seed or in furrow treatment to protect seedling crops from piercing-sucking and chewing insects. All neonicotinoids bind agonistically to the post-synaptic nicotinic acetylcholine receptors (nAChR) in the invertebrate central nervous system, thus competing with the natural neurotransmitter acetylcholine (ACh). Toxicity studies with arthropods suggest that the binding to these receptors is long-lasting (Tennekes, 2010a), and lethal effects are typically delayed (Beketov and Liess, 2008a) such that repeated or chronic exposure can lead to cumulative effects over time (Tennekes and Sánchez-Bayo, 2013). For many aquatic invertebrates with long larval aquatic stages, exposure to neonicotinoids is expected to be prolonged due to either repeated pulse events and/or low level chronic exposures. Many invertebrates are extremely sensitive to these compounds, including non-target aquatic species (Alexander et al., 2007; Beketov and Liess, 2008a; EFSA, 2013; Liess and Beketov, 2011; Pestana et al., 2009; Roessink et al., 2013; Sánchez-Bayo and Goka, 2006; Stoughton et al., 2008) and terrestrial pollinators such as bumble bees and honey bees (Decourtye and Devillers, 2010; Sanchez-Bayo and Goka, 2014; Whitehorn et al., 2012). Consequently, the persistence and movement of neonicotinoids into aquatic ecosystems could pose a risk to sensitive aquatic invertebrates upon which vertebrate wildlife depend for food (Gibbons et al., 2014; Goulson, 2013; Tennekes, 2010b). The objective of this review is to summarize the available data on different neonicotinoid concentrations in surface waters worldwide and to cohesively synthesize and compare these values to the growing body of data from laboratory, field and mesocosm studies on the concentrations observed to cause lethal and sub-lethal toxicity to aquatic invertebrates. Finally, based on probabilistic analyses, we provide recommended aquatic invertebrate effect thresholds to aid in the development of appropriate water quality reference values for the range of neonicotinoids.

1.2. Chemical properties and environmental fate

All neonicotinoids exhibit high water solubility that makes them amenable for use as systemic insecticides. In addition, they also have long half-lives in soil and in water, where they are resistant to hydrolysis at neutral or acidic pH and under anaerobic conditions; although some of them are subject to rapid photodegradation under favorable conditions (i.e. shallow waters with greater light penetration; Table 1). Their chemical properties, particularly their high water solubility and partitioning properties (low log K_{OW}) and low soil adsorption (log K_{OC}), promote movement of these insecticides through surface and subsurface runoff (CCME, 2007; EFSA, 2008) and result in extended persistence under simulated environmental conditions (Tisler et al., 2009). Local environmental conditions can modify the persistence of neonicotinoids in water (e.g., increasing pH and turbidity enhances persistence) (Sarkar et al., 2001). The major transport routes to aquatic ecosystems include surface runoff after rain events (Armbrust and Peeler, 2002), soluble or insoluble fractions transported via snowmelt (Main et al., 2014), leaching into groundwater (Lamers et al., 2011) with associated subsurface discharge into wetlands and other surface waters (PMRA, 2001), talc and graphite dust associated with seeding drills at the time of planting (Krupke et al., 2012; Nuyttens et al., 2013), decay of systemically treated plants in water bodies (Kreutzweiser et al., 2008), and deposition of

Table 1
Chemical properties (solubility, log K_{OW} and K_{OC}) and environmental persistence (DT_{50} for soil and aqueous photolysis and hydrolysis) of neonicotinoid insecticides. Where available, field degradation studies were selected.[a]

Compound	Molecular Mass (Da)[b]	Water Solubility (mg/L) @ 20 °C	Lipophilicity (log K_{OW})	Soil Affinity (log K_{OC})	Soil Persistence (DT_{50} in days)[c]	Water Photolysis (DT_{50} in days)	Water Hydrolysis (DT_{50} in days)[d]
Acetamiprid	222.7	2950	0.80	2.3	2–20	34	Stable; 420 (pH 9)
Clothianidin	249.7	340	0.91	2.08	13–1386	<1	Stable; 14.4 (pH 9)
Dinotefuran	202.2	39,830	−0.55	1.41	50–100	<2	Stable
Imidacloprid	255.7	610	0.57	2.19–2.90	104–228	<1	Stable; >1 yr (pH 9)
Nitenpyram	270.7	590,000	−0.66	1.78	1–15	NA	Stable; 2.9 (pH 9)
Thiacloprid	252.7	184	1.26	3.67	9–27	10–63	Stable
Thiamethoxam	291.7	4100	−0.13	1.75	7–72	2.7–39.5	Stable; 11.5 (pH 9)

[a] Data sources: Pesticide Products Database (PPDB) University of Hertfordshire; 2006–2013 and Hazardous Substances Data Bank (HSDB) Accessed Feb. 5 2014. Available at: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB.
[b] Da = Dalton = g/mol.
[c] Under anaerobic conditions, compounds are much more stable in water and soil.
[d] Under acidic or neutral pH conditions, compounds are stable to hydrolysis, whereas under alkaline conditions (pH 9) hydrolysis can occur.

treated seeds, soil or spray drift into water bodies or depressions. The majority of surface water contamination is expected to be through runoff after major precipitation events (Chiovarou and Siewicki, 2008).

Persistence in soil, and thus the likelihood of neonicotinoid movement into receiving waters, is largely dependent on factors such as application rate, pH, temperature, the presence or absence of crop or plant cover, crop rotation, soil type and organic content, and use of fertilizers. Field dissipation studies where imidacloprid was applied to various crops such as corn, tomatoes and turf at an application rate of 0.5 lb/acre report field half-lives in soil of 7, 53, and 61–107 days respectively (SERA, 2005), but half-lives up to 228 days have been reported (Miles Inc. 1992 in Fossen, 2006). Other neonicotinoids such as clothianidin can have half-lives in soil much longer (up to 1386 days) with residues persisting under some conditions for over 4600 days (DT_{90}) (PMRA, 2004). Scholz and Spiteller (1992) found that imidacloprid dissipation time was more rapid in soils with cover crops (48 days) than in bare soils (190 days). Interestingly, applications of fertilizer and use of formulated products have been reported to alter imidacloprid persistence in soil. For example, increases in soil organic carbon through application of organic fertilizers and manure can increase persistence (Rouchaud et al., 1994). Fertilizers have also been shown to decrease soil adsorption and further enhance the mobility and leaching of imidacloprid due to competition between the pesticide and organic matter for soil binding sites (Flores-Cespedes et al., 2002). In contrast, aged pesticide soil residues are more tightly bound leading to increased sorption and reduced transport down the soil profile, but may still move with particulates in solution to surface waters (Cox et al., 1998).

The features which influence soil retention and persistence are also known to influence leaching of neonicotinoids into groundwater. In the absence of light, neonicotinoids can persist in soil and be transported vertically into groundwater. Leachate concentrations may reach depths of 105 cm (Felsot et al., 1998) and concentrations of 0.005–1.32 μg/L (Gupta et al., 2008), 1–5 μg/L (Larsbo et al., 2013), and 100–400 μg/L (Felsot et al., 1998). Consequently, several studies have detected neonicotinoids in groundwater at maximum concentrations ranging from 1.93 μg/L (imidacloprid) to 8.93 μg/L (thiamethoxam) (Table A.1). Concentrations of thiamethoxam in irrigation water sourced from groundwater in a potato growing region of Wisconsin ranged from 0.31 to 0.58 μg/L, and state-wide sampling revealed noteworthy groundwater concentrations for clothianidin (0.21–3.43 μg/L), imidacloprid (0.26–3.34 μg/L), and thiamethoxam (0.20–3.34 μg/L) (Huseth and Groves, 2014). This suggests that shallow infiltration of neonicotinoids may move horizontally as groundwater and discharge into surface waters such as streams and wetlands.

When entering surface waters, neonicotinoids exhibit peak concentrations within 24 h post-application and breakdown following first-order kinetics: rapid initial loss over the first few days followed by a slower second phase (Armbrust and Peeler, 2002). Most field studies on the fate of neonicotinoids in water have focussed on experimental applications of imidacloprid in rice paddy plantations. Experimental applications at standard rates of 45 and 250 g/ha produced maximum paddy water concentrations of 0.18 μg/L (Kanrar et al., 2006) and 52.9 μg/L (La et al., 2014). At higher application rates of 10,000 g/ha, Thuyet et al. (2011) found that water concentrations peaked at similar levels for treatments applied before (30.2 μg/L) or after (3 μg/L) sowing crops. Rapid initial dissipation of imidacloprid in water in these field studies suggests losses through multiple pathways including dilution, infiltration, photolysis, microbial degradation, plant uptake and, to a much lesser extent, sorption to soil and sediment. The half-lives of imidacloprid in water generally appear to be relatively short (days) (Table 1), but measurable and ecotoxicologically relevant concentrations (0.1 or 0.2 μg/L), can still be detected up to a year after treatment (Kanrar et al., 2006; La et al., 2014), with prolonged persistence under specific environmental conditions such as low temperatures and low pH (Guzsvany et al., 2006) and with the use of the formulated products (Sarkar et al., 2001).

2. Evidence of surface water contamination

Our survey of the water monitoring literature suggests that of the 29 studies identified from 9 countries, neonicotinoids were detected in most surface waters sampled, including puddled water, irrigation channels, streams, rivers, and wetlands in proximity to, or receiving runoff from, agricultural cropland (Fig. 1, Table A.1). The concentrations of individual neonicotinoids from this dataset indicated a geometric mean for average surface water concentrations of 0.13 μg/L (n = 19 studies) and a geometric mean for peak surface water concentrations of 0.63 μg/L (n = 27 studies). Although pesticide monitoring data frequently reports means and maxima, these are usually from grab or spot samples which often underestimate peak concentrations by 1–3 orders of magnitude and average concentrations by 50% (Xing et al., 2013). Depending on the timing of water sampling, particularly in relation to rainfall events, this has major limitations for interpreting the actual peak and average concentrations that are relevant for estimating exposure to aquatic species.

About half of the available water monitoring studies reported detectable imidacloprid concentrations given its longer use history and breadth of applications. Detectable concentrations of imidacloprid ranged from 0.001 (>LOD) to 320 μg/L. Other neonicotinoids are detected at similar water concentrations ranging from 0.008 to 44.1 μg/L for acetamiprid, 0.003 to 3.1 μg/L for clothianidin, and 0.001 to 225 μg/L for thiamethoxam. Where water concentrations were higher, not surprisingly, detection frequencies were also higher. Some of the highest reported concentrations in aquatic systems include imidacloprid in Dutch agricultural surface waters at concentrations up to 320 μg/L (Van Dijk et al., 2013), and thiamethoxam and acetamiprid in playa wetlands of the Texas high plains of up to 225 μg/L (Anderson et al.,

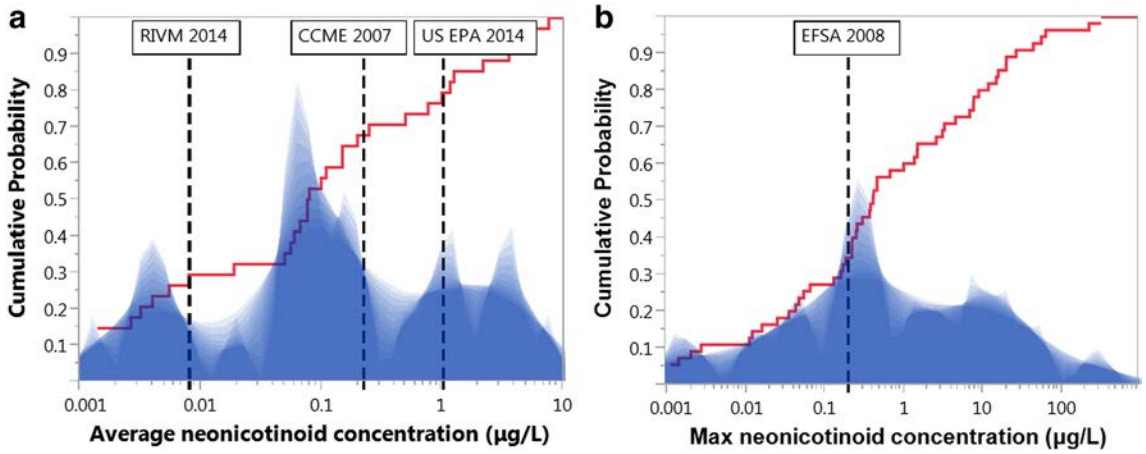

Fig. 1. Shadow histogram of a) average and b) maximum individual neonicotinoid concentrations (log scale, µg/L) reported from water monitoring studies. Overlaid is the cumulative distribution probability (red ascending line) using all available surface water monitoring data showing proportion of data below any given neonicotinoid concentration. Vertical dashed lines illustrate multiple ecological quality reference values set for average imidacloprid water concentrations (RIVM, 2014: 0.0083 µg/L, CCME, 2007: 0.23 µg/L, and US EPA: 1.05 µg/L) or for maximum imidacloprid water concentrations (EFSA, 2008: 0.2 µg/L).

2013). Water samples collected since the mid-1990s from Eastern Canada revealed that imidacloprid was increasingly detected in stream waters draining potato fields after rainfall events, reaching concentrations up to 11.9 µg/L (Denning et al., 2004 in CCME, 2007). In Sydney, Australia, rivers draining horticulture and vegetable growing regions contained five different neonicotinoids with detections in 27–93% of samples and concentrations reaching 4.6 µg/L (imidacloprid) and 1.4 µg/L (thiacloprid) after rainfall events (Sanchez-Bayo and Hyne, 2014). In California, 89% of surface water samples collected from agricultural regions contained imidacloprid with concentrations of up to 3.29 µg/L (Starner and Goh, 2012). Main et al. (2014) found that wetlands in the Canadian Prairies sampled four times over a one year period had maximum concentrations detected in early summer (3.1 µg/L clothianidin and 1.5 µg/L thiamethoxam) and detection frequencies of 36–91%. While not formally considered water bodies, puddles collected on the surface of neonicotinoid seed-treated corn fields in Quebec, Canada have also been found to contain maximum concentrations of 55.7 µg/L clothianidin and 63.4 µg/L thiamethoxam (Samson-Robert et al., in press).

Although no regional patterns were apparent for neonicotinoid detections, wetlands and rivers directly draining or receiving runoff from agricultural crops appear most susceptible. Neonicotinoids, however, have also been frequently detected in water draining urban environments at similar concentrations (Sanchez-Bayo and Hyne, 2014). Importantly, multiple neonicotinoids have been detected in single water samples (Main et al., 2014; Sanchez-Bayo and Hyne, 2014) and often outside of the growing season (Main et al., 2014; Starner and Goh, 2012) suggesting long-term persistence, repeated transport to surface water bodies, or degradation to persistent metabolites (ie. thiamethoxam to clothianidin).

Existing water monitoring data are too scarce to make inferences about the fate of surface water contamination from neonicotinoids in relation to land use and water body features. Frequent detection in water is predicted given the unique properties of this class of insecticides which are highly water soluble, stable to hydrolysis and often slowly degraded. As for other pesticides, water concentrations will be determined by the abiotic and biotic features of the water body and the surrounding land which facilitates transport, retention and degradation (Goldsborough and Crumpton, 1998; Sarkar et al., 1999). Main et al. (2014) reported no statistical differences in average concentrations of neonicotinoids in wetlands surrounded by different cereal and canola crops, although wetlands near canola fields had a higher detection frequency and all contained significantly higher concentrations than wetlands surrounded by grassland. In Texas, playa wetlands in or near grasslands were contaminated with acetamiprid at levels comparable to the cropland, although the frequency of detection was lower (Anderson et al., 2013). As neonicotinoid use increases and more monitoring is conducted, the frequency of detection and the peak and average concentrations of neonicotinoid residues are expected to rise. Equally, as sensitive analytical methods become more widely available, the detection limits also come more in line with toxicity thresholds which, for many sensitive aquatic invertebrates, are typically in the part per billion (µg/L) or part per trillion (ng/L) range (Sánchez-Bayo et al., 2013).

3. Aquatic invertebrate toxicity

3.1. Acute and chronic toxicity of neonicotinoids to aquatic invertebrates

Although the acute toxicity of neonicotinoids to mammals, fish, and birds is generally reported as being lower than for many other insecticides (but see Mineau and Palmer, 2013), extremely low concentrations appear to exert measurable toxicity to a wide range of arthropods, especially insects and some crustaceans. The neonicotinoids have been selected for their specific ability to bind, and activate, the post-synaptic nicotinergic acetylcholine receptors (nAChR) in the insect central nervous system. The neonicotinoid molecule remains bound to the nAChR in insects, holding the channel open and effectively causing continuous nervous system stimulation. In mammals and other vertebrates, the lesser affinity of neonicotinoids for their nAChR appears to be related to the different configuration of the subunits that make up this receptor, so the insecticide binding is weak and/or does not last as long as in insects (Yamamoto et al., 1995). Receptor binding affinity and specificity to the nAChR appears equivalent among different neonicotinoids and is known to be highly conserved across several insect species examined (Zhang et al., 2000). Therefore, differences in toxicity among terrestrial insect species and neonicotinoids have been attributed largely to molecule structure. Neonicotinoid molecules contain either an electronegative nitro- (e.g. imidacloprid, clothianidin, dinotefuran, thiamethoxam) or cyano- (e.g. acetamiprid thiacloprid) substituted heterocyclic group that confers a higher detoxification potential of the latter as reported in bees (Iwasa et al., 2004). Differences in hydrophobicity of the compounds may also affect uptake (penetration across the cuticle and membrane) and thus insecticidal activity (Yamamoto et al., 1998), but this may not be as critical to aquatic invertebrate species. Receptor binding in invertebrates appears to be near irreversible; thus, permanent effects are cumulative with exposure time (Tennekes, 2010a; Tennekes and Sánchez-Bayo, 2011) (but see response by Maus

and Nauen (2010) and rebuttal by Tennekes (2011)), and may therefore exhibit delayed toxicity (Beketov and Liess, 2008a). This trait, in combination with high among-species variability in neonicotinoid toxicity, suggests that current toxicological endpoints commonly used in the regulatory process (i.e., 48-h acute tests for single species) may be inappropriate for this class of insecticides and will lead to an underestimation of the true toxic potential of these insecticides (Beketov and Liess, 2008a; Brock and Wijngaarden, 2012; Tennekes and Sánchez-Bayo, 2011). However, short-term tests still dominate the toxicity literature.

Here, we reviewed over 214 toxicity tests, including acute and chronic tests for imidacloprid, acetamiprid, clothianidin, dinotefuran, thiacloprid, and thiamethoxam with 49 different aquatic arthropod species spanning 12 orders (Table A.2). We conducted a full review of toxic endpoints for aquatic invertebrates following on and updating the work of Goulson (2013), Mineau and Palmer (2013) and Vijver and van den Brink (2014) among others, through searches on the ISI Web of Science for published peer-reviewed studies, but also included industry studies and government reports. Studies included tests with six different neonicotinoids, but predominantly imidacloprid (66%, n = 141 tests), acute studies of ≤96 h duration (83%, n = 178 tests), and (sub)chronic studies of 7 to 39 days duration (17%, n = 36 tests). We only included toxicity tests reporting LC_{50} values (64%, n = 137 tests) and EC_{50} values (36%, n = 77 tests) and excluded those reporting only No Observable Effect Concentrations (NOECs) or Lowest Observable Effect Concentrations (LOECs) because of inconsistency in interpretation. We further considered 16 additional chronic, multi-species field or mesocosm studies to incorporate field-realistic effects on aquatic invertebrate communities (Table A.3). Toxicity data, where combined for the different neonicotinoids are presented as molar equivalents (μmol/L) given the known differences in molecular weights. Back-conversions to concentrations (μg/L) may be approximated by multiplying the molar concentration by the molecular mass of the compound shown in Table 1.

Not surprisingly, neonicotinoid insecticides can exert significant lethal and sub-lethal effects on many aquatic invertebrate populations. In general, acute and chronic toxicity of the neonicotinoids varies greatly among aquatic arthropods (i.e., LC_{50} values range from <1 to >100,000 μg/L, 6 orders of magnitude), with species belonging to the class Insecta typically being the most sensitive (e.g. Alexander et al., 2008), and with cladocerans (Branchiopoda) having the broadest range of sensitivity (Fig. 2). In particular, the Ephemeroptera, Trichoptera and several Diptera, particularly the Chironomidae (midges), were consistently the most sensitive taxa. Many of these species exhibit short-term lethal effects at water concentrations often below 1 μg/L. Sub-lethal endpoints in chronic studies were frequently an order of magnitude or more below the acute tests. For example, Beketov and Liess (2008b) found that downstream drift of aquatic invertebrates (an ecologically relevant endpoint) occurred at concentrations at least nine times lower than corresponding LC_{50} values.

The most widely tested species, *Daphnia magna*, represented 34 studies, or 16% of all neonicotinoid toxicity tests reviewed. This is largely because *D. magna* is considered the global industry standard invertebrate species for most (82%) chemicals tested (Sanchez-Bayo, 2006). However, several authors Ashauer et al. (2011), Beketov and Liess (2008a) and Jemec et al. (2007) reported that this species is by far the least sensitive test species for acute and chronic neonicotinoid studies (Fig. 2). The short-term L[E]C50 for *D. magna* ranges from 4100 to >1,000,000 μg/L, with a geometric mean of 43,927 μg/L (175.8 μmol/L), a value that is at least two to three orders of magnitude higher than the geometric means for most other aquatic invertebrate species (Fig. 2, Table 2). By comparison, Roessink et al. (2013) examined acute and chronic toxicity of imidacloprid to a comprehensive range of aquatic insects and other crustaceans and found that mayflies (Ephemeroptera) and caddisflies (Trichoptera) were the most sensitive species in both acute and chronic tests, with LC_{50} and EC_{50} values in the range of 0.1–0.3 μg/L; other studies have shown midges (Chironomidae) and some other Diptera also to have similar sensitivity (Fig. 2, Table A.2).

While LC_{50} values dominate the hazard assessment for these compounds and allow for direct comparisons of sensitivity among species, several sub-lethal endpoints (growth, reproduction, immobility, feeding, swimming behavior, and emergence) are all responsive to neonicotinoid exposures. Alexander et al. (2007) found that short (12 h) exposure pulses of ≥1 μg/L imidacloprid caused feeding inhibition in mayflies. Even pulse exposures as low as 0.1 μg/L affected the size of adults at emergence (Alexander et al., 2008). Feeding inhibition from imidacloprid exposure similarly appeared to be responsible for decreases in growth and body size for the shredder, *Gammarus pulex* (Ashauer et al., 2011). Immobility of mayflies and caddisflies after a 96 hour exposure to imidacloprid was reported at concentrations in the range of 0.1 to 0.2 μg/L (Roessink et al., 2013). Beketov and Liess (2008b) reported increased downstream drift of macroinvertebrates in a stream microcosm within 2–4 h of exposure to thiacloprid, imidacloprid and acetamiprid. Downstream drift appears to be a sensitive and ecologically relevant measure of imidacloprid effects to several aquatic invertebrate species (Berghahn et al., 2012).

3.2. Relative toxicity of different neonicotinoids and mixtures

Consistent with reported water monitoring data, most of the toxicity research to date has focused on imidacloprid, with relatively few published studies on other neonicotinoids. Based on limited data, however,

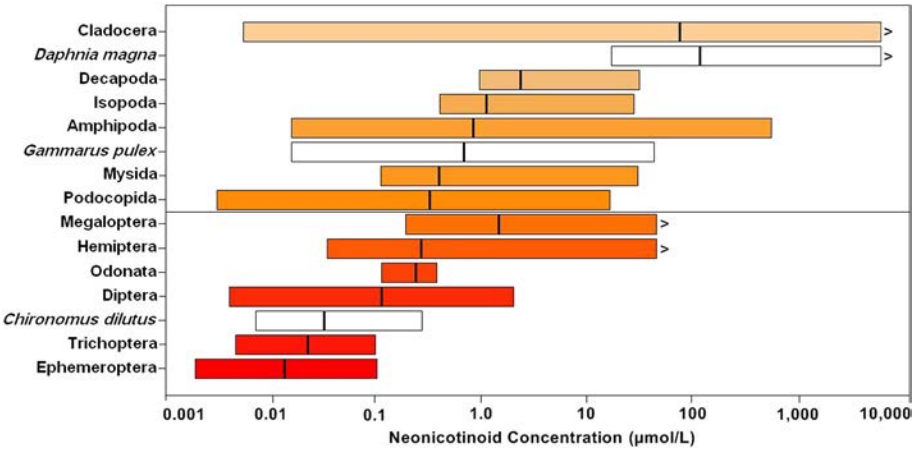

Fig. 2. Range of neonicotinoid toxicity (L[E]C_{50}: 24–96 h in μmol/L) among all tested aquatic invertebrate orders. For context, three of the most common test species (open bars) for the orders Cladocera (*Daphnia magna*), Amphipoda (*Gammarus pulex*) and Diptera (*Chironomus dilutus*) are shown to illustrate differences in sensitivity by species. Vertical lines within bars represent geometric means of test values (see also Table 2).

Table 2
Geometric means by concentration (in μg/L) and by molecular weight (μmol/L) derived from acute toxicity test values (24–96 h L[E]C_{50}) by taxonomic group and by neonicotinoid active ingredient in order of increasing relative toxicity.

Order	Taxa	Geometric mean (μg/L)	Geometric mean (μmol/L)	Active Ingredient	Geometric mean (μg/L)	Geometric mean (μmol/L)
Crustaceans	Cladocera	23,690.0	94.2	Dinotefuran	37,753.1	186.7
	Daphnia magna	43,926.5	175.8	Thiamethoxam	8864.5	30.4
	Decapoda	1562.2	6.87	Acetamiprid	1271.4	5.71
	Isopoda	464.8	1.83	Clothianidin	842.3	3.37
	Amphipoda	235.8	0.93	Thiacloprid	614.8	2.43
	Gammarus pulex	258.7	1.02	Imidacloprid	587.0	2.30
	Mysida	106.2	0.42			
	Podocopida	73.6	0.29			
Aquatic Insects	Megaloptera	711.3	2.78	Dinotefuran	229.8	1.14
	Hemiptera	64.9	0.25	Thiamethoxam	44.8	0.15
	Odonata	55.2	0.22	Acetamiprid	44.4	0.20
	Diptera	32.9	0.13	Imidacloprid	26.8	0.11
	Chironomus dilutus	9.3	0.04	Clothianidin	25.3	0.10
	Trichoptera	6.9	0.03	Thiacloprid	9.6	0.04
	Ephemeroptera	3.9	0.02			

it appears that differences in relative toxicity among the various individual neonicotinoids are minor. For example, the overlap in toxicity ranges of the different neonicotinoids is considerable, and differences among taxonomic groups are greater than those observed among different neonicotinoids (Fig. 3). Therefore, we combined the toxicity L[E]C_{50} values of individual neonicotinoids into a single dataset.

Mineau and Palmer (2013) contend that any apparent differences among neonicotinoids are likely artifacts of data availability rather than any real differences in toxicity. For two species of crustaceans, *Americamysis bahia* and *G. pulex*, and one insect species, *Chironomus riparius*, LC_{50} values are available for multiple neonicotinoids, although not necessarily from the same lab or research group nor identical test conditions. For *A. bahia*, the relative order of toxicity was thiacloprid (LC_{50} = 31–50 μg/L) ≥ clothianidin (LC_{50} = 51 μg/L) ≥ imidacloprid (LC_{50} = 34–159 μg/L) ≥ acetamiprid (LC_{50} = 66 μg/L) > dinotefuran (LC_{50} = 790 μg/L), >thiamethoxam (LC_{50} = 6900 μg/L). Some differences were also apparent for *G. pulex* where relative toxicity ordered acetamiprid (LC_{50} = 50 μg/L) > imidacloprid (LC_{50} = 350 μg/L) ≥ thiacloprid (LC_{50} = 190–9520 μg/L). While some differences in toxicity among neonicotinoids appear to exist for these two crustaceans, in reviewing data for an insect species, *C. riparius*, the data show fewer differences: imidacloprid (LC_{50} = 20 μg/L) ≥ clothianidin (EC_{50} = 22 μg/L) > thiamethoxam (EC_{50} = 35 μg/L). Differences in molecular weights of the various neonicotinoids range from 202.2 to 291.7 Da (Table 1), which may account for some apparent differences in the relative toxicity for certain aquatic species. For example, for *C. riparius*, the above effect levels expressed as molar concentrations are even more similar across neonicotinoids 0.08–0.12 μmol/L.

Neonicotinoids are known to be additively or synergistically toxic when they occur together, or when combined with certain fungicides that are potent cytochrome P450 monooxygenase enzyme inhibitors (Andersch et al. 2010; Iwasa et al., 2004). For example, the combination of clothianidin and the fungicide trifloxystrobin (as in the canola seed treatment formulation PROSPER™) resulted in a 150-fold increase in kill rate to leaf beetle (Phaedon) larvae over clothianidin alone (Wachendorff-Neumann et al., 2012). Bayer Crop Science has patented several combinations of two neonicotinoids demonstrating synergism of insecticidal activity. For example, individual treatments with 0.8 ppm of thiacloprid or 0.8 ppm clothianidin destroyed 25% and 0% of aphid populations after 6 days, but combined at the same doses, the kill rate rose to 98% (Andersch et al. 2010). Binary mixtures of imidacloprid and thiacloprid have been tested on *D. magna* where effects on reproduction, growth and survival most closely followed patterns of synergism or concentration addition (Pavlaki et al., 2011). Neonicotinoids also may interact synergistically with other pesticides, or other inert formulation ingredients commonly present in aquatic systems in agricultural areas (Alexander et al., 2013; Chen et al., 2010; LeBlanc et al., 2012; Vijver and van den Brink, 2014). In contrast, the influence of prior exposure to other xenobiotics, including common-use herbicides, has been shown to provide mosquitos (*Aedes aegypti*) greater co-tolerance to imidacloprid (Riaz et al., 2009), through up-regulation of the P450 monooxygenase genes (CYP enzymes) involved

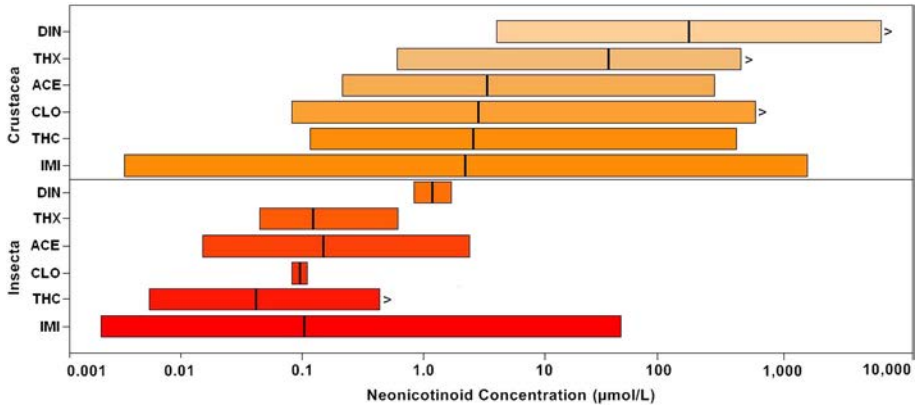

Fig. 3. Range of neonicotinoid toxicity (L[E]C_{50}: 24–96 h in μmol/L) among crustaceans (upper) and aquatic insects (lower) for six different neonicotinoid active ingredients: dinotefuran (DIN), thiamethoxam (THX), acetamiprid (ACE), clothianidin (CLO), thiacloprid (THC), and imidacloprid (IMI). The width of each bar represents the range of standard L[E]C_{50} values (μg/L) and vertical lines within bars represent the geometric mean of the tests (see also Table 2). Note that data are more limited for compounds other than imidacloprid and thiacloprid.

in detoxification (Daborn et al., 2002). Tolerance may also occur at a community level through survival of only the resistant species — known as pollution-induced community tolerance (PICT) (Blanck, 2002).

We compared the species sensitivity distribution curves of imidacloprid (slope = 0.75) to that of thiacloprid (slope = 0.94) and all other neonicotinoids (slope = 0.97). Although a reduction in slope was apparent for imidacloprid, the other neonicotinoids were near parallel and the overall curve shapes were very similar (Fig. 4). Differences in slopes should ideally be less than 10% to assume the same mode of action and an additivity model (de Zwart and Posthuma 2005), but we noted that this subtle difference was influenced by the right-weighting of the upper end of the imidacloprid curve by the large number of studies on the insensitive *D. magna*. In a comprehensive review of mixtures in aquatic environments, Rodney et al. (2013) determined that the concentration addition of individual compounds is typically recommended. This is further supported by Deneer (2000) who found that in 90% of pesticide mixture studies, concentration additivity accurately predicted effect concentrations within a factor of two. Therefore, given the existing limited data showing a high degree of overlap in toxicity among neonicotinoids and the fact that the mechanism of action of different neonicotinoids is the same, we speculate that toxicity thresholds should be reasonably similar and predicted to be at least additive when in mixtures.

3.3. Toxicity of neonicotinoid metabolites

Degradation of neonicotinoids in water through photolysis and hydrolysis produces primary and secondary metabolites that may also exert toxic effects. Most published data on degradation and toxicity of metabolites are for imidacloprid. Although relatively stable to hydrolysis, the major metabolite of imidacloprid in water from hydrolysis is 1-[(6-chloro-3-pyridinyl) methyl]-2-imidazolidone (Zheng and Liu, 1999). Photolysis is the main degradation pathway and has been shown to produce up to nine different metabolites in water. The five most prominent include a cyclic guanidine derivative, a cyclic urea, an olefinic cyclic guanidine, and two fused ring products. In a radiotracer study following 2h of radiation, these five metabolites together accounted for 48% of the radio carbon label and the parent compound accounted for 23% of the radio label (Roberts and Hutson, 1999).

It appears that for those metabolites tested, their relative toxicity to aquatic invertebrates is typically lower than that of the parent compounds, at least under acute 24-h or 48-h exposure conditions (Malev et al., 2012) (Table A.4). The only exception is for thiamethoxam which readily breaks down to clothianidin, an active ingredient itself in several formulated products exhibiting high toxicity to sensitive aquatic taxa (Fig. 3). Most of our knowledge of metabolite toxicity to invertebrates is from bee studies which indicate that some neonicotinoid metabolites can contribute to the observed toxicity (Decourtye et al., 2003; Nauen et al., 2001; Suchail et al., 2001) with the exception of acetamiprid which has no reported toxic metabolites (Iwasa et al., 2004). Most studies for bees have been conducted on metabolites of imidacloprid demonstrating that those with a nitroguanidine-group (olefin-, hydroxy-, and dihydroxy-imidacloprid) were more toxic (oral LD_{50}) than the urea-metabolite and 6-chloronicotinic acid (Nauen et al., 2001). Only three aquatic test species have been used to evaluate toxicity of neonicotinoid metabolites. *D. magna* was tolerant to a range of metabolites, *C. riparius* was somewhat sensitive to the clothianidin metabolite thiazolylnitroguanidine (TMG) (28 day LC_{50} < 18 ug/L) (USEPA OPP Pesticide Ecotoxicity Database) while *Gammarus fossarum* exhibited effects on behavior (24-h LOEC ≤ 62.8) and antioxidant enzyme activity from imidacloprid's degradation product, 6-chloronicotinic acid (6-CNA) (24-h LOEC ≤ 157.7 μg/L) (Malev et al., 2012). Thiamethoxam's metabolite, N-(2-chlorothiazol-5-ylmethyl)-N′-methyl-N′-nitroguanidine (CGA-322704), also exhibits a relatively low NOEC of 0.67 μg/L in a 28-d toxicity test with *C. riparius* (European Commission, 2006).

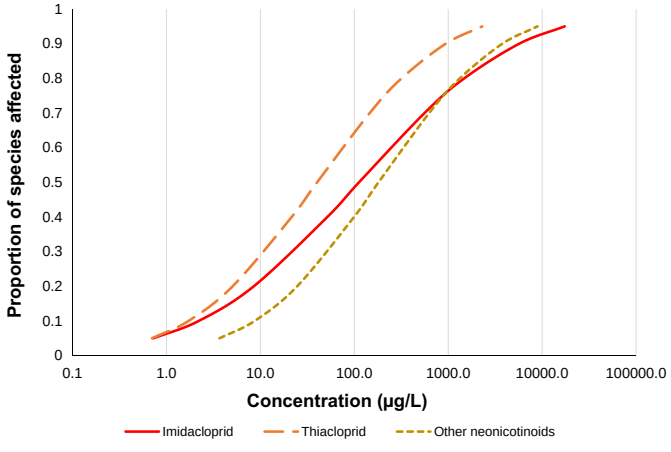

Fig. 4. Comparison of acute LC_{50} species sensitivity distribution curves of imidacloprid with thiacloprid and other neonicotinoids (acetamiprid, clothianidin, dinotefuran, thiamethoxam) combined. Data were insufficient to compare all individual neonicotinoids separately.

Neonicotinoid metabolites therefore represent a potentially lower, although still relevant, toxicity concern, however current water monitoring data do not routinely quantify these metabolites in their analyses. Therefore, we have little information on the prevalence or persistence of these metabolites for exposure assessments. Although toxicity data for aquatic invertebrates are also limited, our current understanding is that, with the exception of thiamethoxam breakdown to the toxic metabolite clothianidin, other neonicotinoid metabolites in water probably contribute relatively less to ecotoxicological effects compared to the parent compounds.

3.4. Analysis of species sensitivity distributions

The use and validation of the SSD approach and HC_5 calculation for neonicotinoid insecticides can be found in Liess and Beketov (2012) and Mineau and Palmer (2013), among others. Here, we used a traditional approach of only including data with similar endpoints of population relevance (LC_{50} or EC_{50}) and only single species laboratory studies. Many other similar analyses reported in the literature have included a mixture of field, mesocosm and laboratory studies, as well as a variety of endpoints (EC_{50}) plus NOECs and LOECs within the same analysis. Other authors, including Maltby et al. (2005), have previously compared single-species acute toxicity data for other pesticides with effects observed in mesocosm studies. They concluded that the lower confidence interval of the HC_5 derived from a SSD based on acute laboratory LC_{50} data was generally protective for aquatic communities in mesocosms, whereas the median HC_5 would require the application of an assessment factor. Guy et al. (2011), in another review of pesticide mesocosm studies, found that one-tenth of the crustacean HC_5 was usually low enough to prevent widespread mortality of different invertebrate taxa. In general, we caution that in extrapolating from a SSD based on laboratory data, an appropriate assessment factor may still be necessary to ensure that no deleterious effects on the ecosystem will occur, particularly for the persistent neonicotinoids demonstrating some level of cumulative action. For example, based on the study of Liess and Beketov (2011), long-term alterations of aquatic community structure were observed at 0.1 μg/L using $SPEAR_{mesocosm}$. This concentration was seven times below the HC_5 threshold identified as a relevant endpoint from a SSD based on acute laboratory LC_{50} information for thiacloprid (Beketov and Liess, 2008a). Presently, no clear standard on the application of assessment factors exists, particularly for SSDs that use only sensitive species (as in the aquatic insects and crustaceans), but typically these range from 3 to 6 as outlined in the European Union surface water guidance document (EFSA, 2013) or from 3 to 10 (RIVM, 2014).

Here, we applied the species sensitivity distribution (SSD) approach to examine neonicotinoid toxicity among aquatic arthropods using the CADDIS Species Sensitivity Distribution Generator v.1 software (US EPA http://www.epa.gov/caddis/da_software_overview.html). Consistent with several other researchers, we calculated HC_5 (Hazardous Concentration) levels at the 5% tail of a log-normal SSD (Postuma et al., 2002) (Fig. 5). Using the acute toxicity data available for all individual neonicotinoids (standardized and weighted by molecular mass to imidacloprid) on 42 different species (geometric mean of multiple tests by species) and based only on lethality as an endpoint (LC_{50} values), we fitted a SSD ($r^2 = 0.95$) which yielded an HC_5 of 0.63 μg/L or 0.002 μmol/L (95% CI: 0.20–2.20 μg/L; 0.001–0.008 μmol/L) (Fig. 5a). The results of the chronic toxicity SSD with 18 test species used in studies of 7–28 days duration where the endpoints included lethality (LC_{50}), and any other sub-lethal endpoints (EC_{50}) such as growth, reproduction, immobility, or emergence ($r^2 = 0.92$) yielded an HC_5 of 0.146 μg/L or 0.001 μmol/L (95% CI: 0.035–0.61 μg/L; 0.00014–0.002 μmol/L) (Fig. 5b). We propose that the lower confidence limit of each of the two HC_5 values would be appropriate acute and chronic exposure thresholds, above which ecologically relevant population-level effects on sensitive aquatic invertebrate species, are likely to occur. Sublethal and community-level effects could still occur during short-term (acute) exposure at concentrations below 0.63 μg/L (HC_5 of acute SSD). Based on short-term toxicity tests reporting sublethal EC_{50} values for 26 species, we estimated an $HC_5 = 0.395$ μg/L or 0.002 μmol/L (95% CI: 0.073–2.13 μg/L; 0.0003–0.008 μmol/L). Thus in setting regulatory thresholds, regulators may need to consider both short-term sublethal effects in addition to lethality under acute and chronic neonicotinoid exposure scenarios to prevent impacts to aquatic communities.

3.5. Impacts on aquatic communities and ecosystems: mesocosm and field studies

Limitations of extrapolating effects from laboratory studies with single species to possible effects in the field have prompted several researchers to assess neonicotinoid effects on multi-species communities. However, such field and mesocosm studies usually suffer from a lack of control over species composition, contaminant exposure, and the role of different environmental variables, thereby limiting their reproducibility. Regardless, more environmentally relevant multi-species community effects are often observed at neonicotinoid concentrations well below single species toxicity thresholds. Our review suggests stream mesocosms exposed to imidacloprid or thiacloprid produced effects on a range of invertebrate taxa at environmentally relevant water concentrations of 0.01 to 24.1 μg/L (e.g. Pestana et al., 2009; Mohr et al., 2012; Berghahn et al., 2012, Boettger et al. 2013); rice mesocosm experiments revealed similar community-level effects at water concentrations ranging from <0.01 to 240 μg/L (Daam et al., 2013; Hayasaka et al., 2012a,b; Jinguji et al., 2013; Sánchez-Bayo and Goka, 2006) (Table A.3). The insect groups most commonly affected belong to the orders Ephemeroptera, Trichoptera and Diptera, as generally predicted by their sensitivity in single species tests. Emergence and other sublethal effects such as growth appear to be a more sensitive endpoint than abundance (Alexander et al., 2008; Mohr et al., 2012).

When considering the ecological effects of pesticides, sensitive community and ecosystem processes and functions, such as important trophic interactions and leaf litter breakdown rates, need to be considered. At concentrations of ~1.0 μg/L, neonicotinoids have been observed to alter predator–prey interactions in experimental aquatic communities (Hayasaka et al., 2012b; Sánchez-Bayo and Goka, 2006). For example, Englert et al. (2012) observed reduced leaf consumption and increased carnivorous behavior by *G. fossarum*, an important shredder species, at thiacloprid concentrations above 0.5–1.0 μg/L. Significant reductions in leaf feeding activity of *G. pulex* have also been observed at concentrations of imidacloprid above 30 μg/L ($EC_{50} = 5.34$ μg/L) with lasting effects on feeding behavior even at the lowest exposure concentrations of 0.81 to 9.0 μg/L (Agatz et al., 2014). Kreutzweiser et al. (2008) found that leaves from maple trees treated with imidacloprid at realistic field concentrations (3–11 mg/kg in trees) did not affect survival of aquatic leaf-shredding insects or litter-dwelling earthworms. However, adverse sub-lethal effects from these exposures were detected; specifically feeding rates of aquatic insects and earthworms were reduced, leaf decomposition (mass loss) was decreased, measurable weight losses occurred among earthworms, and aquatic and terrestrial microbial decomposition activity was significantly inhibited.

Of particular concern for field relevance is that toxic effects may be amplified at concentrations lower than observed in short-duration laboratory experiments, and that they may be delayed until after exposure ceases thereby delaying population recovery (Beketov and Liess, 2008a; EFSA, 2013; Song et al., 1997). For example, in a multi-generation microcosm study, populations of the mosquito larvae *Culex pipiens* exposed to thiacloprid pulses were found to decline and failed to recover in the presence of the more pesticide tolerant competitor, *D. magna* (Liess et al., 2013). Also, in a multi-year mesocosm study, Liess and Beketov (2011) found that species with low intrinsic sensitivity to thiacloprid showed only short-term effects at 100 μg/L, but species with high intrinsic sensitivity showed effects at 3.3 μg/L, and particularly sensitive univoltine (1 brood/yr) species showed long-term effects at 0.1 μg/L, with several species disappearing from the community. These effect levels were up to 70 times below the lowest laboratory, short-term LC_{50} for single species. Three processes may be responsible for this mismatch. First, in the field, additional natural and anthropogenic stressors are widely known to lower effective thresholds for toxicants. Liess and Beketov (2011) concluded that those species characterized by vulnerable traits in the presence of natural stressors (e.g., intra- and interspecific competition), were affected more strongly by thiacloprid than non-stressed species. Thus, sensitivity was more than an order of magnitude greater when additional stress was present. Second, field exposure scenerios generally include repeated pulses of neonicotinoids or other chemical stressors. Such sequential pulses of neonicotinoids may act cumulatively to exert stronger effects than single exposures (Liess et al., 2013). Third, the persistence of neonicotinoids in water under certain field conditions, such as high turbidity, acidity, depth, and filamentous algal or other shading, will increase chemical persistence thereby increasing the duration of aquatic organism exposure. This suggests that even with short-term pulse exposures, standard laboratory toxicity tests may not capture the range of lethal or sub-lethal effects that can continue to occur and thus impede population and community recovery. Chronic or repeated neonicotinoid exposure conditions appear more probable in nature than single acute exposures, and natural environmental conditions and stressors can inherently enhance toxicity.

3.6. Water quality reference values for protection of aquatic life

Current ecological water quality guidelines vary widely by country and several are presently under review (Table 3). Despite the controversy over this class of insecticides, few water quality reference values presently exist for (ecologically) acceptable levels of neonicotinoids in surface waters; these are predominantly limited to the most widely studied compound, imidacloprid (Table 3). Recommended water quality reference values have been derived primarily from available acute and chronic laboratory toxicity tests with standard test organisms using a mixture of LC_{50}s, EC_{50}s, NOECs and LOECs as toxicity endpoints. Few have considered multispecies or field-realistic long-term exposure scenarios beyond standard 48 to 96 h, and 14 to 28 day tests. The most recent reference values (e.g. Netherlands (RIVM, 2014; Smit et al., 2014)) were derived using a probabilistic (SSD) approach and incorporated a large range of toxicity data, including mesocosm and field studies, to obtain a reference value of 0.0083 μg/L for imidacloprid. By contrast, the U.S. EPA (2014) has set the "Aquatic Life Benchmark"

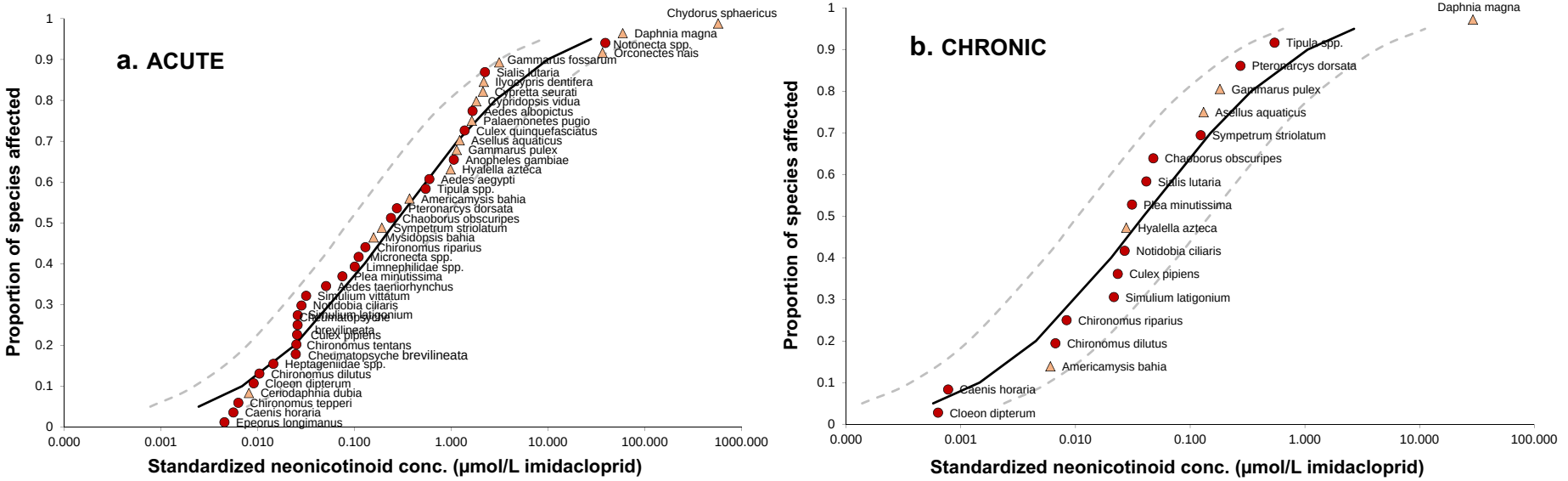

Fig. 5. Species sensitivity distributions for a) 137 acute (LC_{50}; 24–96 h) laboratory toxicity tests with 42 different aquatic invertebrate species, and b) 36 chronic (L[E]$C_{50;}$ 7–39 day) laboratory toxicity studies with 18 aquatic invertebrate species. Red circles represent aquatic insects and orange triangles represent crustaceans. Distributions are based on test values of multiple neonicotinoid compounds with concentrations standardized to imidacloprid molecular mass. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Summary of published ecological quality reference values for neonicotinoids (imidacloprid except this review) in freshwater environments against which average (chronic or long-term) or maximum (acute or peak) exposure concentrations are to be compared.

Source	Reference value (μg/L)	Justification
EPA (2014) (USA)	1.05 (average) 35.0 (maximum)	Aquatic life benchmark — methodology uncertain
CCME (2007) (Canada)	0.23	EC_{15} for the most sensitive of two freshwater species tested with assessment factor of 10 applied.
EFSA (2008) (Europe)	0.2 (maximum)	No Observable Effect Concentration (NOEC) (0.6 μg/L) from a 21 d German microcosm study to which an assessment factor of 1–3 has been applied based on expert deliberations
RIVM (2008) (Netherlands)	0.067 (average)	Maximum permissible concentration (MPC) for long term exposure derived from the lowest NOEC value for chronic toxicity studies with assessment factor of 10 applied.
RIVM (2014) (Netherlands)	0.0083 (average)	Updated MPC for long-term exposure derived from chronic studies using species sensitivity distribution (SSD) approach and Hazard Concentration (HC_5) applied to NOEC/LC_{10}/EC_{10} values with assessment factor of 3 applied.
Mineau and Palmer (2013)	0.0086 or 0.029 (average)	The higher of two empirically-determined acute–chronic ratios applied to the most sensitive of 8 aquatic species tested to date; or HC_5 from SSD applied using NOECs from chronic studies of 7 single species and 1 species assemblage.
This review	0.035 (average) 0.2 (maximum)	Lower confidence interval of HC_5 from SSDs generated using 137 acute (LC_{50}) and 36 chronic (L[E]C_{50}) toxicity tests considering all neonicotinoid compounds weighted and standardized to imidacloprid and all available test species.

for imidacloprid at 1.05 μg/L for invertebrate chronic (average) exposure and 35 μg/L for acute (maximum) exposure using methods that are unclear, though likely based on species such as *D. magna*. Canada has published a single value for imidacloprid of 0.23 μg/L as a "Water Quality Guideline for the Protection of Aquatic Life" (CCME, 2007). Under the European Water Framework Directive, a Maximum Permissible Concentration (MPC) of 0.067 μg/L is used for chronic or average imidacloprid concentrations, while a Maximum Acceptable Concentration (MAC) of 0.20 μg/L is used for short-term or peak concentrations (RIVM, 2008). Until recently, the lowest reference value reported is that of the Dutch regulatory body which has adopted a Maximum Permissible Risk (MPR) level for protection of ecosystems of 0.013 μg/L. In 2014, the Netherlands released an update recommending that the MPC for imidacloprid be lowered to 0.0083 μg/L, while the MAC would remain at 0.2 μg/L (RIVM, 2014). Fig. 1 demonstrates how many of the water monitoring data (means and maxima) reported worldwide would exceed these published reference values. For example, 79% (15/19) of studies reported "average" neonicotinoid concentrations that would have exceeded the most recent RIVM (2014) threshold of 0.0083 μg/L, while 81% (22/27) of studies reporting "peak" neonicotinoid concentrations found levels that would have exceeded the 0.2 μg/L imidacloprid reference value set by EFSA (2008).

Reference values for other neonicotinoids in surface waters are not well established although, consistent with our findings, Mineau and Palmer (2013) suggested that guidelines for other neonicotinoids should be similar to that for imidacloprid. Currently, the US EPA has established one acute benchmark for thiamethoxam of 17 μg/L and 18.9 μg/L for thiacloprid; however, derivation methods for these values and for their imidacloprid value (1.05 μg/L) are unclear and insufficiently protective given the available evidence and the lack of inclusion of chronic exposure data. We note that considerable variability exists in the reference values themselves and in how they are derived (Table 3). In some cases, acceptable levels are derived from single species and/or mesocosm studies using the lowest L[E]C_{50}, others add assessment factors of 3–10, and still others have applied SSDs to derive the HC_5 or HC_{15} followed by a range of assessment factors. The wide discrepancy in water quality reference values is not unique to imidacloprid or the neonicotinoid insecticides more generally. Guy et al. (2011) reported several examples of widely divergent reference values and argued that the majority were insufficiently protective, at least based on field and mesocosm data. Here we take the approach that, based on a very large number of neonicotinoid studies using consistent LC_{50} and EC_{50} endpoints and applying the lower confidence interval of our HC_5 calculation, threshold values of 0.2 μg/L for maximum (peak, short-term) neonicotinoid concentrations and 0.035 μg/L for average (longer-term) neonicotinoid concentrations represent minimally protective thresholds for sensitive aquatic invertebrates to which safety factors might need to be applied as we further elucidate mechanisms of cumulative action, field level responses, and recovery patterns for this class of insecticides.

3.7. Proposed approaches for addressing neonicotinoid mixtures in water

The toxicity of these compounds is predicted to be additive in nature through cumulative agonistic binding at the same receptor type. However, to our knowledge, this assumption has not been tested formally using binary or mixture aquatic toxicity studies. Based on this assumption that all neonicotinoids have the same mechanism of action, relatively equivalent toxicity, and predicted additive toxicity, we pooled all toxicity data for different neonicotinoid compounds weighted and standardized to imidacloprid molecular mass when estimating our HC_5 values. In doing so, we propose that where multiple neonicotinoids are present in water, the sum of all neonicotinoid concentrations corrected for molecular mass (total neonicotinoids) may be used as an approximation for predicting additivity of toxic effects. Other approaches may be more appropriate, such as standardizing individual neonicotinoid concentrations to imidacloprid based on toxic equivalency. However, this would require more detailed knowledge of the comparative toxicity of the different neonicotinoids. Ignoring neonicotinoid mixtures may greatly underestimate the threshold exceedances and thus we currently advocate for the simple molar concentration summation approach as an approximation until further experimental work using neonicotinoid mixtures confirms a more mechanistic or comprehensive method. Ultimately, the reference values proposed here based on individual compound exposures and assuming equivalent neonicotinoid toxicity may need to be revised when new mixture and comparative neonicotinoid data becomes available.

4. Conclusions and recommendations

We conclude based on comprehensive species sensitivity distribution analysis of 214 toxicity tests of 48 species that any long-term neonicotinoid concentrations in water exceeding 0.035 μg/L or short term peak exposures exceeding 0.2 μg/L can affect sensitive aquatic invertebrate populations. By comparison, this 0.035 μg/L value is consistent with the Vijver and van den Brink (2014) suggested threshold of 0.013–0.067 μg/L for imidacloprid, but higher than that proposed by Mineau and Palmer (2013) (0.0086 μg/L) and by the Netherlands MPC (RIVM, 2014) (0.0083 μg/L) (Table 3). Given the uncertainty of the ecological safety of these pesticides and their long-term persistence in the natural environment, we concede that additional safety factors may be appropriate. Our analysis shows that 74% (14/19) of surface water studies reporting average individual neonicotinoid residues exceeded 0.035 μg/L. Furthermore, exceedance of our proposed 0.2 μg/L peak

threshold would thus occur for 81% (22/27) of monitoring studies reporting maximum water concentrations of individual neonicotinoids. That exceedance would be expected to increase if multiple neonicotinoids were summed during monitoring and presented as "total neonicotinoids" given the likelihood of additive effects.

A recent surge in the number of published toxicity studies with neonicotinoid insecticides and aquatic invertebrates has produced a mass of new and useful data, but often with confusing results. This appears to be largely due to 1) vast differences in species sensitivity of test organisms which ranges several orders of magnitude, 2) differences in species, duration, conditions and reporting of toxicity tests, and 3) apparent differences between laboratory studies and field or mesocosm studies representing varying levels of field realism. This can often impede the ultimate goal of setting regulatory threshold concentrations that are protective. Generally speaking, environmental risk assessments that follow a tiered approach of increasing complexity and environmental relevance have received considerable support (Brock and Wijngaarden, 2012; EFSA, 2013) and are recommended for the different neonicotinoids. In addition, environmental monitoring data suggest that multiple neonicotinoids are frequently and repeatedly transported into water bodies, or are persisting for durations well beyond the commonly used 48 to 96 hour duration of acute toxicity tests. Given this exposure profile, chronic studies (28 days or longer) and mesocosm studies should be the primary tests guiding regulatory decision making. Equally, many of the mayfly (Ephemeroptera), caddisfly (Trichoptera), and midge (Diptera) species that are critical for supporting numerous aquatic and terrestrial food webs, appear highly sensitive to neonicotinoids, but are not as extensively tested as some standard test species (e.g., *D. magna*) that appear to be up to 100,000 times less sensitive. Adverse indirect effects of imidacloprid on food webs including insectivorous birds have already been reported from areas draining Dutch farmlands (Hallmann et al., 2014).

Despite the ongoing advances in technology, extensive experimental toxicity data and strict regulation of pesticides in many developed countries, recent data for rivers in the European Union suggests that pesticides (not including neonicotinoids) still account for 87% of organic pollutant exceedances of acute risk thresholds based on aquatic invertebrates (Malaj et al., 2014). The neonicotinoid insecticides represent a significant additional pesticide threat to surface and ground waters because of their broad use, high water solubility, environmental persistence and very high non-target toxicity, and thus require scientifically robust approaches to accurately determine risk. Existing information presented here suggests that stricter regulations and use of neonicotinoid insecticides are warranted to protect aquatic ecosystems and the broader biodiversity they support.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2014.10.024.

Acknowledgments

We gratefully acknowledge advice and/or data from Jeroen van der Sluijs, Paul Kozak, Valerie Fournier, and Isabelle Giroux along with 3 anonymous reviewers. Assistance with collating and validating toxicity data and references was provided by Coleen Macdonald and Midori Matsuzaki. The authors declare no competing financial interest. This work was funded by a Natural Science and Engineering Research Council (NSERC) Strategic Project Grant to C.A.M. (STPGP 430138 – 12).

References

Agatz, A., Ashauer, R., Brown, C.D., 2014. Imidacloprid perturbs feeding of *Gammarus pulex* at environmentally relevant concentrations. Environ. Toxicol. Chem. 33, 648–653.
Alexander, A.C., Culp, J.M., Liber, K., Cessna, A.J., 2007. Effects of insecticide exposure on feeding inhibition in mayflies and oligochaetes. Environ. Toxicol. Chem. 26, 1726–1732.
Alexander, A.C., Heard, K.S., Culp, J.M., 2008. Emergent body size of mayfly survivors. Freshw. Biol. 53, 171–180.
Alexander, A., Luis, A., Culp, J., Baird, D., Cessna, A., 2013. Can nutrients mask community responses to insecticide mixtures? Ecotoxicology 22, 1085–1100.
Andersch, W, Jeschke, P, Thielert, W. Combination of methiocarb and one or more compounds selected from thiacloprid, thiamethoxam, acetamiprid, nitenpyram, and dinotefuran; effective animal pests control and for plant seed dressing. Google Patents. United States: Bayer CropScience AG; 2010.
Anderson, T.A., Salice, C.J., Erickson, R.A., McMurry, S.T., Cox, S.B., Smith, L.M., 2013. Effects of landuse and precipitation on pesticides and water quality in playa lakes of the southern high plains. Chemosphere 92, 84–90.
Armbrust, K.L., Peeler, H.B., 2002. Effects of formulation on the run-off of imidacloprid from turf. Pest Manag. Sci. 58, 702–706.
Ashauer, R., Hintermeister, A., Potthoff, E., Escher, B.I., 2011. Acute toxicity of organic chemicals to *Gammarus pulex* correlates with sensitivity of *Daphnia magna* across most modes of action. Aquat. Toxicol. 103, 38–45.
Beketov, M.A., Liess, M., 2008a. Acute and delayed effects of the neonicotinoid insecticide thiacloprid on seven freshwater arthropods. Environ. Toxicol. Chem. 27, 461–470.
Beketov, M.A., Liess, M., 2008b. Potential of 11 pesticides to initiate downstream drift of stream macroinvertebrates. Arch. Environ. Contam. Toxicol. 55, 247–253.
Berghahn, R., Mohr, S., Huebner, V., Schmiedichе, R., Schmiedling, I., Svetich-Will, E., Schmidt, R., 2012. Effects of repeated insecticide pulses on macroinvertebrate drift in indoor stream mesocosms. Aquat. Toxicol. 122, 56–66.
Blanck, H., 2002. A critical review of procedures and approaches used for assessing Pollution-Induced Community Tolerance (PICT) in biotic communities. Hum. Ecol. Risk. Assess. 8, 1003–1034.
Boettger, R., Feibicke, M., Schaller, J., Dudel, G., 2013. Effects of low-dosed imidacloprid pulses on the functional role of the caged amphipod Gammarus roeseli in stream mesocosms. Ecotoxicol. Environ. Saf. 93, 93–100.
Brock, T.C.M., Wijngaarden, R.P.A., 2012. Acute toxicity tests with *Daphnia magna*, *Americamysis bahia*, *Chironomus riparius* and *Gammarus pulex* and implications of new EU requirements for the aquatic effect assessment of insecticides. Environ. Sci. Pollut. Res. Int. 19, 3610–3618.
CCME, 2007. Canadian Water Quality Guidelines: Imidacloprid. Scientific Supporting Document. Canadian Council of Ministers of the Environment, Winnipeg.
Chen, X.D., Culbert, E., Hebert, V., Stark, J.D., 2010. Mixture effects of the nonylphenyl polyethoxylate, R-11 and the insecticide, imidacloprid on population growth rate and other parameters of the crustacean, *Ceriodaphnia dubia*. Ecotoxicol. Environ. Saf. 73, 132–137.
Chiovarou, E.D., Siewicki, T.C., 2008. Comparison of storm intensity and application timing on modeled transport and fate of six contaminants. Sci. Total Environ. 389, 87–100.
Cox, L., Koskinen, W.C., Yen, P.Y., 1998. Changes in sorption of imidacloprid with incubation time. Soil Sci. Soc. Am. J. 62, 342–347.
Daam, M.A., Santos Pereira, A.C., Silva, E., Caetano, L., Cerejeira, M.J., 2013. Preliminary aquatic risk assessment of imidacloprid after application in an experimental rice plot. Ecotoxicol. Environ. Saf. 97, 78–85.
Daborn, P.J., Yen, J.L., Bogwitz, M.R., Le Goff, G., Feil, E., Jeffers, S., Tijet, N., Perry, T., Heckel, D., Batterham, P., Feyereisen, R., Wilson, T.G., 2002. ffrench-Constant, RH. A single P450 allele associated with insecticide resistance in *Drosophila*. Science 297, 2253–2256.
Decourtye, A., Devillers, J., 2010. Ecotoxicity of neonicotinoid insecticides to bees. In: Hervé Thany, S. (Ed.), Insect Nicotinic Acetylcholine Receptors. Springer-Verlag, Berlin.
Decourtye, A., Lacassie, E., Pham-Delègue, M.-H., 2003. Learning performances of honeybees (*Apis mellifera* L) are differentially affected by imidacloprid according to the season. Pest Manag. Sci. 59, 269–278.
Deneer, J.W., 2000. Toxicity of mixtures of pesticides in aquatic systems. Pest Manag. Sci. 56, 516–520.
de Zwart, D., Posthuma, L., 2005. Complex mixture toxicity for single and multiple species: proposed methodologies. Environ Toxicol Chem. 24, 2665–2676.
Elbert, A., Haas, M., Springer, B., Thielert, W., Nauen, R., 2008. Applied aspects of neonicotinoid uses in crop protection. Pest Manag. Sci. 64, 1099–1105.
Englert, D., Bundschuh, M., Schulz, R., 2012. Thiacloprid affects trophic interaction between gammarids and mayflies. Environ. Pollut. 167, 41–46.
Eskenazi, B., Bradman, A., Castorina, R., 1999. Exposures of children to organophosphate pesticides and their potential adverse health effects. Environ. Health Perspect. 107 (Suppl. 3), 409–419.
European Commission (EU), 2006. Review Report for the Active Substance Thiamethoxam. European Commission Standing Committee on the Food Chain and Animal Health: Health and Consumer Protection Directorate-General.
European Food Safety Authority (EFSA), 2008. Conclusion regarding the peer review of the pesticide risk assessment of the active substance imidacloprid. European Food Safety Authority Scientific Report. European Food Safety Authority.
European Food Safety Authority (EFSA), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA JournalEFSA Panel on Plant Protection Products and their Residues.
Felsot, A.S., Cone, W., Yu, J., Ruppert, J.R., 1998. Distribution of imidacloprid in soil following subsurface drip chemigation. Bull. Environ. Contam. Toxicol. 60, 363–370.
Flores-Cespedes, F., Gonzalez-Pradas, E., Fernandez-Perez, M., Villafranca-Sanchez, M., Socias-Viciana, M., Urena-Amate, M.D., 2002. Effects of dissolved organic carbon on sorption and mobility of imidacloprid in soil. J. Environ. Qual. 31, 880–888.
Fossen, M., 2006. Environmental Fate of Imidacloprid. Environmental Monitoring Department of Pesticide Regulation, Sacramento, California.
Gibbons, D., Morrissey, C., Mineau, P., 2014. A review of the direct and indirect effects of neonicotinoids and fipronil on vertebrate wildlife. Environ. Sci. Pollut. Res. 1–16.
Goldsborough, L.G., Crumpton, W.G., 1998. Distribution and environmental fate of pesticides in Prairie wetlands. Great Plains Res. 8, 73–95.
Goulson, D., 2013. Review: an overview of the environmental risks posed by neonicotinoid insecticides. J. Appl. Ecol. 50, 977–987.

Gupta, S., Gajbhiye, V.T., Gupta, R.K., 2008. Soil dissipation and leaching behavior of a neonicotinoid insecticide thiamethoxam. Bull. Environ. Contam. Toxicol. 80, 431–437.
Guy, M., Singh, L., Mineau, P., 2011. Using field data to assess the effects of pesticides on crustacea in freshwater aquatic ecosystems and verifying the level of protection provided by water quality guidelines. Integr. Environ. Assess. Manag. 7, 426–436.
Guzsvany, V., Csanádi, J., Gaal, F., 2006. NMR study of the influence of pH on the persistence of some neonicotinoids in water. Acta Chim. Slov. 53, 52–57.
Hallmann, C.A., Foppen, R.P.B., van Turnhout, C.A.M., de Kroon, H., Jongejans, E., 2014. Declines in insectivorous birds are associated with high neonicotinoid concentrations. Nature 511, 341–343.
Hayasaka, D., Korenaga, T., Sánchez-Bayo, F., Goka, K., 2012a. Differences in ecological impacts of systemic insecticides with different physicochemical properties on biocenosis of experimental paddy fields. Ecotoxicology 21, 191–201.
Hayasaka, D., Korenaga, T., Suzuki, K., Saito, F., Sánchez-Bayo, F., Goka, K., 2012b. Cumulative ecological impacts of two successive annual treatments of imidacloprid and fipronil on aquatic communities of paddy mesocosms. Ecotoxicol. Environ. Saf. 80, 355–362.
Huseth, A.S., Groves, R.L., 2014. Environmental fate of soil applied neonicotinoid insecticides in an irrigated potato agroecosystem. PLoS One 9, e97081.
Iwasa, T., Motoyama, N., Ambrose, J.T., Roe, R.M., 2004. Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, *Apis mellifera*. Crop. Prot. 23, 371–378.
Jemec, A., Tisler, T., Drobne, D., Sepcic, K., Fournier, D., Trebse, P., 2007. Comparative toxicity of imidacloprid, of its commercial liquid formulation and of diazinon to a non-target arthropod, the microcrustacean *Daphnia magna*. Chemosphere 68, 1408–1418.
Jeschke, P., Nauen, R., 2008. Neonicotinoids—from zero to hero in insecticide chemistry. Pest Manag. Sci. 64, 1084–1098.
Jeschke, P., Nauen, R., Schindler, M., Elbert, A., 2010. Overview of the status and global strategy for neonicotinoids. J. Agric. Food Chem. 59, 2897–2908.
Jinguji, H., Thuyet, D., Uéda, T., Watanabe, H., 2013. Effect of imidacloprid and fipronil pesticide application on *Sympetrum infuscatum* (Libellulidae: Odonata) larvae and adults. Paddy Water Environ. 11, 277–284.
Kanrar, B., Ghosh, T., Pramanik, S.K., Dutta, S., Bhattacharyya, A., Dhuri, A.V., 2006. Degradation dynamics and persistence of imidacloprid in a rice ecosystem under west Bengal climatic conditions. Bull. Environ. Contam. Toxicol. 77, 631–637.
Kreutzweiser, D.P., Good, K.P., Chartrand, D.T., Scarr, T.A., Thompson, D.G., 2008. Are leaves that fall from imidacloprid-treated maple trees to control Asian longhorned beetles toxic to non-target decomposer organisms? J. Environ. Qual. 37, 639–646.
Krupke, C.H., Hunt, G.J., Eitzer, B.D., Andino, G., Given, K., 2012. Multiple routes of pesticide exposure for honey bees living near agricultural fields. PLoS One 7, e29268.
La, N., Lamers, M., Bannwarth, M., Nguyen, V., Streck, T., 2014. Imidacloprid concentrations in paddy rice fields in northern Vietnam: measurement and probabilistic modeling. Paddy Water Environ. 1–13.
Lamers, M., Anyusheva, M., La, N., Nguyen, V.V., Streck, T., 2011. Pesticide pollution in surface- and groundwater by paddy rice cultivation: a case study from northern Vietnam. CLEAN 39, 356–361.
Larsbo, M., Löfstrand, E., de Veer, DvA, Ulén, B., 2013. Pesticide leaching from two Swedish topsoils of contrasting texture amended with biochar. J. Contam. Hydrol. 147, 73–81.
LeBlanc, H.M.K., Culp, J.M., Baird, D.J., Alexander, A.C., Cessna, A.J., 2012. Single versus combined lethal effects of three agricultural insecticides on larvae of the freshwater insect *Chironomus dilutus*. Arch. Environ. Contam. Toxicol. 63, 378–390.
Liess, M., Beketov, M., 2011. Traits and stress: keys to identify community effects of low levels of toxicants in test systems. Ecotoxicology 20, 1328–1340.
Liess, M., Beketov, M., 2012. Rebuttal related to "Traits and stress: Keys to identify community effects of low levels of toxicants in test systems" by Liess and Beketov (2011). Ecotoxicology 21, 300–303.
Liess, M., Foit, K., Becker, A., Hassold, E., Dolciotti, I., Kattwinkel, M., Duquesne, S., 2013. Culmination of low-dose pesticide effects. Environ. Sci. Technol. 47, 8862–8868.
Main, A.R., Headley, J.V., Peru, K.M., Michel, N.L., Cessna, A.J., Morrissey, C.A., 2014. Widespread Use and frequent detection of neonicotinoid insecticides in wetlands of Canada's Prairie Pothole Region. PLoS One 9, e92821.
Malaj, E., von der Ohe, P.C., Grote, M., Kühne, R., Mondy, C.P., Usseglio-Polatera, P., Brack, W., Schäfer, R.B., 2014. Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale. Proc. Natl. Acad. Sci. 111, 9549–9554.
Malev, O., Klobučar, R.S., Fabbretti, E., Trebše, P., 2012. Comparative toxicity of imidacloprid and its transformation product 6-chloronicotinic acid to non-target aquatic organisms: microalgae *Desmodesmus subspicatus* and amphipod *Gammarus fossarum*. Pestic. Biochem. Physiol. 104, 178–186.
Maltby, L., Blake, N., Brock, T.C.M., Van den Brink, P.J., 2005. Insecticide species sensitivity distributions: importance of test species selection and relevance to aquatic ecosystems. Environ. Toxicol. Chem. 24, 379–388.
Maus, C., Nauen, R., 2010. Response to the publication: Tennekes, H.A. (2010): "The significance of the Druckrey-Küpfmüller equation for risk assessment: the toxicity of neonicotinoid insecticides to arthropods is reinforced by exposure time". Toxicology 280, 176–177.
Mineau, P., Palmer, C., 2013. Neonicotinoid insecticides and birds: the impact of the nation's most widely used insecticides on birds. American Bird Conservancy.
Mohr, S., Berghahn, R., Schmiediche, R., Hubner, V., Loth, S., Feibicke, M., Mailahn, W., Wogram, J., 2012. Macroinvertebrate community response to repeated short-term pulses of the insecticide imidacloprid. Aquat. Toxicol. 110, 25–36.
Nauen, R., Ebbinghaus-Kintscher, U., Schmuck, R., 2001. Toxicity and nicotinic acetylcholine receptor interaction of imidacloprid and its metabolites in *Apis mellifera* (Hymenoptera: Apidae). Pest Manag. Sci. 57, 577–586.
Nuyttens, D., Devarrewaere, W., Verboven, P., Foque, D., 2013. Pesticide-laden dust emission and drift from treated seeds during seed drilling: a review. Pest Manag. Sci. 69, 564–575.
Pavlaki, M.D., Pereira, R., Loureiro, S., Soares, A., 2011. Effects of binary mixtures on the life traits of *Daphnia magna*. Ecotoxicol. Environ. Saf. 74, 99–110.
Pest Management Regulatory Agency (PMRA), 2001. Imidacloprid: Regulatory Note — REG2001–11. Pesticide Management Regulatory Agency, Health Canada.
Pest Management Regulatory Agency (PMRA), 2004. Clothianidin Poncho 600 Seed Treatment Insecticide: Regulatory Note — REG2004–06 revision. Pesticide Management Regulatory Agency, Health Canada.
Pestana, J.L.T., Alexander, A.C., Culp, J.M., Baird, D.J., Cessna, A.J., Soares, A., 2009. Structural and functional responses of benthic invertebrates to imidacloprid in outdoor stream mesocosms. Environ. Pollut. 157, 2328–2334.
Postuma, L., Suter II, G.W., Traas, T.P., 2002. Species Sensitivity Distributions in Ecotoxicology. CRC Press, Boca Raton, Florida.
Riaz, M.A., Poupardin, R., Reynaud, S., Strode, C., Ranson, H., David, J.-P., 2009. Impact of glyphosate and benzo[a]pyrene on the tolerance of mosquito larvae to chemical insecticides. Role of detoxification genes in response to xenobiotics. Aquat. Toxicol. 93, 61–69.
RIVM, 2008. Environmental Risk Limits for Imidacloprid. In: Posthuma-Doodeman C.J.A.M. (Ed.), National Institute for Public Health and the Environment Bilthoven, Netherlands.
RIVM, 2014. Water Quality Standards for Imidacloprid: Proposal for an Update According to the Water Framework Directive. In: Smit, C.E. (Ed.), National Institute for Public Health and the Environment, Bilthoven, Netherlands.
Roberts, T.R., Hutson, D.H., 1999. Imidacloprid. Metabolic Pathways of Agrochemicals — Part 2: Insecticides and Fungicides. The Royal Society of Chemistry, Cambridge, UK.
Rodney, S.I., Teed, R.S., Moore, D.R.J., 2013. Estimating the toxicity of pesticide mixtures to aquatic organisms: a review. Hum. Ecol. Risk. Assess. 19, 1557–1575.
Roessink, I., Merga, L.B., Zweers, H.J., Van den Brink, P.J., 2013. The neonicotinoid imidacloprid shows high chronic toxicity to mayfly nymphs. Environ. Toxicol. Chem. 32, 1096–1100.
Rouchaud, J., Gustin, F., Wauters, A., 1994. Soil biodegradation and leaf transfer of insecticide imidacloprid applied in seed dressing in sugar beet crops. Bull. Environ. Contam. Toxicol. 53, 344–350.
Samson-Robert, O., Labrie, G., Chagnon, M., Fournier, V., 2014. Neonicotinoid-contaminated puddles of water represent a risk of intoxication for Honey bees. PLoS One (in press).
Sanchez-Bayo, F., 2006. Comparative acute toxicity of organic pollutants and reference values for crustaceans. I. Branchiopoda, Copepoda and Ostracoda. Environ. Pollut. 139, 385–420.
Sánchez-Bayo, F., Goka, K., 2006. Ecological effects of the insecticide imidacloprid and a pollutant from antidandruff shampoo in experimental rice fields. Environ. Toxicol. Chem. 25, 1677–1687.
Sanchez-Bayo, F., Goka, K., 2014. Pesticide residues and bees — a risk assessment. PLoS One 9, e94482.
Sanchez-Bayo, F., Hyne, R.V., 2014. Detection and analysis of neonicotinoids in river waters — development of a passive sampler for three commonly used insecticides. Chemosphere 99, 143–151.
Sánchez-Bayo, F., Tennekes, H.A., Goka, K., 2013. Impact of systemic insecticides on organisms and ecosystems. In: Trdan, S. (Ed.), Insecticides — Development of Safer and More Effective Technologies. Intech Open access (Available at: http://www.intechopen.com/books/insecticides-development-of-safer-and-more-effective-technologies/impact-of-systemic-insecticides-on-organisms-and-ecosystems).
Sarkar, M.A., Biswas, P.K., Roy, S., Kole, R.K., Chowdhury, A., 1999. Effect of pH and type of formulation on the persistence of imidacloprid in water. Bull. Environ. Contam. Toxicol. 63, 604–609.
Sarkar, M.A., Roy, S., Kole, R.K., Chowdhury, A., 2001. Persistence and metabolism of imidacloprid in different soils of West Bengal. Pest Manag. Sci. 57, 598–602.
Scholz, K., Spiteller, M., 1992. Influence of groundcover on the degradation of ^{14}C-imidacloprid in soil. Brighton Crop Protection Conference — Pests and Diseases. Brighton, England.
Smit, C.E., Posthuma-Doodeman, C.J.A.M., Van Vlaardingen, P.L.A., De Jong, F.M.W., 2014. Ecotoxicity of imidacloprid to aquatic organisms: derivation of water quality standards for peak and long-term exposure. Hum. Ecol. Risk. Assess. http://dx.doi.org/10.1080/10807039.2014.964071.
Song, M.Y., Stark, J.D., Brown, J.J., 1997. Comparative toxicity of four insecticides, including imidacloprid and tebufenozide, to four aquatic arthropods. Environ. Toxicol. Chem. 16, 2494–2500.
Starner, K., Goh, K., 2012. Detections of the Neonicotinoid insecticide imidacloprid in surface waters of three agricultural regions of California, USA, 2010–2011. Bull. Environ. Contam. Toxicol. 88, 316–321.
Stoughton, S.J., Liber, K., Culp, J., Cessna, A., 2008. Acute and chronic toxicity of imidacloprid to the aquatic invertebrates *Chironomus tentans* and *Hyalella azteca* under constant- and pulse-exposure conditions. Arch. Environ. Contam. Toxicol. 54, 662–673.
Suchail, S., Guez, D., Belzunces, L.P., 2001. Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in *Apis mellifera*. Environ. Toxicol. Chem. 20, 2482–2486.
Syracuse Environmental Research Associates (SERA), 2005. Imidacloprid: Human Health and Ecological Risk Assessment — Final Report. SERA TR 05-43-24-03a. Syracuse Environmental Research Associates, New York.
Tennekes, H.A., 2010a. The significance of the Druckrey–Kupfmuller equation for risk assessment-the toxicity of neonicotinoid insecticides to arthropods is reinforced by exposure time. Toxicology 276, 1–4.
Tennekes, H.A., 2010b. The Systemic Insecticides: A Disaster in the Making. ETS Nederland BV.
Tennekes, H.A., 2011. The significance of the Druckrey–Küpfmüller equation for risk assessment—the toxicity of neonicotinoid insecticides to arthropods is reinforced

by exposure time: responding to a Letter to the Editor by Drs. C. Maus and R. Nauen of Bayer CropScience AG. Toxicology 280, 173–175.
Tennekes, H.A., Sánchez-Bayo, F., 2011. Time-dependent toxicity of neonicotinoids and other toxicants: implications for a new approach to risk assessment. J. Environ. Anal. Toxicol. S4, 001.
Tennekes, H.A., Sánchez-Bayo, F., 2013. The molecular basis of simple relationships between exposure concentration and toxic effects with time. Toxicology 309, 39–51.
Thuyet, D.Q., Watanabe, H., Motobayashi, T., 2011. Effect of formulations and treatment methods of nursery boxes applied with insecticide on the behavior of imidacloprid in rice paddy fields. J. Pestic. Sci. 36, 9–15.
Tisler, T., Jemec, A., Mozetic, B., Trebse, P., 2009. Hazard identification of imidacloprid to aquatic environment. Chemosphere 76, 907–914.
U.S. Environmental Protection Agency (USEPA), 2014. OPP Pesticide Toxicity Database. http://www.epa.gov/oppefed1/ecorisk_ders/aquatic_life_benchmark.htm accessed July 2014.
U.S. Geological Survey (USGS), 2012. Annual Pesticide Use Maps 1992–2011. NAWQA Pesticide National Synthesis Project. U.S. Geological Survey.
Van Dijk, T.C., Van Staalduinen, M.A., Van der Sluijs, J.P., 2013. Macro-invertebrate decline in surface water polluted with imidacloprid. PLoS One 8, e62374.
Vijver, M.G., van den Brink, P.J., 2014. Macro-invertebrate decline in surface water polluted with imidacloprid: a rebuttal and some new analyses. PLoS One 9.
Wachendorff-Neumann, U, Mauler-Machnik, A, Erdelen, C, Ohtake, H. Synergistic mixture of trifloxystrobin and imidacloprid. Google patents. United States: Bayer Cropscience AG; 2012.
Whitehorn, P.R., O'Connor, S., Wackers, F.L., Goulson, D., 2012. Neonicotinoid pesticide reduces bumble bee colony growth and queen production. Science 336, 351–352.
Xing, Z.S., Chow, L., Rees, H., Meng, F.R., Li, S., Ernst, B., Benoy, G., Zha, T.S., Hewitt, L.M., 2013. Influences of sampling methodologies on pesticide-residue detection in stream water. Arch. Environ. Contam. Toxicol. 64, 208–218.
Yamamoto, I., Yabuta, G., Tomizawa, M., Saito, T., Miyamoto, T., Kagabu, S., 1995. Molecular mechanism for selective toxicity of nicotinoids and neonicotinoids. J. Pestic. Sci. 20, 33–40.
Yamamoto, I., Tomizawa, M., Saito, T., Miyamoto, T., Walcott, E.C., Sumikawa, K., 1998. Structural factors contributing to insecticidal and selective actions of neonicotinoids. Arch. Insect Biochem. Physiol. 37, 24–32.
Zhang, A., Kaiser, H., Maienfisch, P., Casida, J.E., 2000. Insect nicotinic acetylcholine receptor: conserved neonicotinoid specificity of [^{3}H]imidacloprid binding site. J. Neurochem. 75, 1294–1303.
Zheng, W., Liu, W., 1999. Kinetics and mechanism of the hydrolysis of imidacloprid. Pestic. Sci. 55, 482–485.

SWAMP
Surface Water Ambient Monitoring Program

Technical Report	2010

Interpreting Narrative Objectives for Biostimulatory Substances for California Central Coast Waters

Karen R. Worcester, Staff Environmental Scientist
David M. Paradies, CCAMP Data Consultant
Mary Adams, Environmental Scientist

July 2010

Central Coast Ambient Monitoring Program
California Central Coast Water Board

Water Boards

www.waterboards.ca.gov/swamp

Interpreting Narrative Objectives for Biostimulatory Substances for California Central Coast Waters

Central Coast Ambient Monitoring Program Technical Report
California Central Coast Water Board
July 1, 2010

Summary

This technical paper describes an approach for interpreting the 1994 California Central Coast Water Quality Control Plan (Basin Plan) narrative language stating that "waters shall not contain biostimulatory substances in concentrations that promote aquatic growths to the extent that such growths cause nuisance or adversely affect beneficial uses." In this approach, Central Coast Water Board staff employed Basin Plan Objectives, U.S. Environmental Protection Agency (U.S. EPA) standards, guideline values from the literature, our own monitoring data, and modeled estimates of potential algal growth and resultant oxygen deficits. The resulting numeric endpoints can be used for regional water quality assessments and to support assessment decisions for the California Integrated Report for addressing Clean Water Act Sections 303(d) and 305(b). To conduct this analysis, we have relied heavily upon data collected by the Central Coast Ambient Monitoring Program (CCAMP). CCAMP conducts monitoring for the Central Coast Water Board and is the Central Coast regional component of the California Surface Water Ambient Monitoring Program. CCAMP data can be viewed at www.ccamp.org.

We identified a pool of long-term monitoring locations, or "sites", from the extensive CCAMP dataset that have always met either warm or cold water oxygen objectives based on both monthly grab samples and 24-hour continuous monitoring. From this dataset, we identified an upper range for dissolved oxygen concentration of 13 milligrams per liter (mg/L), over which site oxygen concentrations rarely or never fell. We established 13 mg/L as an upper limit for oxygen, to address the U.S. EPA 'Gold Book' (1986) water quality standard for excessive gas saturation. We identified a reference subset of the initial set of sites that showed no other signs of eutrophication, such as oxygen levels over 13 mg/L, water column chlorophyll a exceeding 15 micrograms per liter (ug/L) or observed floating algal cover exceeding 50%.

We examined nutrient characteristics of data from this reference set to identify a proposed screening criterion of 1.0 mg/L nitrate as nitrogen (mg/L NO3-N) to protect aquatic life. This number represents the 95th percentile of the reference data set. We then used the California Benthic Biomass Tool (Tetratech, 2007), or "Benthic Biomass Tool", to evaluate individual monitoring sites in terms of predicted oxygen deficits, maximum benthic algal biomass and benthic chlorophyll a concentrations. These modeled outputs can be evaluated against the "presumed impaired" thresholds identified in the "Technical Approach to develop Nutrient Numeric Endpoints for California" (Creager, 2006), to characterize the risk of eutrophication associated with specific conditions at a given site or water body.

Based on this analysis, we will designate water bodies as impaired for aquatic life use when nitrate concentrations exceed 1.0 mg/L NO3-N and there is additional evidence of eutrophication, including depressed or supersaturated dissolved oxygen concentrations, pH over 9.5, floating algal mats over 50%, water column chlorophyll *a* concentrations over 15 ug/L, predicted oxygen deficits over 1.25 mg/L, and predicted benthic algal biomass or predicted benthic chlorophyll *a* concentrations over levels recommended by the "Technical Approach to develop Nutrient Numeric Endpoints for California" (Creager et al., 2006).

Background

Nitrate is regulated as a toxicant in California, because of its impacts on the public water supply and human health. The drinking water standard is set at 10 mg/L NO3-N to protect against methemoglobinemia ("blue baby syndrome"), and more recent research implies that lower levels may be required to protect against thyroid cancer (Ward et al., 2010) and other health concerns. Also, a growing body of research on aquatic toxicity recommends even lower thresholds for protection of aquatic life; for example, Camargo et al. (2005) recommends 2.0 mg/L NO3-N for protection of sensitive aquatic species. However, no numeric standards for nitrate are currently in place in California for protection of aquatic life, either for direct toxicity or for indirect effects as a biostimulatory substance. The purpose of this document is to address nitrate and related biostimulatory indicators as they relate to aquatic life beneficial uses.

In some environmental conditions, excessive nutrient concentrations in stream systems stimulate algal growth, which can create nuisance conditions for several beneficial uses including irrigation, industrial supply and recreational use. More importantly, excessive algae can remove oxygen from water, creating conditions unsuitable for many aquatic life forms. This condition is called "eutrophication". Some algal blooms are also toxic to aquatic life, wildlife, and even humans. Waters that contain excessive algal growth are characterized by wide swings in dissolved oxygen concentrations, typically dropping below concentrations set to protect for aquatic life at night, and often rising above fully saturated levels during daytime (U.S. EPA, 2000b). Low oxygen conditions can result in fish kills and harm to other aquatic life. Some species, such as trout, are particularly sensitive to low oxygen conditions, which is why more rigorous standards are set to support cold water fish habitat.

Supersaturated oxygen conditions can be indicative of excessive algal photosynthetic activity and can be exacerbated by rapid increases in water temperature. Total gas supersaturation can cause direct harm to fish when total dissolved gas saturation increases enough to cause "gas bubble trauma". This is a sometimes fatal condition which occurs when gas bubbles, primarily nitrogen and/or oxygen, are released into the bloodstream and accumulate in the skin, eyes, and gills of fish (Weitkamp, 2008). It is usually considered a problem for fish in discharge waters from dams, but can also be associated with eutrophication (Canadian Council of Ministers of the Environment, 1999; Fidler and Miller, 1994). Edsall and Smith (2008) showed gas bubble trauma could be induced with

oxygen supersaturation alone. U.S. EPA (1986) has recommended an upper limit of 110% total dissolved gas saturation to protect fish from gas bubble trauma.

Regulatory Setting - The 1994 Central Coast Water Quality Control Plan (Basin Plan) contains narrative language stating that "waters shall not contain biostimulatory substances in concentrations that promote aquatic growths to the extent that such growths cause nuisance or adversely affect beneficial uses." Similar narrative language is used throughout California. In the past, states lacked guidance on how to "translate" this narrative language into quantifiable endpoints, making it difficult to apply this objective in a regulatory setting.

In 2000, the U.S. Environmental Protection Agency (EPA) released technical guidance for developing numeric nutrient criteria for the Xeric west (U.S. EPA, 2000a). This guidance recommended states follow one of three approaches:

1) Adopt nutrient criteria that reflect local conditions, either as numeric criteria or as procedures to translate a narrative criterion into quantifiable endpoints, following EPA Technical guidance (U.S. EPA, 2000b).
2) Adopt EPA Section 304(a) criteria, described in the technical guidance (U.S. EPA, 2000a), either as numeric criteria or as procedures to translate a narrative criterion into quantifiable endpoints.
3) Develop criteria capable of protecting beneficial uses using other scientifically defensible methods and data.

EPA technical guidance recommended two approaches to setting nitrogen reference conditions. The preferred approach was to use the 75th percentile of data from a set of reference sites. The other approach was to use the 25th percentile of all data. Using the second approach, EPA derived a reference value of 0.38 mg/L total nitrogen (TN) for the xeric west (which includes the Central Coast Region), and also identified a subregional value of 0.5 mg/L TN for the Central and Southern California Chaparral Ecoregion (U.S. EPA, 2000a).

California convened a Technical Advisory Group to develop its own approach to development of nutrient endpoints, following approach #1 above, by translating narrative criteria into quantifiable endpoints through the California Nutrient Numeric Endpoint Approach.

California Nutrient Numeric Endpoint Approach - The "Technical Approach to Develop Nutrient Numeric Endpoints for California" (Creager, et al., 2006), or "California NNE Approach", was developed by Tetratech, Inc. for the State Water Resources Control Board (SWRCB), in order to interpret the biostimulatory narrative objective, and to support development of numeric criteria for nutrients to protect for aquatic life beneficial uses. The California NNE Approach utilizes predicted benthic algae biomass and benthic chlorophyll *a* concentrations as "response variables" or "secondary indicators" to define Beneficial Use Risk Categories that can serve as preliminary numeric targets. These numeric targets are set at a conservative (protective) level to account for uncertainty and to be applicable throughout California. The California NNE Approach

recommends numeric boundaries between three categories of risk in cold and warm water streams: "presumptive unimpaired", "potentially impaired", and "presumptive impaired". The recommended numeric boundary for benthic algal biomass between risk categories of "potentially impaired" and "presumptive impaired" are: 200 milligrams chlorophyll *a* per square meter (mg/m^2) for warm water streams and 150 mg/m^2 for cold water streams, and corresponding benthic algal biomass of 80 $grams/m^2$ ash free dry weight (AFDW) in warm water streams and 60 $grams/m^2$ AFDW in cold water streams. The boundary established by the California NNE approach between these two risk categories for pH is 9.5 pH units, which is well over the upper Basin Plan standard of 8.5.

Tetratech Inc. developed the Benthic Biomass Tool as a companion tool to the California NNE Approach, to predict instream benthic algal density and other secondary endpoints, in response to a number of inputs. Data on nutrient concentrations (minimums, maximums, and mean average), as well as latitude, canopy cover, stream depth and velocity is input into the Benthic Biomass Tool, to generate several model outputs. These include benthic biomass and benthic chlorophyll *a* concentrations for both cold and warm water streams. The tool predicts these outputs for five models and seven different methods taken from the scientific literature. The models and their application are described extensively in Appendix 3 of the California NNE Approach (Crieger, 2006). They include empirical models (Dodds, 1997 and 2002) and the QUAL2K simulation models (Chapra and Pelletier, 2003), including the standard model, a revised model that provides a better fit to Dodd's empirical data, and a revised model that adjusts for algae accrual time between scour events (this is especially important in areas with summer rain events). The revised QUAL2K simulation model also predicts the anticipated maximum algal contribution to oxygen deficit. This is the maximum amount of dissolved oxygen expected to be removed from the water as a result of predicted benthic algal growth. The outputs can then be evaluated using the numeric targets for secondary indicators, established by the California NNE Approach to determine the risk of impairment at a given site from nutrient over-enrichment.

The Water Quality Control Policy (WQCP) for developing California's Clean Water Act Section 303(d) list (SWRCB, 2004), or "Listing Policy", describes the process by which the SWRCB and Regional Water Boards will comply with the listing requirements of Section 303(d) of the federal Clean Water Act. Section 6.1.3 "Evaluation Guideline Selection Process" provides the requirements for a proposed guideline before it can be accepted for use as part of the 303(d) listing process. According to SWRCB staff analysis, the California NNE Approach does meet these requirements, namely it is:

- Applicable to the beneficial use
- Protective of the beneficial use
- Linked to the pollutant under consideration
- Scientifically-based and peer reviewed
- Well described, and
- Identifies a range above which impacts occur and below which no or few impacts are predicted.

Central Coast Water Board staff has used the California NNE Approach and Benthic Biomass Tool, paired with an empirical evaluation of Central Coast reference data, to develop a nitrate guideline value and supporting evidence to assess whether aquatic life uses show negative effects associated with eutrophication.

Establishing Characteristics of Unimpaired Sites

Oxygen Reference Range remaining above Basin Plan objectives and below 13 mg/L – Waters that have large amounts of algae or other plant material present can show widely ranging diel oxygen concentrations (U.S. EPA, 2000b). Water can "supersaturate" during daylight hours because plant photosynthetic activity releases oxygen to the water and in some circumstances that oxygen is trapped beneath the surface tension of the water's surface. Also, water can become oxygen depleted during dark hours because plant respiration (and decay) removes oxygen from the water column. The resulting widely ranging oxygen concentrations are a primary indication of eutrophication and one of the resulting outcomes that is deleterious to aquatic life.

Central Coast Water Board staff evaluated Central Coast Ambient Monitoring Program (CCAMP) diel oxygen data collected from 105 sites where dissolved oxygen recording probes were deployed for 20 or more hours during summer months. CCAMP collects this data to determine if oxygen levels drop during the highest risk time of day, which is pre-dawn. This is important because monitoring staff conducts routine monthly grab sampling between 9 a.m. and 4 p.m., when oxygen levels are typically highest.

From the combined dataset of grab samples and diel data, we established two data sets for potential reference sites. The first set was from the 32 sites where dissolved oxygen concentrations never dropped below 7.0 mg/L, the cold water aquatic life standard. The second was for the 59 sites where dissolved oxygen concentrations never dropped below 5.0 mg/L, the warm water aquatic life standard. We examined oxygen concentrations of both diel and monthly grab sample data for these sites for each hour of the day (Figures 1 and 2). For the 32 sites that met the cold water objective, 29 sites never exceeded 13 mg/L at any time. Of the 644 grab samples taken at these 32 sites, only 6 samples (or 1.0%) exceeded 13 mg/L. For the 59 sites that met the warm water objective, 43 sites never exceeded 13 mg/L at any time. Of the 1,695 grab samples taken at these 43 sites, only 32 samples (or 1.9%) exceeded 13 mg/L. We determined that 13 mg/L is an appropriate upper value to screen both warm and cold water sites for oxygen super-saturation outside of reference ranges.

Water column chlorophyll *a* concentrations remaining under 15 ug/L - This value has been used for a number of years as a CCAMP screening value. The state of North Carolina has set a maximum acceptable chlorophyll *a* standard of 15 ug/L for cold water (lakes, reservoir, and other waters subject to growths of macroscopic or microscopic vegetation designated as trout waters), and 40 ug/L for warm water (lakes, reservoir, and other waters subject to growths of macroscopic or microscopic vegetation not designated as trout waters) (North Carolina Administrative Code 15A NCAC 02B .0211 (3) (a)). Oregon uses an average chlorophyll *a* concentration of >15 ug/L as a criterion for

nuisance phytoplankton growth in lakes and rivers (OAR, 2000). A chlorophyll *a* concentration of 8 ug/L is recommended as a threshold of eutrophy for plankton in EPA's Nutrient Criteria Technical Guidance Manual for Rivers and Streams (2000b). The Central Coast Region has used 40 ug/L as stand-alone evidence to support chlorophyll *a* listing recommendations for the 303(d) Impaired Water Bodies list. However, we are using 15 ug/L as supporting evidence of nutrient over-enrichment, based on a review of existing and recommended limits used elsewhere.

Floating algal cover not exceeding 50% of the water's surface - Typical nuisance criteria cited in the literature for filamentous algal cover range from 40 to 55% (Stevenson, et al., 1996). The State of Nevada uses 50% cover as a screening threshold for filamentous algal cover to identify possible algae related problems (NDEP, 2007). CCAMP documents the percent surface coverage of floating algal mats at each monthly site visit and has associated photographs supporting these observations. We are using 50% floating algal cover as supporting evidence of excessive algal growth and nutrient over-enrichment. Floating algal cover is defined as filamentous algae that is sufficiently long and thick that it breaks the water's surface and creates nuisance algal mats.

Establishing a guideline value for nitrate-N

We evaluated CCAMP data for characteristics of sites meeting warm and cold water oxygen objectives that do not show evidence of eutrophication. These sites remained below the limits of characteristics described above, related to oxygen range, water column chlorophyll a concentrations, and algal cover. Twenty of the 32 original sites that met the cold water objective also met all of these conditions, and twenty-six of the original 59 sites that met the warm water oxygen objective also met all of these conditions. These sites are considered "reference".

No sites from the cold water data reference set and only one site from the warm water reference data set had nitrate-N concentrations that exceeded 1.0 mg/L NO3-N as an average (Figures 3 and 4). The single site that exceeded this value was located below a dam, and was well oxygenated as a result.

One approach U.S. EPA (2000) recommended for setting nitrogen criteria was at the 75^{th} percentile of reference data. One mg/L NO3-N represents the 95^{th} percentile of our reference data set. We set 1.0 mg/L NO3-N as the tentative guideline value to screen for aquatic life use protection. We recognize that this is a higher concentration threshold than that derived using the EPA approach, but we believe it is more applicable for the central coast of California because reference conditions here tend to be found in higher gradient waters of small coastal streams, whereas most land uses in the Region occur around lower gradient systems with wide, flat floodplains, where nutrient levels can be expected to be naturally higher (Franklin, et al., 2002).

Application of the California Benthic Biomass Tool to CCAMP Data

Staff submitted summary data for 209 CCAMP sites, collected between 1998 and 2006, for water body minimums, maximums, and means for nitrate, nitrite, ammonium, ortho-phosphate, total nitrogen, total phosphorus and water temperature into the Benthic Biomass Tool. To screen data for probable effects, we utilized the recommended NNE warm water threshold values of 200 mg/m^2 for benthic chlorophyll *a* and 80 grams/m^2 ash-free dry weight (AFDW) for algal density, and the cold water threshold values of 150 mg/ m^2 for benthic chlorophyll *a* and 60 grams/m^2 AFDW for algal density. We used a latitude of 35 degrees and a canopy cover of 80% as model inputs. Our assumption of a relatively dense canopy cover produces an estimate of probable effects that conservatively (less frequently) identifies problem conditions. We used default values in the Benthic Biomass Tool for several other model inputs, including stream velocity of 0.3 meters per second and stream depth of 0.5 meters. Resulting outputs provided estimates of benthic algal biomass, benthic chlorophyll *a* concentration, and estimated oxygen deficit for each water body.

The Benthic Biomass Tool provides a sensitivity analysis that examines how varying model inputs alter model outputs. We examined the sensitivity of model outputs to default values. For example, reducing stream velocity or depth defaults by half produced minor increases in predicted algal biomass estimates. However, reducing canopy cover by half (from 80 to 40%) produced large increases in predicted biomass. By allowing the default value for canopy to remain at 80% (the highest value the Tool allows), we will typically underpredict algal cover, and thus can use outputs that exceed the "probable impairment" level as reliable evidence of a problem.

Predicted Oxygen Deficit – We used the Benthic Biomass Tool to further evaluate these sites in terms of predicted "maximum algal contribution to oxygen deficit". This is the amount of oxygen predicted to be removed from the water column as a result of benthic algal biomass, and is an output of the QUAL2K model (Chapra and Pelletier, 2003) embedded in the Tool. Staff evaluated resulting individual site outputs for all CCAMP data from 209 sites. The Benthic Biomass Tool generated an estimated oxygen deficit for each site based on predicted algal biomass. Based on the nitrate concentrations associated with CCAMP sites that do not show evidence of biostimulation, staff evaluated the oxygen deficit associated with sites that had average nitrate concentrations of 1.0 mg/L NO3-N or lower. The maximum contribution of algae to oxygen deficit at this nitrate concentration was approximately 1.25 mg/L (Figure 5). All of the cold water reference sites and most of the warm water reference sites fall within this level of predicted oxygen deficit (Figures 6 and 7). We identified 1.25 mg/L oxygen deficit as a threshold below which risk of eutrophication is minimized. It should be recognized that the actual oxygen deficit at any given location may vary significantly from this modeled estimate, because of other variables such as vertical stratification, water residence time, transparency and distance downstream from pollution sources.

Reference Site Performance - We examined the performance of our reference sites relative to NNE biomass predictions. The twenty reference sites that met cold water

standards and showed no other evidence of eutrophication had predicted benthic chlorophyll *a* values that remained well under the NNE threshold of "presumptive impaired", of 150 mg/m^2 (Figure 8). In fact, for all but one of these sites, the predicted values were at around 50 mg/m^2 or lower. Similarly, all warm water reference sites remained under the NNE warm water "presumptive impaired" threshold of 200 mg/m^2; all but two of these sites remained under 100 mg/m^2. Not only do these sites show no empirical evidence of eutrophication, the model outputs show they fall into the NNE Categories of "presumptive unimpaired" (below 100 and 150 mg/m2 for cold and warm water habitats, respectively), as should be expected for reference conditions.

Using Nitrate Screening Criterion to Develop Lines of Evidence

Nitrate and other nutrients are treated as "toxins" by the Listing Policy (SWRCB, 2004). Consequently, in developing Lines of Evidence for the 2008 Integrated Report, Central Coast Water Board staff evaluated nitrate data using the binomial distribution established for toxic pollutants in Table 3.1 of the Listing Policy, based on exceedance of 1.0 mg/L NO3-N. We provided further evidence of eutrophication using supporting data and Benthic Biomass Tool model outputs. These included predictions of benthic algal biomass and/or benthic chlorophyll *a* concentrations exceeding model thresholds of "probable impairment", evaluation of model prediction of algal contribution to oxygen deficit relative to our established 1.25 mg/L threshold, as well as parameters measured in the field, including floating algal mats exceeding 50% of the water surface, water column chlorophyll *a* concentrations over 15 ug/L, pH over 9.5, and evidence of oxygen depression (concentrations less than the appropriate Basin Plan standard) and/or super-saturation (concentrations greater than 13 mg/L),.

Conclusions

In this technical paper, we have developed an integrated approach for interpreting the Central Coast Water Quality Control Plan's "narrative objective for biostimulatory substances", that will protect aquatic life beneficial uses from the consequences of nutrient over-enrichment and resulting eutrophication. We have used this approach to develop decisions related to water body impairment for the 2010 Integrated Report for addressing Clean Water Act Sections 303(d) and 305(b). In this approach we have relied on empirical data evaluation along with simulation models, guideline values from the scientific literature, regional water quality objectives, and EPA standards, to develop multiple lines of reasoning that can support regulatory decision-making.

We screen sites for evidence of eutrophication by evaluating data for exceedance of 1.0 mg/L nitrate (as N), using a binomial distribution according to Table 3.1 of the Listing Policy (2004). We further support our decisions with other evidence of eutrophication, using supporting data and Benthic Biomass Tool model outputs. These include predictions of benthic algal biomass and/or benthic chlorophyll *a* concentrations exceeding model thresholds of "probable impairment", evaluation of model prediction of algal contribution to oxygen deficit relative to our established 1.25 mg/L threshold, as well as parameters measured in the field, including floating algal mats exceeding 50% of

the water surface, water column chlorophyll *a* concentrations over 15 ug/L, pH over 9.5and evidence of oxygen depression (concentrations less than the appropriate Basin Plan standard) and/or super-saturation (concentrations greater than 13 mg/L).

An EPA draft document entitled "Empirical Approaches for Nutrient Criteria Derivation" was released for review in August, 2009. This document recommended a "stressor–response" statistical approach that quantifies the relationship between nutrients and biological response measures, in this case for macroinvertebrates. In an April, 2010 peer review of this document, the U.S. EPA Scientific Advisory Board (August 12. 2009) recommended against stand-alone statistical methods such as the one used by U.S. EPA, because of the challenges associated with proving cause and effect. Instead, the SAB recommended a weight of evidence approach to criteria development. The SAB also stressed the importance of recognizing downstream impacts associated with excessive nutrients. The SAB suggested that the omission of dissolved oxygen as a response variable in the EPA approach was a significant omission, since it is clearly related to nutrient over-enrichment, whereas macroinvertebrate species diversity is not as well supported scientifically as a response variable. We feel the approach we used addresses a number of the SAB's concerns by using multiple lines of reasoning and by including dissolved oxygen response as an important line of evidence. Our approach also addresses potential downstream impacts through its combination of both empirical and risk-based evidence.

We acknowledge that field conditions, including benthic algal biomass, benthic chlorophyll a concentration and algal contribution to oxygen deficit, may vary considerably from modeled values, depending on a number of variables including stream substrate type, streambed profile, vertical stratification, residence time, absolute temperatures and irradiance (transparency). For this reason, field evidence of widely ranging oxygen, pH or excessive algal cover or chlorophyll a concentrations is preferable for confirming impairments to the aquatic life beneficial use. However, modeled outputs also help characterize risk to downstream environments where site level characteristics may be more conducive to algal growth, and thus should be included as part of the overall weight of evidence of impairment. Our use of a relatively high default value for canopy closure ensures that we are likely to be underprotective with our modeled results, making their use as additional lines of reasoning more supportable.

In order to use this approach to develop guideline values for the Integrated Report, we are required to meet several criteria:

- Applicable to the beneficial use
- Protective of the beneficial use
- Linked to the pollutant under consideration
- Scientifically-based and peer reviewed
- Well described, and
- Identifies a range above which impacts occur and below which no or few impacts are predicted.

We believe that our approach meets these criteria. This document has been peer reviewed through the SWAMP document review process, and because our approach represents an innovative use of the California NNE, we also sent a draft of this document to one of the primary authors. He responded as follows:

> *I think the approach you are using is an interesting one. Indeed, it is an approach that is well suited to the nature of the simple screening tool we created. Rather than making quantitative predictions about the status of an individual site, you are using cumulative results across multiple sites to determine an appropriate level of nitrate to protect DO criteria from algal impacts. In essence, you are using the tool predictions to rank the sites relative to their observed DO data - which is likely to be more reliable than predicting specific conditions at individual sites. This becomes one line of evidence supporting a basin plan objective of 1 mg/L NO3-N. I presume you have also done a direct comparison of nitrate concentrations versus DO excursions and found it noisier. In essence, the tool is being used to smooth the relationship and filter out other co-factors that may be controlling response at individual sites. I suspect, however, that stakeholders may ask you to provide some more direct evidence of actual harm associated with nitrate greater than 1 mg/L, so fleshing out other lines of evidence may be important. I think eutrophication effects will likely govern here.*
>
> *- Dr. Jon Butcher, Tetratech Associate Director*

References

Camargo, J.A., A. Alonso, A. Salamanca. 2005. Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates. Chemosphere, 58:1255-1267.

Canadian Council of Ministers of the Environment. 1999. Canadian water quality guidelines for the protection of aquatic life: Dissolved gas supersaturation. In: Canadian environmental quality guidelines, 1999, Canadian Council of Ministers of the Environment, Winnipeg.

Chapra, S. and G. Pelletier. 2003. QUAL2K: A Modeling Framework for Simulation River and Stream Water Quality: Documentation and Users Manual. Civil and Environmental Engineering Dept., Tufts University, Medford, MA.

Creager, C., J. Butcher, E. Welch, G. Wortham, and S. Roy. July 2006. Technical Approach to develop Nutrient Numeric Endpoints for California. Tetratech, Inc. Prepared for U.S. EPA Region IX and State Water Resources Control Board.

CCRWQCB (Central Coast Water Quality Control Board). September 1994. Central Coast Water Quality Control Plan, Central Coast Basin (Basin Plan).

Dodds, W.K., V.H. Smith, and B. Zander. 1997. Developing nutrient targets to control benthic chlorophyll levels in streams: A case study of the Clark Fork River. *Water Research*, 31(7): 1738-1750.

Dodds, W.K., V.H. Smith, and K. Lohman. 2002. Nitrogen and phosphorus relationships to benthic algal biomass in temperate streams. *Canadian Journal of Fisheries and Aquatic Sciences*, 59: 865-874.

Edsall, D. A. and C.E. Smith. Oxygen-induced gas bubble disease in rainbow trout, *Oncorhynchus mykiss* (Walbaum). 2008. US Fish and Wildlife Service, Fish Technology Center, Bozeman, Montana, USA. *Aquatic Research*, 22(2): 135 – 140.

Fidler, L.E. and S.B. Miller. British Columbia Water Quality Guidelines for Dissolved Gas Supersaturation. 1994. Environment Canada. Aspen Applied Sciences Ltd. Valemount, BC V0E 2Z0

Franklin, D.H., J.L. Steiner, M.L.Cabrera, and E.L. Usery. 2002. Distribution of Inorganic Nitrogen and Phosphorus Concentrations in Stream Flow of Two Southern Piedmont Watersheds. Journal of Environmental Quality 31:1910-1917.

Nevada Department of Environmental Protection (NDEP). 2007. Nutrient Assessment Protocols for Wadeable Streams in Nevada.

North Carolina Administrative Code 15A NCAC 02B .0211. North Carolina Department of Environment and Natural Resources, Division Of Water Quality. Surface Waters and Wetlands Standards. August 1, 2004.

Stevenson, R.J., M.L. Bothwell, R.J. Lowe. *Algal Ecology: Freshwater Benthic Ecosystems*. Academic Press, 1996. 753 pages.

Oregon Administrative Rules (OAR). 2000. Nuisance Phytoplankton Growth. Water Quality Program Rules, 340-041-0150.

SWRCB, 2004. Water Quality Control Policy for Developing California's Section 303(d) List (Listing Policy). Resolution No. 2004-0063. State Water Resources Control Board, California Environmental Protection Agency. Sacramento, CA.

Tetratech, Inc. California Benthic Biomass Spreadsheet Tool, Version 13. User Guide and Documentation. February 28, 2007.

U.S. EPA, 1986. Quality Criteria for Water (The Gold Book). May 1, 1986. EPA440/5-86-001.

U.S. EPA, 2000a. Ambient Water Quality Criteria Recommendations: Information Supporting the Development of State and Tribal Nutrient Criteria. Rivers and Streams in Nutrient Ecoregion III. U.S. Environmental Protection Agency, Office of Water, Washington D.C. EPA 822-B-00-016.

U.S. EPA, 2000b. Nutrient Criteria Technical Guidance Manual, Rivers and Streams. U.S. Environmental Protection Agency, Office of Water, Washington D.C., EPA-822-00-002.

U.S. EPA, 2009 (draft). Empirical Approaches for Nutrient Criteria Derivation. United States Environmental Protection Agency, Office of Water, Office of Science and Technology Science Advisory Board Review Draft, August 17, 2009.

U.S. EPA Science Advisory Board Review of "Empirical Approaches for Nutrient Criteria Derivation. U.S Environmental Protection Agency. April 27, 2010.

Ward, M. H., B.A. Kilfoy, P.J. Weyer, K.E. Anderson, A.R. Folsom, J.R. Cerhan. 2010. Nitrate Intake and the Risk of Thyroid Cancer and Thyroid Disease. Epidemiology: 21(3): 389-395.

Weitkamp, D.E. Total Dissolved Gas Supersaturation Biological Effects: Review of Literature, 1980-2007. Parametrix 411 108th Ave. NE, Suite 1800 Bellevue, Washington 98004-5571 June 2008.

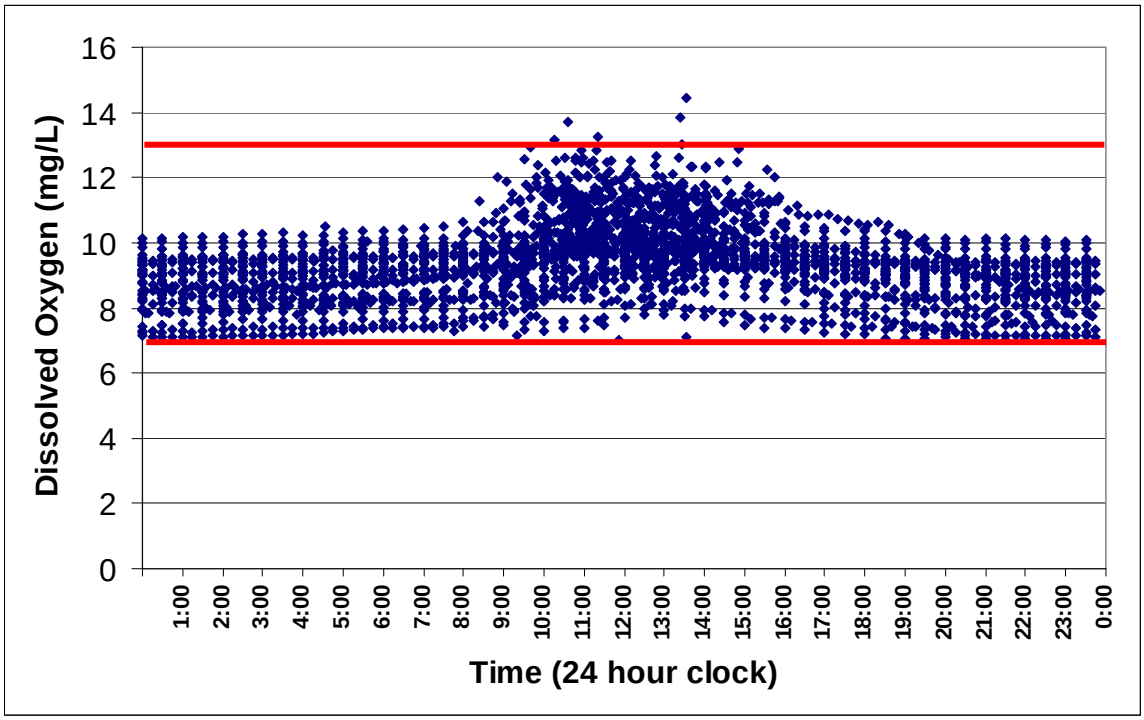

Figure 1. Hourly dissolved oxygen at 32 CCAMP sites that always meet the cold water aquatic life criterion (CCAMP data, 1998 – 2008). Includes 24-hour probe and monthly grab sample data. The cold water dissolved oxygen criterion (7.0 mg/L) and proposed upper screening limit (13.0 mg/L) are shown.

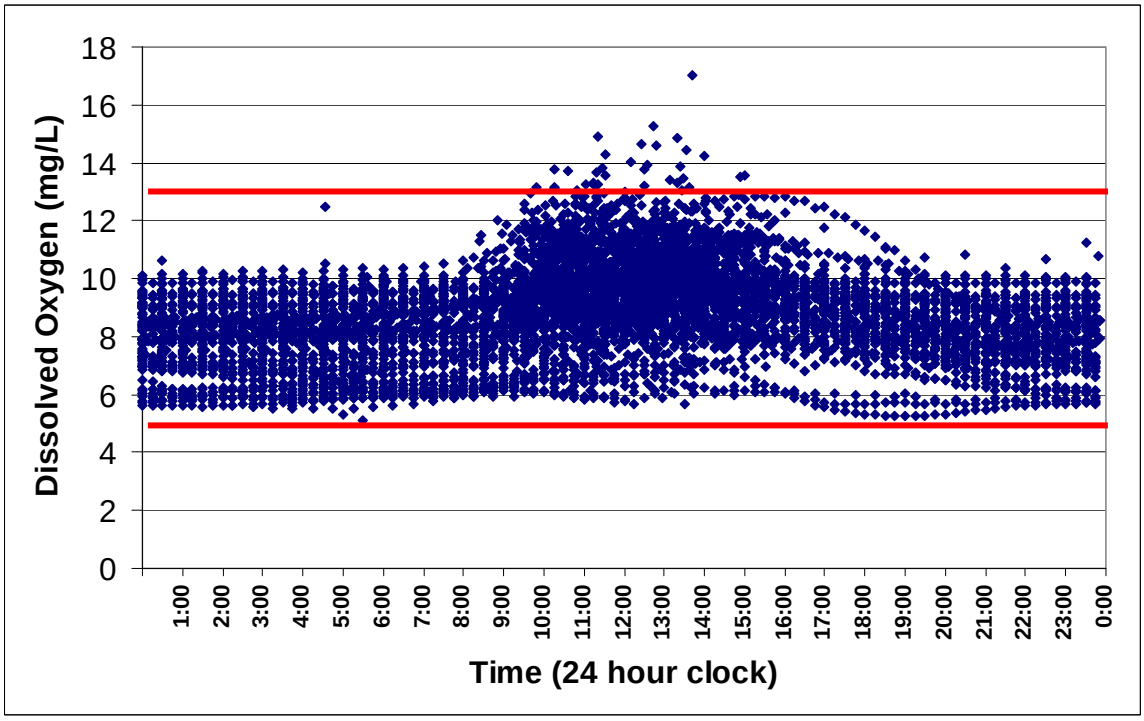

Figure 2. Hourly dissolved oxygen at 59 CCAMP sites that always meet the warm water aquatic life criterion (CCAMP data, 1998 – 2008). Includes 24-hour probe and monthly grab sample data. The warm water dissolved oxygen criterion (5.0 mg/L) and proposed upper screening limit (13.0 mg/L) are shown.

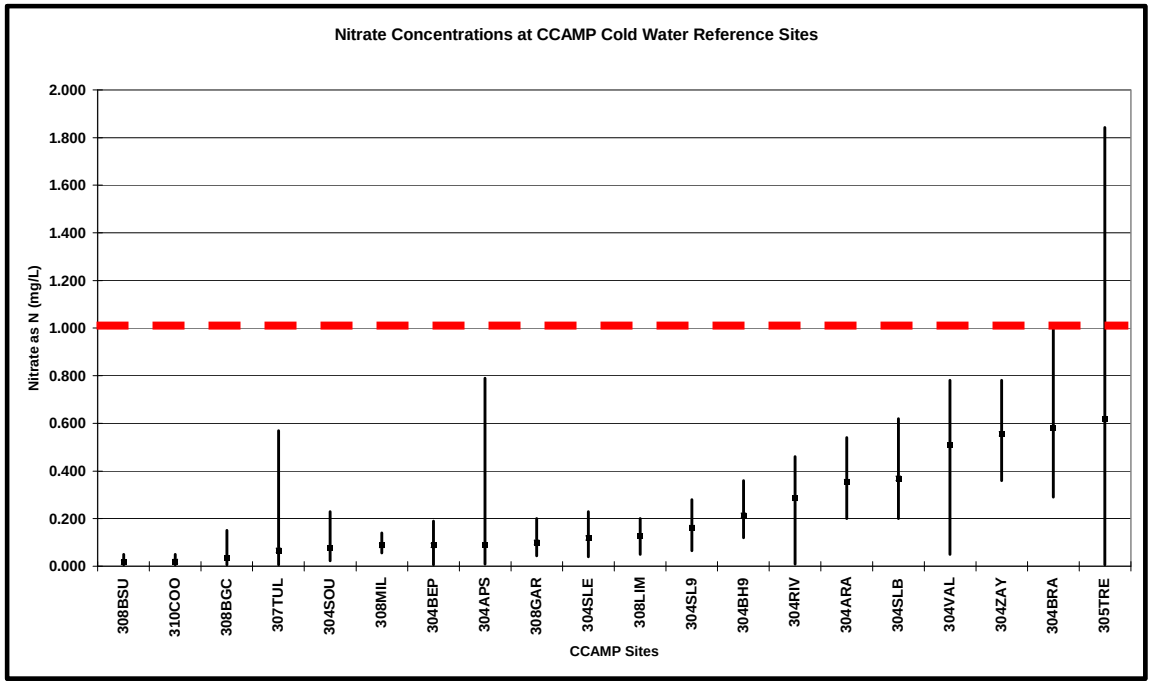

Figure 3. Mean Nitrate concentrations (mg/L-N) at twenty CCAMP sites that do not violate the Cold Water Oxygen Objective (7 mg/L) and do not exceed several screening criteria for indicators of eutrophication. Proposed guideline value of 1.0 mg/L is indicated.

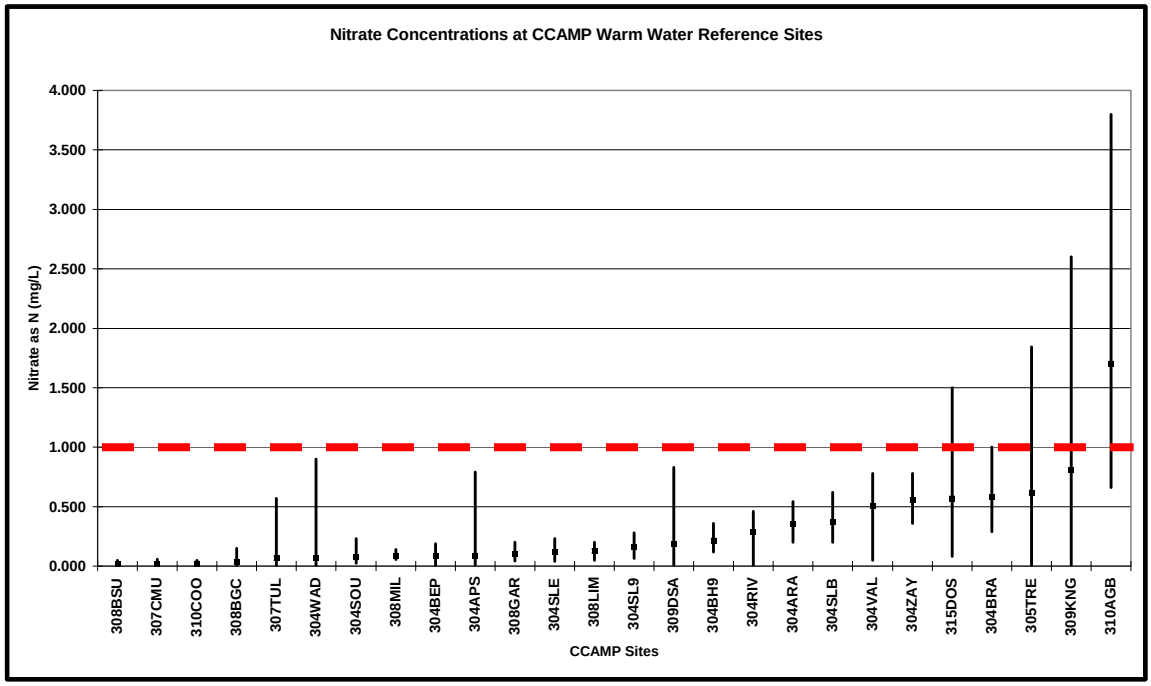

Figure 4. Mean Nitrate concentrations (mg/L-N) at CCAMP sites that do not violate the Warm Water Oxygen Objective (5 mg/L) and do not exceed several screening criteria for indicators of eutrophication. Proposed guideline value of 1.0 mg/L is indicated.

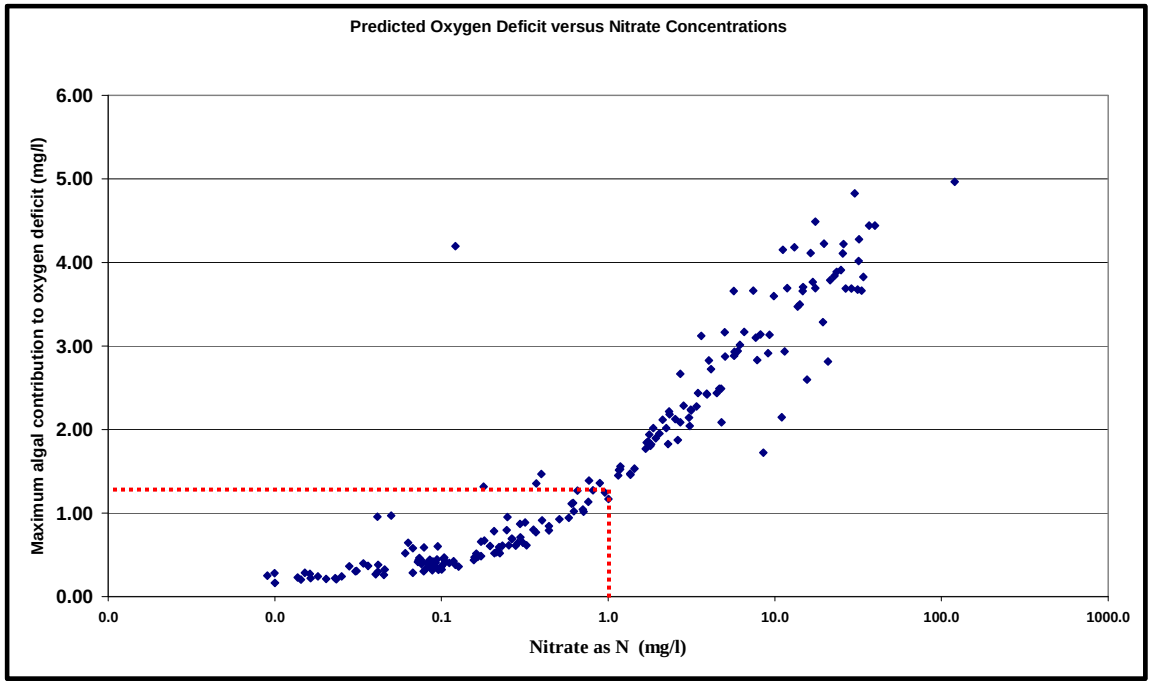

Figure 5. Relationship between average site nitrate concentrations (mg/L-N) and predicted oxygen deficit (mg/L). An average nitrate concentration of 1.0 mg/L-N predicts an estimated maximum algal contribution to oxygen deficit of approximately 1.25 mg/L, based on the California Benthic Biomass Tool (2007).

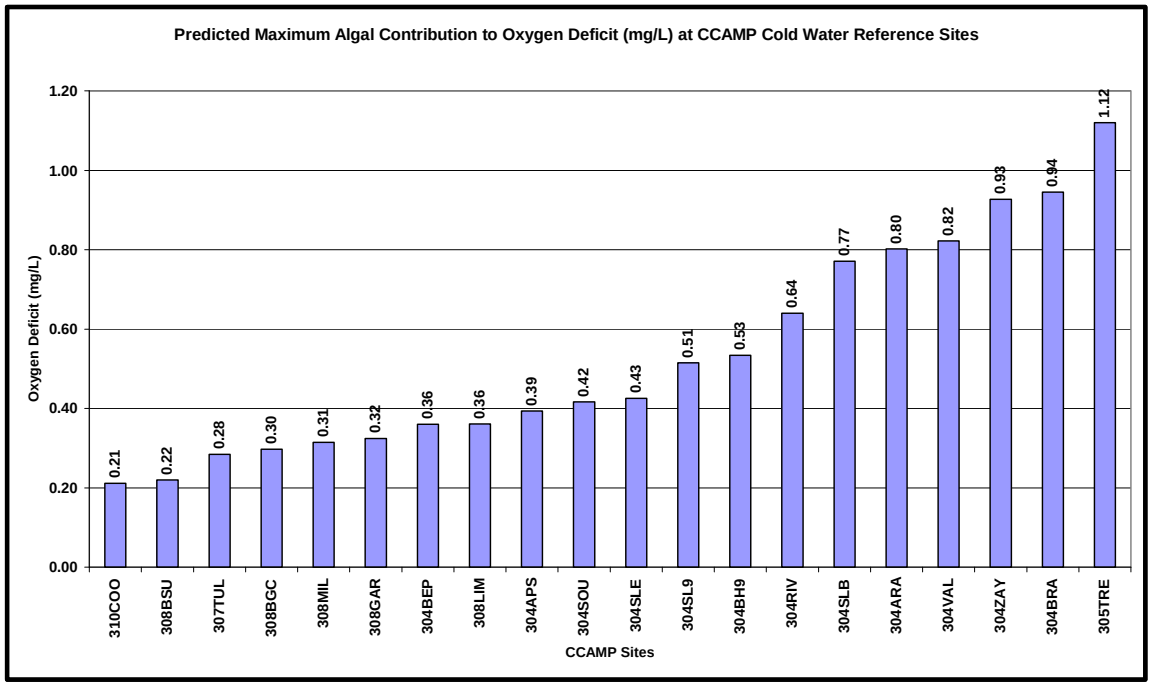

Figure 6. Predicted Maximum Algal Contribution to Oxygen Deficit (mg/L) at CCAMP sites that do not violate the Cold Water Oxygen Objective (7 mg/L) and do not exceed other eutrophication screening criteria. Proposed screening value for oxygen deficit is 1.25 mg/L.

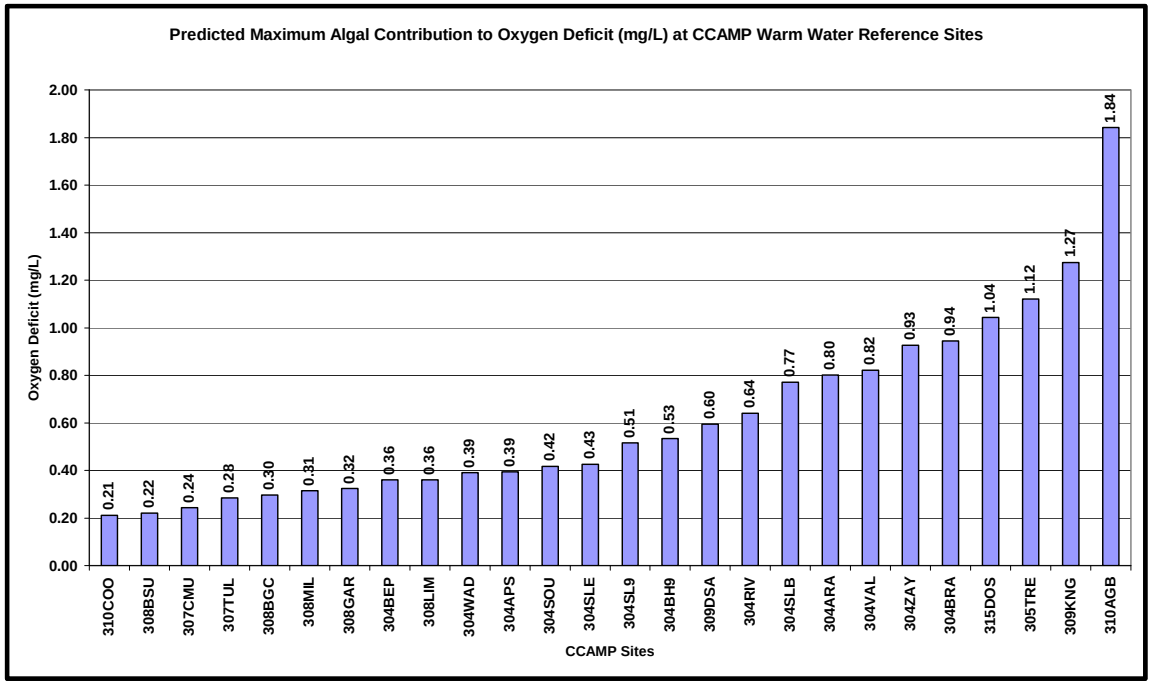

Figure 7. Predicted Maximum Algal Contribution to Oxygen Deficit (mg/L) at CCAMP sites that do not violate the Warm Water Oxygen Objective (5 mg/L) and do not exceed other eutrophication screening criteria. Proposed screening value for oxygen deficit is 1.25 mg/L.

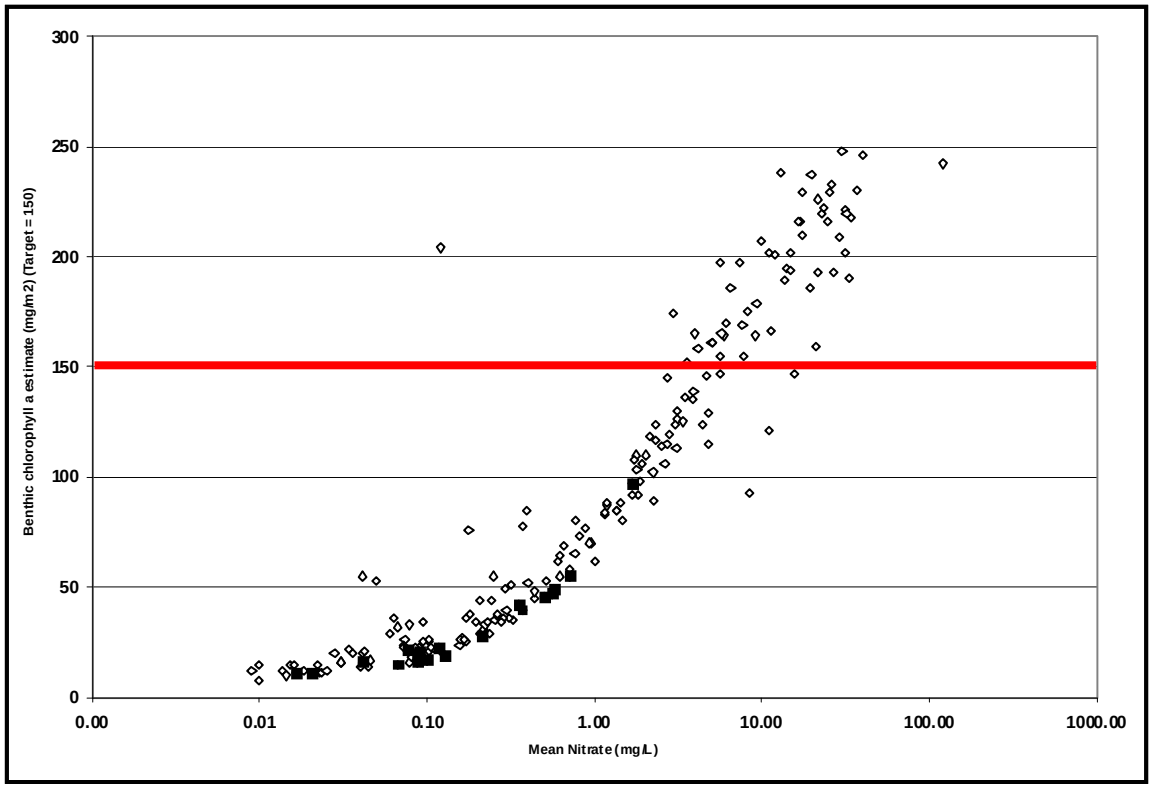

Figure 8. Benthic chlorophyll a predictions (mg/m^2) using the California Benthic Biomass Tool (2007), relative to average nitrate concentrations for 209 CCAMP sites (including cold water reference sites shown as black squares). California NNE recommended threshold for cold water is 150 mg/m^2.