

DETECTING THE TRANSPORT OF TOXIC PESTICIDES FROM GOLF COURSES INTO WATERSHEDS IN THE PRECAMBRIAN SHIELD REGION OF ONTARIO, CANADA

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Abstract—Golf courses impact the environment through alterations to habitat and through the release of nutrients and pesticides. The Precambrian Shield region of central Ontario, Canada, which is a major recreational area, is especially susceptible to the impacts of golf courses as a result of the geology and hydrology of the region. In a monitoring program at two golf courses in the Muskoka region conducted during the spring, summer, and fall of 2002, semipermeable membrane devices (SPMDs) were deployed into streams that drain the golf courses. The extracts from the SPMDs were tested for toxicity using bioassays with early life stages of an aquarium fish, the Japanese medaka (*Oryzias latipes*). Toxicity was assessed using a scoring system developed for the present study. The bioassays with medaka indicated that toxicity was highest in extracts from SPMDs deployed during the spring and the fall. The peaks in toxicity for the SPMDs deployed at the two golf courses corresponded with the presence in the SPMD extracts of pentachloronitrobenzene (PCNB) at concentrations up to 334 ng/SPMD. Quintozene is the turfgrass fungicide in which PCNB is the active ingredient. Pentachlorothioanisole, an anaerobic degradation product of PCNB, also was detected in the SPMDs deployed during the spring. Extracts prepared from SPMDs with high toxicity contained residues of a surfactant used in pesticide formulations, nonylphenol, at concentrations up to approximately 20 $\mu\text{g}/\text{SPMD}$. Overall, these data indicate that some pesticides applied to golf courses in the Precambrian Shield of central Ontario may have the potential to cause toxic impacts to aquatic organisms in adjacent watersheds.

Keywords—Golf course Pesticide Japanese medaka Semipermeable membrane device

INTRODUCTION

There has been interest in evaluating the potential environmental impacts of the construction and operation of golf courses [1] and, in particular, the potential for transport of pesticides and nutrients into groundwater and surface water adjacent to golf courses [2–5]. Maintenance of turf on golf courses often requires intensive applications of chemicals for turf maintenance, both because high-quality turf conditions are expected by the users and because the turf must withstand low-mowing and heavy traffic. Suzuki et al. [4] studied the persistence of several pesticides applied to golf courses in Japan and concluded that golf courses have a “high pollution potential for pesticides relative to agricultural areas.”

Modern construction techniques for golf courses generally involve deforestation and removal of the topsoil, followed by application of a sand substrate and laying of turf. The U.S. Golf Association recommends a root zone mixture for the turf that includes at least 90% sand. This practice maintains maximum infiltration and percolation of water through the root zone, but a high potential exists for movement of some pesticides into groundwater or for surface water contamination [3]. Petrovic and Larsson-Kovach [6] determined that sand substrates were more likely to allow leaching of the herbicide mecoprop. Sand is more porous than other soils, and it also has lower organic matter content, resulting in fewer adsorptive sites. In addition, it has a low cation-exchange capacity and provides a poor environment for the microorganisms responsible for degradation. Pore sizes are larger in sand, and the low surface tension in the pores results in greater downward

movement of water [7]. Primi et al. [8] found that groundwater in Long Island, New York, USA, became contaminated with pesticides from golf courses when the soil was coarse with low organic matter content and when pesticides had a low organic partitioning coefficient (K_{oc}), resulting in little retention in soil.

The Precambrian Shield region, which is north of the high-density urban region of southern Ontario, Canada, is being rapidly developed for golf enthusiasts. Several golf courses in this area are becoming international attractions. These very large golf complexes can be in excess of 300 ha and may include hotel and condominium accommodations. Because of the unique geology and hydrological conditions of the Precambrian Shield, golf courses in this area may be highly sensitive to the impacts of construction and operations. The shield area is characterized as having many lakes, rivers, and streams. Once precipitation or irrigation water saturates the substrate on top of silicate bedrock, the water is transported along the surface of the bedrock to lower elevations. The practice of depositing a sandy substrate before establishment of turf adds to the potential for saturation of the substrate above the granite and may result in overland transport during heavy rain events. In golf courses of this region, heavy fertilizer use was shown to result in the transport of nutrients into the watershed [9], which significantly altered benthic invertebrate communities [10].

The present study was designed to determine whether organic substances that are toxic to early life stages of fish are transported from golf courses in the Precambrian Shield of Ontario and whether toxic compounds occur in the watersheds of golf courses at times that coincide with the application of pesticides to golf courses and conditions conducive to surface

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runoff. Two golf courses in the Muskoka region of central Ontario were monitored over the period from May to November of 2002. Passive samplers (i.e., semipermeable membrane devices [SPMDs]) were deployed within the golf course watersheds at monthly intervals, and the retrieved devices were extracted for toxicity testing using early life stages of Japanese medaka (*Oryzias latipes*). The extracts also were analyzed for concentrations of model toxic compounds, which included the fungicide pentachloronitrobenzene (PCNB) and its microbial metabolite, pentachloroaniline (PCA), and a contaminant in the fungicide technical mixture, hexachlorobenzene (HCB), as well as the alkylphenol surfactants nonylphenol (NP) and octylphenol (OP). These model compounds were selected because records provided by golf courses in the area indicated that they were applied in large quantities, would be expected to accumulate in SPMDs, and have been shown to be toxic to aquatic organisms. In addition, the analytical methods and instrumentation were available for analysis. The study results are discussed in terms of the potential for the model compounds and other contaminants from golf courses to impact aquatic organisms in adjacent watersheds

MATERIALS AND METHODS

Monitoring

Two golf courses in the Muskoka region of central Ontario were selected for monitoring. The old course is a private course associated with a hotel and condominium complex. It is a small (8-ha), nine-hole course with clay substrate that drains directly into an oligotrophic lake. The original topsoil was not removed during course construction, which occurred approximately 20 years ago. Two drainage ponds are located on the course, with a stream flowing from outside the course through the two ponds and discharging into the lake. The fairways are mowed to within 1 m of the stream. A site on the old course that drains a watershed area of approximately 0.3 km² was monitored during the spring, summer, and fall of 2002. The new course is a large (121-ha), 18-hole golf course that was constructed in 2000 and 2001. A site on the new course that drains nine holes, comprising a watershed area of approximately 1.5 km², was sampled in the spring, summer, and fall of 2002. During construction, much of the topsoil from the new course was removed down to the bedrock, and sand was applied up to 3 m depth and shaped for course development. A reference site, which is upstream of the new course and located on the downstream margin of a wetland, was used as the reference location. The reference location drains a watershed area of approximately 2.2 km². To monitor the new golf course before it became operational, SPMDs were deployed at the new course and at the reference site during the period from June to September of 1999 (i.e., preconstruction) and during the period from May to August of 2000 (i.e., during construction).

Semipermeable membrane devices were deployed at the monitoring sites on both golf courses and at the reference site at monthly intervals from May to October of 2002. The SPMDs were prepared and deployed as described by Metcalfe et al. [11]. Briefly, SPMDs were constructed of 40-cm sections of polyethylene layflat tubing containing 1 ml of the synthetic lipid triolein. The SPMDs were transported to the sites on ice, secured in shrouds constructed of galvanized metal stovepipe, and placed into the flowing stream. The SPMDs were retrieved 28 d after deployment, immediately placed into solvent-washed amber jars, and transported on ice. In the laboratory, the jars were stored at -10°C until samples were prepared for

analysis. Three SPMDs were deployed at each site, of which one was for toxicity testing, one was for analysis of substituted chlorobenzenes, and one was for analysis of alkylphenol surfactants. At each monitoring location, a single trip blank SPMD was exposed to the air during both deployment and retrieval. The trip blank SPMD was sealed and stored at -10°C for the interval between deployment and retrieval. Contaminants of medium to low polarity (i.e., $K_{ow} > 3$) are accumulated and retained in SPMDs at concentrations that are in equilibrium with the contaminant concentrations in the surrounding water [11–13].

The SPMDs were extracted as described by Metcalfe et al. [11]. Briefly, each SPMD bag was cleaned and dialyzed into hexane for 24 h. The dialysate was passed through anhydrous sodium sulfate and evaporated to an appropriate volume, which depended on whether they were to be used for toxicity testing or analysis of model compounds. The efficiencies of extraction of the model compounds from the SPMDs were greater than 80%.

Toxicity testing

The toxicity of SPMD dialysates to early life stages of Japanese medaka was determined using a static nonrenewal assay, and toxicity was assessed by calculating a toxicity score for medaka exposed to the extracts. The toxicity scores were calculated to maximize the quantitative data generated from both the acute and subacute endpoints observed with this *in vivo* model. A similar approach to the development of a scoring system for toxicity to early life stages of medaka was used by Shi and Faustman [14]. A high toxicity score indicates that compounds in the extracts affect the survival, hatch, and development of early life stages of medaka.

The early life-stage toxicity testing was conducted essentially as described by Metcalfe et al. [11]. Different dilutions of SPMD extracts, plus a reagent blank control and a trip blank control, all at a volume of 100 µl, were placed in 2-ml glass vials, and the acetone solvent was evaporated off before adding 1 ml of embryo-rearing medium. The SPMD extracts were diluted by factors of 100, 300, and 1,000 in the toxicity test medium; these dilutions were identified in toxicity tests by the notations of 0.01, 0.003, and 0.001, respectively. Newly fertilized eggs were collected from female medaka, separated, and individually placed in exposure vials. The number of exposure replicates for each test concentration varied between 10 and 20, depending on the availability of eggs.

The medaka in exposure vials were incubated at 25°C, and the embryos were examined daily under a dissecting microscope to monitor mortality and embryo development from day 0 (i.e., fertilization) to day 17 (i.e., normal point of total yolk resorption). The developmental endpoints that were monitored included the number of days to hatch, the presence of toxicopathic lesions (e.g., hemorrhage, cardiac edema, and blood stasis), and developmental anomalies (e.g., tube heart, eye deformities, and tail deformities) among the medaka.

Toxicity was evaluated using a scoring system developed specifically for the present study. This scoring system was developed to provide quantitative information on the various subacute responses that were observed with the test model. According to the scoring system, endpoints of toxicity were divided into acute and subacute endpoints. Acute end points described different times to mortality. The subacute end points described different degrees of delayed hatch and the incidence of various toxicopathic lesions and developmental anomalies

Table 1. Toxic endpoints observed in early life stages of Japanese medaka (*Oryzias latipes*) exposed to extracts from semipermeable membrane devices deployed at golf courses in the Muskoka region of Ontario, Canada, and the severity ranks assigned to each end point to calculate toxicity scores for the effects of these extracts^a

Endpoints	Criteria	Severity rank
Acute		
Death at <5 d (before hatch)	Mortality very early in development	14
Death at >5 d (before hatch)	Mortality early in development	13
Death within 2 d of hatch	Mortality later in development, directly after hatch	12
Death within 4 d of hatch	Mortality later in development, before the end of the assay	10
Moribund after hatch, and death within 4 d of hatch	Mortality later in development before the end of the assay, with moribund sac fry before death	11
Subacute		
No hatch by end of assay	No hatch, but developing larvae live to the end of the assay; larvae will not survive further	9
Moribund after hatch, and no death	Hatch, but developing sac fry are moribund after hatch; fry live to the end of the assay, but will not survive further	8
Delayed hatch after control	Hatch, but delayed; sac fry live to the end of the assay but are unlikely to survive though swim-up	7
Pericardial edema	Developing larvae and/or sac fry have pericardial edema but live to the end of the assay; fry are unlikely to survive though swim-up	6
Eye deformity	Developing larvae and/or sac fry have eye deformity (teratogenesis) but live to the end of the assay; fry are unlikely to survive through swim-up	5
Blood stasis	Developing larvae and/or sac fry have blood stasis but live to the end of the assay; fry are unlikely to survive through swim-up	4
Tube heart	Developing larvae have tube heart but live to the end of the assay; fry may recover and survive through swim-up	3
Tail deformity	Developing larvae and/or sac fry have tail deformity (teratogenesis) but live to the end of the assay; fry are likely to survive through swim-up	2
Hemorrhage	Developing larvae and/or sac fry have hemorrhage but live to the end of the assay; fry are likely to recover and survive through swim-up	1

^a Severity ranks were assigned values between 1 and 14 using the criteria described in the table.

in the early life stages of medaka. The endpoints were ranked from 1 to 14, according to their severity (Table 1). The severity ranks were developed subjectively from a practical knowledge regarding the impact of the toxic endpoint on the development and survival of the medaka. With this scoring system, both acute and subacute end points may apply to the same fish. The formula used to calculate the toxicity score for each affected fish calculates a separate average score for five acute toxicity endpoints as the acute toxicity score and calculates a separate average score for nine subacute end points as the subacute toxicity score. The overall scores were tabulated for each fish as the sum of the acute toxicity scores (ATS) and the subacute toxicity scores (SATS). The average toxicity score per fish was calculated as the sum of the overall scores divided by the number of fish:

$$\text{average toxicity score} = \frac{\text{ATS} + \text{SATS}}{\text{number of fish}}$$

where

$$\text{ATS} = \sum \frac{\text{acute severity ranks}}{\text{number of endpoints}}$$

$$\text{SATS} = \sum \frac{\text{subacute severity ranks}}{\text{number of endpoints}}$$

Analysis of model compounds

Alkylphenols. Extracts from SPMDs were prepared for analysis of NP and OP as described by Bennett and Metcalfe [12]. Briefly, lipids and other coextractives were removed by gel

permeation chromatography (GPC) using Biobeads SX-3 (Bio-Rad, Toronto, ON, Canada). The extracts were then derivatized (i.e., acetylated) using triple-distilled acetic anhydride. After acetylation, the organic layer was passed through anhydrous sodium sulfate, and the combined extracts were evaporated to 1 ml. The combined extract was cleaned up by silica gel chromatography using aminopropyl silica from Sigma (Toronto, ON, Canada). The column was eluted with 3 ml of hexane (discarded) and then with 6 ml of 10% isopropyl alcohol in hexane. The isopropyl alcohol/hexane eluent was evaporated to 0.1 ml for analysis by gas chromatography–mass spectrometry (GC-MS).

The alkylphenols were analyzed using a Varian (Palo Alto, CA, USA) 3800 gas chromatograph coupled to a Saturn 2200 ion-trap mass selective detector (Varian) with electron-impact ionization. The GC was equipped with a Varian DB-5 column (length, 30 m; inner diameter, 0.25 mm; film thickness, 0.25 μm). Helium was used as the carrier gas. Instrument conditions were as follows: Injector temperature, 250°C; ion-trap temperature, 120°C; manifold temperature, 50°C; and transfer-line temperature, 150°C. The column oven program was an initial temperature of 70°C, hold for 1.5 min, ramp from 150°C at 10°C/min, ramp from 190°C at 2°C/min, ramp to 290°C at 25°C/min, and then a final hold for 10 min.

For quantitation of the analytes by GC-MS in selected-ion mode, single ions were monitored, and confirmation of the analytes was done by monitoring the ratios of one to three qualifier ions. For NP, the quantitation ion was m/z 191, and the qualifier ions were m/z 163, 149, and 121. For OP, the

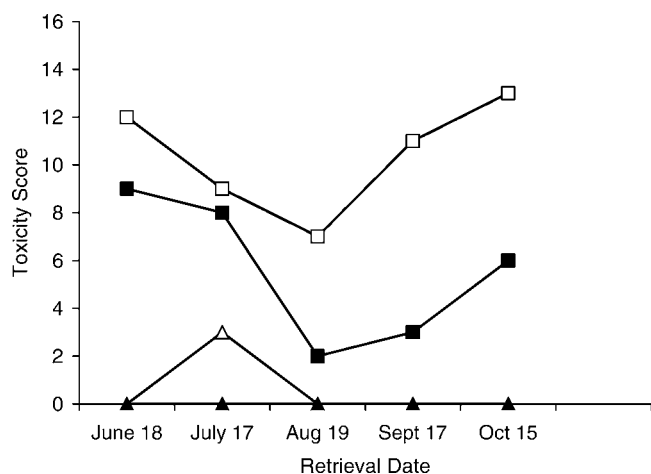


Fig. 1. Toxicity scores calculated for Japanese medaka (*Oryzias latipes*) exposed to extracts from semipermeable membrane devices deployed at the old golf course and reference site in the Muskoka region of Ontario, Canada, during the spring, summer, and fall of 2002. Data are shown for extracts with a 0.001 dilution (lowest concentration; ■ = old course; ▲ = reference site) and a 0.003 dilution (medium concentration; □ = old course; △ = reference site).

quantitation and qualifier ions were m/z 238 and 135, respectively. The analytes were quantified based on peak area responses relative to the total areas of the peaks generated in total ion chromatograms for analytical standards. Note that only one chromatographic peak occurs for OP and that NP generates multiple peaks. The calibration standard was prepared from NP and OP purchased from Sigma. The purities of OP and NP were greater than 98%. The concentrations of the analytes in the samples were calculated on the basis of the total amount in the SPMD (i.e., ng/SPMD). Limits of quantitation were 50 and 10 ng/SPMD for NP and OP, respectively.

Substituted chlorobenzenes. Extracts were analyzed for substituted chlorobenzenes, including the fungicide PCNB and its microbial metabolite, PCA, and a contaminant in the fungicide technical mixture, HCB. The extracts were prepared for GC-MS analysis as described by Metcalfe and Metcalfe [13]. Lipids and other coextractives were removed from the extracts by GPC using Biobeads SX-3. After GPC, the extracts were further cleaned up by silica column chromatography. Elution with 40 ml of hexane produced fraction A, containing the substituted chlorobenzene analytes (i.e., PCNB, PCA, and HCB). Sulfur coextractives were removed from fraction A by precipitation with prewashed copper powder. The volume of the combined extract was reduced to 0.1 ml for analysis by GC-MS.

The substituted chlorobenzene compounds, PCNB, PCA, and HCB, were analyzed with a Varian 3800 gas chromatograph coupled to a Saturn 2200 ion-trap, mass-selective detector and equipped with a Varian DB-5 column (length, 60 m; inner diameter, 0.25 mm; film thickness, 0.25 μ m). Helium was used as the carrier gas. Instrument conditions were as follows: Injector temperature, 250°C; ion-trap temperature, 150°C; manifold temperature, 50°C; and transfer-line temperature, 170°C. The column oven program was an initial temperature of 70°C, hold for 2 min, ramp to 260°C at 15°C/min, hold for 10 min, ramp to 290°C at 20°C/min, and a final hold for 10 min.

For quantitation of the analytes by GC-MS in selected ion mode, single ions were monitored, and confirmation of the analytes was done by monitoring the ratios of two to three

qualifier ions. For PCNB, the quantitation ion was m/z 265 and the qualifier ions were m/z 230 and 203. For PCA, the quantitation ion was m/z 295, and the qualifier ions were m/z 265, 237, and 214. For HCB, the quantitation ion was m/z 284, and the qualifier ions were m/z 249 and 214. The analytes were quantified against a standard prepared in the laboratory from material purchased from Sigma. The purities of PCNB, PCA, and HCB were greater than 98%. The concentrations of the analytes in the samples were calculated on the basis of the total amount in the SPMD (i.e., ng/SPMD). The limits of quantitation for the analytes were in the range of 0.5 to 1.5 ng/SPMD.

Subsequent to these analyses for target chlorinated benzenes (i.e., HCB, PCA, and PCNB), a fraction containing relatively high concentrations of these analytes was analyzed for unknown chlorobenzene compounds by full-scan GC-MS. Analyses were conducted with the Varian 3800 gas chromatograph coupled to a Saturn 2200 ion-trap mass spectrometer under the operating conditions described above except that the instrument was operated in full-scan mode over the mass range of m/z 70 to 300. Major peaks detected in the total ion chromatograms were putatively identified using the National Institute of Standards mass spectral library available on the Varian ChemStation® software.

Statistical analysis

Data regarding the toxicity scores for extracts at the 1:300 dilution (i.e., 0.003) prepared from SPMDs retrieved over five dates in 2002 were compiled for each of the monitoring sites (i.e., old course, new course, and reference). The data were tested for differences in toxicity scores between the sites over the total monitoring period ($n = 5$) using the Kruskal–Wallis nonparametric analysis of variance (ANOVA) at a level of significance of $\alpha \leq 0.05$. Similarly, analytical data for extracts from SPMDs retrieved from each site over the monitoring period ($n = 5$) were analyzed for differences using the Kruskal–Wallis nonparametric ANOVA. The ANOVA tests were performed using SPSS® 9.0 for Windows (SPSS, Inc., Chicago, IL, USA). Linear-regression analysis to determine relationships between the toxicity scores (i.e., 0.003 dilution) and the analytical data for extracts from SPMDs deployed at the golf courses were conducted using Excel® software (Microsoft, Redmond, WA, USA).

RESULTS

Trends in the toxicity of SPMD extracts were monitored during 2002 at the old and new golf courses and at the reference site. Extracts from SPMDs were diluted by 100-, 300-, and 1,000-fold in the toxicity test medium (i.e., 0.01, 0.003, and 0.001, respectively) so that subacute effects on the early life stages could be observed throughout the assay. Some toxicity was detected in undiluted extracts that were prepared from trip blank SPMDs (up to 10-fold dilution), which probably can be attributed to the presence of coextractives, such as a toxic impurity of triolein (i.e., oleic acid) that has been identified in the SPMD dialysate [15].

At the old golf course, a seasonal trend in toxicity was observed for 2002. High toxicity scores were observed in medaka exposed to extracts prepared from SPMDs retrieved in the spring and early summer (June and July) and in the fall (September and October), with toxicity declining in the extracts from SPMDs retrieved in August (Fig. 1). Note that toxicity score data are shown for only the tests at the lowest

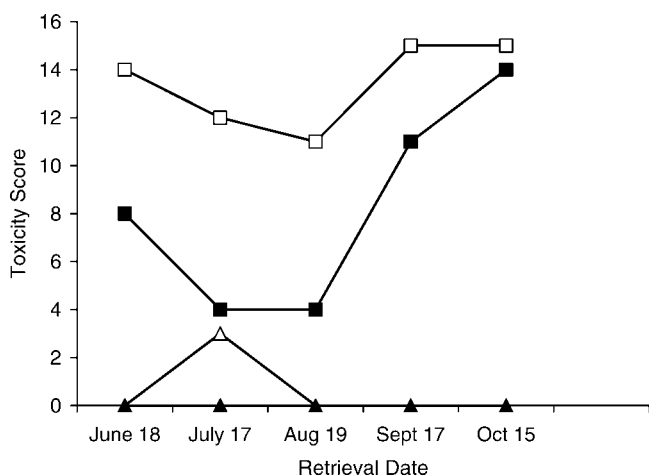


Fig. 2. Toxicity scores calculated for Japanese medaka (*Oryzias latipes*) exposed to extracts from semipermeable membrane devices deployed at the new golf course and the reference site in the Muskoka region of Ontario, Canada, during the spring, summer, and fall of 2002. Data are shown for extracts with a 0.001 dilution (lowest concentration; ■ = new course; ▲ = reference site) and a 0.003 dilution (medium concentration; □ = new course; △ = reference site).

dilution (i.e., 0.001) and the medium dilution (i.e., 0.003), because the highest dilutions tended to give toxicity scores of greater than 18 for all extracts tested. Minimal background toxicity was observed for the SPMDs deployed at the reference site (Fig. 1). At the new golf course, a seasonal trend of high toxicity in the SPMDs retrieved in June and in September of 2002 also was observed (Fig. 2). This golf course opened in the fall of 2001. In previous years, SPMDs also were deployed at this site from June to September in 1999 (preconstruction) and from May to August in 2000 (during construction). In extracts prepared from the SPMDs deployed in these previous years, no toxicity was associated with the extracts at the dilutions tested. As shown in Figure 2, toxicity was observed in the extracts prepared from the SPMD retrieved in 2002, after the golf course had become operational. No toxicity was observed for the SPMDs retrieved from the reference site in 1999, 2000, and 2002. The medaka used for toxicity testing in all three of these years originated from the same broodstock, so it is unlikely that these results were caused by variability in the sensitivity of the test organism.

The substituted chlorobenzenes, PCNB, HCB, and PCA, were detected at the monitoring sites on the old and new golf courses in extracts prepared from SPMDs deployed during the spring and fall of 2002 (Table 2). For PCNB, the highest concentration was 334 ng/SPMD, which was detected in the SPMD retrieved on June 18, 2002, at the old golf course. In comparison to the SPMDs retrieved from the old golf course in the spring, lower concentrations of PCNB were detected in the SPMDs retrieved from the monitoring site at the new golf course in June 2002 (Table 2). Comparable concentrations of PCNB, however, were detected in SPMDs deployed at both the old and new golf courses in September and October of 2002. Pentachloroaniline was detected at concentrations of less than 30 ng/SPMD (Table 2), which is consistent with this compound being present as a degradation product of PCNB.

In the SPMDs deployed at the old and new golf courses, HCB often was detected at concentrations that were comparable to or exceeded the concentrations of PCNB (Table 2). Although HCB is a contaminant of the quintozone formulation,

Table 2. Amounts of hexachlorobenzene (HCB), pentachloroaniline (PCA), and pentachloronitrobenzene (PCNB) detected in semipermeable membrane devices (SPMDs) retrieved in the Muskoka region of Ontario, Canada^a

SPMD retrieval date	Analyte	Old golf course (ng/SPMD)	New golf course (ng/SPMD)	Reference site (ng/SPMD)
June 18, 2002	HCB	22.3	0.6	ND
	PCA	28.7	2.3	ND
	PCNB	334.0	17.2	ND
July 17, 2002	HCB	8.4	ND	ND
	PCA	ND	ND	ND
	PCNB	ND	ND	ND
August 19, 2002	HCB	1.5	0.8	ND
	PCA	0.7	ND	ND
	PCNB	2.7	2.7	ND
September 17, 2002	HCB	12.7	9.7	P
	PCA	2.3	ND	ND
	PCNB	12.6	12.8	ND
October 16, 2002	HCB	51.0	9.1	P
	PCA	5.1	ND	ND
	PCNB	25.6	21.4	ND

^a Data are presented for analysis by gas chromatography–mass spectrometry. ND = not detected; P = present but below the limits of quantitation.

it also may be present in SPMD samples because it is a contaminant of other pesticide formulations. None of the analytes was detected at the reference site except for trace amounts of HCB (i.e., present but below the limits of quantitation), which probably were present as a result of atmospheric inputs.

In both of the samples from the old and new golf courses, but not in the sample from the reference site, a large peak was identified at a chromatographic retention time of 18.3 min. According to the National Institute of Standards mass spectral library, the full-scan mass spectrum for this compound corresponded with high probability (>90%) to pentachloroaniline. Quantitative analysis of this compound was not possible, because it was not available commercially in purified form.

For the SPMDs deployed in 2002, OP was not detected in any of the extracts; however, NP was clearly detected in extracts from SPMDs deployed at the old and new golf courses. As shown in Table 3, NP was detected in microgram amounts in the SPMDs that were retrieved from both golf courses in June, July, and October of 2002. No NP was detected in any of the SPMDs deployed at the reference site or in the SPMDs deployed at the old and new golf courses in August and September of 2002 (Table 3).

Analysis of variance of combined data ($n = 5$ per site) for each site monitored in 2002 indicated that the toxicity of the SPMD extracts (i.e., toxicity scores at 0.003 dilution) varied

Table 3. Amounts of nonylphenol detected in semipermeable membrane devices (SPMDs) retrieved in the Muskoka region of Ontario, Canada^a

SPMD retrieval date	Old golf course ($\mu\text{g}/\text{SPMD}$)	New golf course ($\mu\text{g}/\text{SPMD}$)	Reference site ($\mu\text{g}/\text{SPMD}$)
June 18, 2002	16.41	1.3	ND
July 17, 2002	12.64	1.2	ND
August 19, 2002	2.59	ND	ND
September 17, 2002	ND	ND	ND
October 16, 2002	16.24	19.83	ND

^a ND = not detected.

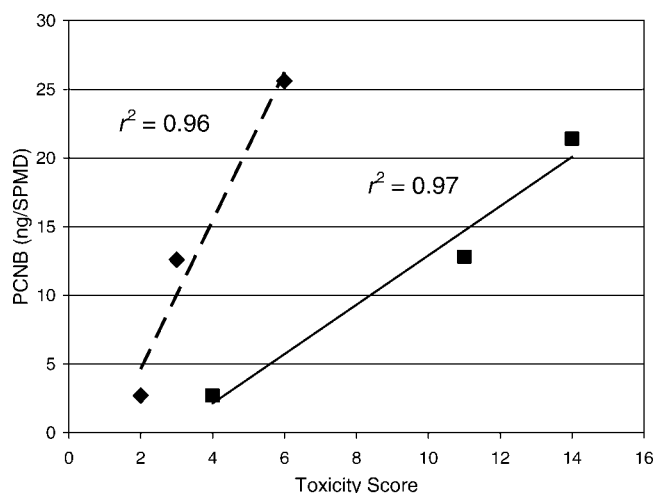


Fig. 3. Relationships between toxicity scores calculated for Japanese medaka (*Oryzias latipes*) exposed to extracts with a 0.003 dilution and the amounts of pentachloronitrobenzene (PCNB) accumulated in the extracts prepared from semipermeable membrane devices (SPMDs) retrieved in August, September, and October of 2002 from the old golf course (◆) and new golf course (■) in the Muskoka region of Ontario, Canada. Data for extracts prepared from SPMDs retrieved in June and July of 2002 were not included in this analysis.

significantly ($\alpha = 0.05$) between the old and new golf courses and the reference site. Similarly, the concentrations of NP in extracts prepared from SPMDs deployed over 2002 ($n = 5$ per site) were significantly different between the monitoring sites. No significant differences were found between the concentrations of PCNB in extracts from the SPMDs deployed at the old and new golf courses, but PCNB concentrations in SPMD extracts from both golf course sites were significantly greater than PCNB concentrations in SPMDs deployed at the reference site. Linear-regression analysis showed no relationships between the toxicity of the SPMD extracts (i.e., toxicity scores at 0.003 dilution) and the concentrations of any of the analytes except for the concentrations of PCNB in extracts from the SPMDs retrieved from the old and new golf courses in August, September, and October of 2002 (Fig. 3).

DISCUSSION

In the present study, temporal trends were detected for toxicity and contaminant loads in SPMDs deployed at two golf courses located on the Precambrian Shield in the district of Muskoka in Ontario. At the old golf course, toxicity was evident in extracts from SPMDs retrieved in both the spring (i.e., June) and the fall (i.e., September and October). A similar trend was observed for the toxicity of extracts prepared from SPMDs deployed at the new course. In all cases, toxicity was lowest in extracts prepared from SPMDs retrieved in late July and August. The trends in toxicity are consistent with the timing of applications of fungicides. Large amounts of fungicides were applied to the golf courses during late fall and early spring, which coincides with the peaks in toxicity in SPMD extracts. In contrast, herbicides and smaller amounts of insecticides were applied primarily to the golf courses for turf maintenance during the summer months. It also is possible that the appearance of toxicity in SPMD extracts was related to runoff events in the streams where the SPMDs were deployed. Unfortunately, the flows in the streams were not monitored over the study period. Gauged hydrograph data for the Magnetawan River in Ontario, which drains a watershed ad-

acent to the two golf courses, showed that in 2002, natural flows spiked to velocities as high as 37 cm/s in the spring (i.e., April 9–23) and 11 cm/s in the early summer (i.e., June 16–22) and remained at basal flows (i.e., <5 cm/s) for the remainder of the year. These natural flow patterns, however, may not reflect runoff into the golf course streams, because heavy irrigation of the courses occurs during the summer and fall.

Fungicides, including PCNB, are applied in the fall to combat the snow mold that develops from infestations of turf by *Typhula* sp. fungus in northern locations of the United States and throughout Canada [16]. Golf course superintendents attempt to make the last application of fungicide immediately before the permanent winter snow cover in the fall (i.e., October and November) and again in the early spring. Quintozene, which is a technical product containing the active ingredient PCNB, is an industry standard for snow mold control throughout North America [17]. According to club records, applications of quintozene in November 2002 amounted to 225 kg at the old course and 2,010 kg at the new course. The previous November (i.e., 2001), applications of quintozene were 315 and 3,150 kg at the old and new courses, respectively. Vincelli [18] conducted simulations of fungicide runoff following applications on turfgrass and predicted that the concentrations of fungicides in runoff, including PCNB, iprodione, chlorothalonil, and azoxystrobin, would exceed lethal concentrations for aquatic organisms under some conditions. Analysis of leachate from a golf course in the State of Washington, USA, showed no detectable levels of PCNB, but the microbial degradation product, PCA, was detected at low parts-per-billion concentrations in all monthly leachate samples [19]. Hexachlorobenzene has been identified as a contaminant in the quintozene technical product [20]. The pentachlorothioanisole identified in the extract from SPMDs deployed in the spring of 2002 at both the old and new golf courses was a major constituent of the sample, probably exceeding the concentration of PCNB by an order of magnitude. Pentachlorothioanisole has been identified previously as a microbial degradation product of PCNB under anaerobic conditions in water [21]. Our previous, unpublished studies showed that SPMDs efficiently accumulate chlorobenzene compounds from water at sampling rates between 4 and 6 L/d and that these compounds are retained in the SPMDs over the deployment period. To our knowledge, no data have been published regarding the toxicity of pentachlorothioanisole, so it is not possible to assess the contribution of this compound to the toxicity of the extract to early life stages of medaka.

We previously showed that a quintozene formulation containing PCNB was acutely toxic to early life stages of medaka, with a nominal median lethal concentration for the test population of 707 ng/ml and a nominal median effective concentration for reduction in hatch of 71 ng/ml [22]. These toxic thresholds are higher than the total amounts of PCNB in the extracts from SPMDs deployed in 2002 (Table 2). Despite the linear relationships noted between toxicity and PCNB concentrations in the SPMDs retrieved between August and October of 2002 (Fig. 3), it is unlikely that PCNB was solely responsible for the toxicity of the SPMD extracts to medaka. Other compounds that are used for turf treatment probably accumulated in the SPMDs and contributed to the observed toxicity. For instance, the fungicide chloroneb was applied in March 2002 at amounts of 60 and 120 kg at the old and new golf courses, respectively. In October 2002, the fungicide ipro-

dione was applied in amounts of 105 and 280 kg to the old and new golf courses, respectively.

The results of analyses for alkylphenols accumulated in SPMDs indicated that NP was present at microgram levels in extracts from SPMDs retrieved at the two courses in June, July, and October. Our previous studies with SPMDs deployed in the Great Lakes showed that these alkylphenol compounds are accumulated efficiently from water and are retained in the passive samplers over the deployment period [20]. No data were provided by the golf courses regarding the application of alkylphenol surfactants, although information was provided that a surfactant called Primer[®] was spot-applied throughout the summer of 2002 on the golf greens to aid the infiltration of water. No information is available regarding what type of surfactant is present in this formulation. The pattern of occurrence of NP in the SPMDs coincides with the periods of heavy pesticide applications on golf courses during the spring and fall. It is difficult, however, to establish which pesticides applied to the golf courses contained NP, because the composition of the pesticide formulations is proprietary. In toxicity tests conducted with early life stages of medaka, we determined that NP caused lethality at a nominal median lethal concentration for the test population of 852 ng/ml and delayed hatch at a nominal median effective concentration of 712 ng/ml. The SPMDs deployed at the old and new golf courses in 2002 accumulated up to 20.9 µg of NP (Table 3). At the 1,000-fold dilutions of the SPMD extracts used in the toxicity tests, however, this would be equivalent to a concentration of 20.9 ng/ml in the test medium. Therefore, the NP accumulated in the SPMDs should not have been solely responsible for the acute toxicity to medaka observed at the dilutions used in the test protocol but probably contributed to the observed toxicity.

Overall, no single compound or class of compounds in the SPMD extracts probably is wholly responsible for the observed toxicity of extracts to early life stages of medaka. A range of herbicides, insecticides, and fungicides likely accumulated in the SPMDs. All these compounds, not just the model compounds analyzed in the extracts, likely contributed to the observed toxicity. Elevated toxicity, however, was observed in tests with extracts prepared from the SPMDs that were deployed during periods of maximum fungicide application (i.e., in the spring and fall).

The present study indicates that the compounds accumulated in passive sampling devices deployed near golf courses are toxic to early life stages of a model fish species (i.e. Japanese medaka). The toxicity of the SPMD extracts was correlated with the presence of two classes of model contaminants in the extracts. It cannot be stated definitively, however, that the contaminants discharged into streams from golf courses are a toxic hazard to fish and other aquatic organisms. Factors such as stream flow and exposure duration will vary the exposure conditions, and aquatic organisms will vary in sensitivity to the contaminants that originate from golf courses. In situ studies are needed to evaluate whether contaminants discharged from golf courses on the Precambrian Shield are affecting aquatic organisms.

Several beneficial management practices can be employed to decrease the potential for movement of pesticides from golf courses into the surrounding aquatic environment [23–27]. The key to reducing the impacts of pesticides applied to golf courses on watersheds, however, is restraint in the use of pesticides. Much of the impetus for using large amounts of pesticides relates to cosmetic standards for golf courses [28]. Golfers

expect to play on aesthetically pleasing golf courses, and they demand trimmed fairways and fast putting surfaces, especially when they are paying high membership fees for private courses. This means that grass is cultured in a more stressful environment and is less tolerant of disease, heat, cold, drought, and excess moisture. These conditions drive golf course superintendents to use large amounts of pesticides to maintain courses. Perhaps education of golfers to lower their cosmetic standards would be the best management strategy.

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