Influence of Suspended Solids on Acute Toxicity of Carbofuran to Daphnia Magna: II. An Evaluation of Potential Interactive Mechanisms

Steven P. Bradbury
Carl Herbrandson, *University of Minnesota - Twin Cities*
Deborah L. Swackhamer

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Influence of suspended solids on acute toxicity of carbofuran to *Daphnia magna*: II. An evaluation of potential interactive mechanisms

Carl Herbrandson[a,1], Steven P. Bradbury[b], Deborah L. Swackhamer[c,*]

[a] *National Biological Service, Minnesota Cooperative Fish and Wildlife Research Unit, 200 Hodson Hall, University of Minnesota, St. Paul, MN 55108, USA*

[b] *USEPA, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, 6201 Congdon Boulevard, Duluth, MN 55804, USA*

[c] *Division of Environmental and Occupational Health, School of Public Health, University of Minnesota, Mayo Mail Code 807, Minneapolis, MN 55455, USA*

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Abstract

It has been demonstrated that simultaneous exposure of *Daphnia magna* to suspended solids and a carbamate pesticide potentiates the toxic response to the pesticide. The toxicodynamics between these stressors were investigated to determine possible mechanisms of interaction. Three experimental series were conducted with *D. magna* to determine: the effect of food availability on carbofuran toxicity; the effect of food availability on jointly administered carbofuran and suspended solids; and changes in the magnitude of effects which can occur with suspended solids of different composition. These experiments demonstrated that both carbofuran toxicity and the joint toxicity of carbofuran and suspended solids to *D. magna* can be modulated by food availability. While it is clear that food dilution could contribute to energy stress, it appears likely that additional interactive processes also contribute to the observed synergism between carbofuran and suspended solids. Additionally, decomposed peat was shown to be less of a stressor to these pelagic invertebrates than inorganic subsoil.

Keywords: Multiple stressors; Synergism; Carbofuran; Suspended solids; *Daphnia magna*; Food stress

1. Introduction

Laboratory toxicity studies generally focus on the effects of a single toxicant on an organism. In natural aquatic systems, organisms are exposed to combinations of chemicals and other potential stressors. The effects of stressors can combine in an additive, a less than additive (antagonistic), or a

* Corresponding author. Tel.: +1-612-626-0435; fax: +1-612-626-4837.

E-mail address: dswack@umn.edu (D.L. Swackhamer).

[1] Present address: Minnesota Department of Health, Division of Environmental Health, 121 E. 7th Place, St. Paul, MN 55101, USA.

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greater than additive (synergistic) manner. Therefore, an understanding of the interactions between stressors is necessary to determine the proper application of laboratory studies to the natural environment.

It has been shown in the accompanying paper that suspended solids increase the toxic response of 72–96-h-old *D. magna* to carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl *N*-methylcarbamate) in a synergist manner in 48 h exposures (Herbrandson et al., 2003). *D. magna* exposed to freely-dissolved carbofuran over a 48-h period without suspended solids resulted in an EC_{50} of 92 μg/l, and a lowest observable effect concentration (LOEC) of 75 μg/l. Suspended solids alone showed no observable effect on test organisms at 10 000 mg/l suspended solids. However, in experiments with *D. magna* exposed to carbofuran, the suspended solids LOEC ranged from < 100 to 10 000 mg/l. Analysis of the results showed that at any freely-dissolved concentration of carbofuran greater than 9.5 μg/l, there is a positive relationship between suspended solids concentration and *D. magna* response. Conversely, for all suspended solids concentrations tested, there is a positive correlation between freely-dissolved carbofuran concentration and *D. magna* response.

While it has been shown that suspended solids potentiates carbofuran toxicity to *D. magna*, the mechanism of that interaction is not clear. Carbofuran is a non-ionic organic insecticide and nematicide with a reported log K_{ow} between 1.63 and 2.32 (Trotter et al., 1991; Howard, 1991) and a K_{oc} of 21.9–160 l/kg (Sukop and Cogger, 1992; Wauchope et al., 1992; Dowling et al., 1994; Howard, 1991). As a consequence, its sorption to particles is very limited (Sharom et al., 1980; Achik et al., 1991a,b), and therefore the bioavailability of carbofuran and its uptake toxicokinetics in *D. magna* is not expected to change significantly when the organism is simultaneously exposed to suspended solids.

Carbofuran is an acetylcholinesterase (AChE) inhibitor and will cause repetitive activity in acetylcholine-based synapses in the central nervous system of invertebrates (Eldefrawi, 1985). Following exposure to an AChE inhibitor (dichlorvos), blood glucose levels in a freshwater prawn (*Macrobrachium lamarrei*) are raised suggesting the mobilization of energy stores (Omkar and Shukla, 1985). And while tissue levels of glucose in exposed freshwater snails are decreased on exposure to methyl parathion, quinalphos, and nuvan (Rambabu and Rao, 1994), glucose metabolism in the nervous system of rats can be increased upon administration of soman (Samson et al., 1984). Since exposure of animals to AChE inhibitors appears to affect an organism's energy budget, it seems reasonable to propose that toxic effects elicited by an inhibitor could be affected by energy availability or the energy stores within an organism. This, in turn, may affect an organism's ability to respond to additional stresses such as exposure to suspended solids.

Suspended solids composition could also affect energy stores through additional mechanisms. Since *D. magna* are non-selective filter feeders (Lampert, 1974; DeMott, 1982; Kerfoot and Kirk, 1991), dilution of food by suspended solids could result in decreased caloric intake. It has also been proposed that the added weight of ingested solids will cause daphnids to sink, requiring a significant increase in energy expenditure to maintain proper buoyancy (Zurek, 1983). If net energy balance affects the toxicodynamics of carbofuran or suspended solids in mixtures, increased energy expenditure will increase toxicity to *D. magna*.

The intent of this series of experiments was to elucidate possible mechanisms whereby suspended solids might potentiate the response of *D. magna* to carbofuran. Initially, the effect of food on *D. magna* response to carbofuran and suspended solids was investigated. In additional experiments, *D. magna* were exposed to suspended solids of varying density to modify buoyancy and energy expenditure necessary to maintain equilibrium in the water column.

2. Materials and methods

All experiments were conducted in the Suspended Solids Testing Apparatus (SSTA), which is described in Herbrandson et al. (1999). The SSTA contains 10 rotating 1-l chambers separated from the main tank by 0.1 μm pore size nylon membranes (MSI, Westboro, MA). The SSTA allows the testing of 10 different suspended solids concentrations while maintaining equal concentrations of freely-dissolved chemical in all chambers (Herbrandson et al., 1999). The suspended solids used in the experiments were prepared as described in Herbrandson et al. (1999) and Herbrandson et al. (2003). Decomposed peat (34.9% organic carbon; density of 1.5 kg/l) and subsoil (0.46% organic carbon; density of 2.1 kg/l) were collected in August 1993. Both soils were dried in a forced draft Leekow furnace at 35 °C. The decomposed peat was ground in a Wiley mill and filtered through a 250 μm stainless steel sieve. The subsoil was ground in a Wisconsin mill and sieved to 43 μm. The soils were stored in sealed containers at 4 °C.

Each experiment described in this report contains mixtures of the two component soils, unless otherwise noted. Thoroughly homogenized mixtures were prepared for each chamber in each experiment. The composition of suspended solids used in each experiment is noted below and in Table 1. The resulting mixtures were representative of suspended solids having organic carbon content ranging from 0.5 to 20%.

Technical grade carbofuran (99.2%) was provided by FMC (Philadelphia, PA). Experimental protocols were similar to those detailed in Herbrandson et al. (2003). Carbofuran exposure concentrations, noted in all figures, tables and text, were determined from triplicate enzyme linked immunosorbant assay (ELISA; RaPID Assay, Ohmicron, Newton, PA). Measured concentrations of freely-dissolved carbofuran in the tank have been shown to provide reasonable estimates of freely-dissolved carbofuran concentrations in chambers with and without suspended solids (Herbrandson et al., 1999). All reported carbofuran concentrations are measured values, with the exception of experiment 1 which are nominal values. ELISA analysis of 6 beakers from experiment 1 showed actual concentrations averaged 108% of the target concentrations (10% CV).

D. magna used in bioassays were 72–96 h old and from 17–22 day-old parentage. *D. magna* in culture tanks were fed 4.8 mg/l day. In standard experiments, *D. magna* were fed 2.5 mg/l dry wt. 75:25 yeast-alfalfa-trout chow:Tetra Conditioning Food (YAT:Tetra; Tetra Conditioning Food was obtained from TetraWerke; Melle, Germany) once at the beginning of each 48 h exposure period. The consistency of *D. magna* cultures was monitored with weekly reference toxicity tests to carbofuran (Herbrandson, 1996).

Statistical analyses on data, including maximum-likelihood probit estimations of concentration–response curves and confidence limits, were calculated using ToxCalc 5.0 (Tidepool Scientific Software, McKinleyville, CA).

3. Experimental design

Experiments 1, 2 and 3 were designed to assess the effect of hunger-induced stress on carbofuran toxicity (Experiment 1); the effect of hunger-induced stress on combined carbofuran and suspended solids toxicity (Experiment 2); and the effect of different soil components on carbofuran-stressed *D. magna* (Experiment 3). Basic protocols used in the experiments described below have been outlined previously (Herbrandson et al., 1999, 2003). Table 1 summarizes the variables for all experiments described in this paper. All experiments were performed in the SSTA, except Experiment 1 was performed in beakers under conditions similar to those in reference toxicity tests (Herbrandson, 1996).

3.1. Food availability and carbofuran toxicity

Experiment 1 was designed to test the hypothesis that increasing food availability would decrease the toxicity of carbofuran. The toxicity of carbofuran was determined at food concentrations of 2.5, 12.5, and 25 mg/l (Experiment 1). For each food concentration, carbofuran dose–response curves were determined for 0, 20, 40, 80, and 160 μg/l carbofuran. *D. magna* were exposed statically to carbofuran in 250 ml beakers (9–15 organisms per beaker). Four replicates were used for each food treatment. The range of carbofuran treatments in Experiment 1 was broad, so as to elicit no effects as well as complete mortality.

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Table 1
Experimental conditions to assess interactive effects of carbofuran and suspended solids

Experiment	Carbofuran (μg/l)	Organic carbon (%)	Subsoil (mg/l)	Decomposed peat (mg/l)	Total suspended solids (mg/l)	Food (mg/l)	Carbofuran EC_{50} (μg/l)	95% C. I.
1	0–160	N/A	N/A	N/A	N/A	2.5	63	57–69
1	0–160	N/A	N/A	N/A	N/A	12.5	97	–*
1	0–160	N/A	N/A	N/A	N/A	25	109	102–117
							Suspended solids EC_{50} (mg/l)	95% C. I.
2A	80	3	0–9264	0–739	0–10 000	25	1030	810–1290
2B	9.4	3	0–9264	0–739	0–10 000	0	6620	5240–8000
2C	65	0.5 and 3	999 and 926	1 and 74	1000	0, 2.5 and 10	N/A	–
3A	47	0.5–20	999–431	1–569	1000	2.5	N/A	–
3B-1	44	35	0	0–739	0–739	0	N/A	–
3B-2	102	35	0	1–1308	1.3–1308	25	430	356–508
3C-1	51, 60	0.5–20	999	1–1308	1000–2307	2.5	N/A	–
3C-2	63	0.5–20	999	1–1308	1000–2307	25	N/A	–

N/A indicates not applicable.

* Confidence limits could not be calculated for this experiment using probit analysis because there was no mortality in the controls. A trimmed Spearman–Karber analysis of this experiment yielded an EC_{50} of 93 with a 95% confidence interval of 78–109.

3.2. Food availability and combined carbofuran and suspended solids toxicity

Experiments 2A, 2B and 2C assessed the effect of hunger-induced stress on the combined toxicity of carbofuran and suspended solids. These experiments were designed to test the hypothesis that food availability significantly decreases the combined toxicity of carbofuran and suspended solids in *D. magna*. Relative effects were explored at carbofuran and suspended solid mixtures previously demonstrated to affect about 50% of test organisms (Herbrandson et al., 2003). Using these exposure levels (i.e. in the linear response range), the sensitivity of the bioassay to different food treatments was maximized.

3.3. Soil components and carbofuran toxicity

The third experimental series assessed whether inorganic or organic suspended solids contributed more to the combined toxicity of carbofuran and solids. Three experiments were performed to test this hypothesis. Experiment 3A was a variation of the standard SSTA protocol. For this experiment the effects of equivalent concentrations of suspended solids (1000 mg/l) with different fractions of organic carbon (f_{oc}) (0.5, 3, 5, 10 and 20%), based on varying ratios of decomposed peat and subsoil, were tested in replicate chambers (Table 1). Relative responses were evaluated with respect to different treatments of decomposed peat ratios.

Experiment 3B-1 was designed to investigate the value of decomposed peat as food for *D. magna*. This experiment was conducted without subsoil and food, but with decomposed peat concentrations varied over the same range as standard SSTA experiments (i.e. 0–739 mg/l suspended solids; Table 1). Since this experiment was designed to determine whether decomposed peat could have a net positive effect on *D. magna* survival, a target carbofuran concentration of 40 μg/l was chosen based on previous results. This carbofuran concentration was expected to affect about 50% of unfed *D. magna* exposed to 0 mg/l suspended solids.

Experiment 3B-2 was designed to assess the effect of decomposed peat (five concentrations from 1.3 to 1308 mg/l) on carbofuran-stressed *D. magna* under high food conditions (25 mg/l) without subsoil present. A carbofuran concentration of 80 μg/l combined with the range of decomposed peat treatments was expected to yield a concentration–response curve of 0–100% affected *D. magna*.

Experiments 3C-1 and 3C-2 were designed to determine if decomposed peat, in the presence of subsoil and carbofuran, would have a positive or negative effect on *D. magna*. Experimental conditions resembled those outlined for 3A, except decomposed peat was varied while maintaining a constant concentration of subsoil. In 3C-2, 25 mg/l food was added, while in the 3C-1 experiments a standard food concentration of 2.5 mg/l was employed. Therefore, while 3A had different amounts of subsoil and decomposed peat totaling the same concentration of suspended solids (1000 mg/l) in each chamber, 3C-1 and 3C-2 were conducted with the same concentration of subsoil (999 mg/l) and different decomposed peat and total suspended solids concentrations. Nominal carbofuran concentrations for 3C-1 and 3C-2 of 46 and 63 μg/l, respectively, were intended to affect 50% of test organisms.

4. Results and discussion

Combinations of stressors may have effects that are considerably different from those of the individual stressors alone (Broderius, 1991). Organisms in an aquatic environment are exposed to a variety of chemical and environmental stressor combinations. Hanazato and Dodson (1995) have shown a synergistic relationship, in *Daphnia pulex*, between carbaryl (1-napthyl methylcarbamate), oxygen stress, and predator kairomones. Other studies have reported relationships between food and chemical toxicant effects in *D. magna* (Kluttgen and Ratte, 1994; Enserink et al., 1995; Adema, 1973) and that pre-exposure of *D. magna* to heat can decrease the toxicity of malathion (*O*,*O*-dimethyl-*S*-1,2-di(carboethoxy) ethyl phosphorodithioate) to *D. magna* (Bond and Bradley, 1995).

Herbrandson et al. (2003) demonstrated that carbofuran toxicity to *D. magna* was potentiated by suspended solids in non-additive manner. Energy utilization, food availability, and suspended solids composition may be important factors in the toxicodynamic interaction of these stressors. The experimental series described here test hypotheses that the increased energy needs of exposed *D. magna* elicit a synergistic interaction between carbofuran and suspended solids. Parameters from these experiments are detailed in Table 1.

4.1. Experimental series 1: food availability significantly decreases carbofuran toxicity

Results of Experiment 1 are shown in Fig. 1. As food availability increased, the carbofuran concentration–response curve shifted to the right, signifying less *D. magna* sensitivity to carbofuran. The resulting 48 h EC_{50}s (Table 1) were 63, 97, and 109 μg/l carbofuran for 72–96-h-old *D. magna* with 2.5, 12.5, and 25 mg/l food, respectively. The results clearly demonstrate a food dependent increase in *D. magna* tolerance to carbofuran intoxication.

Food additions also affected the toxicity of carbofuran in reference toxicity tests (Section 2). Weekly reference toxicity tests were performed with test organisms from different broods and tanks that were raised under similar conditions. *D. magna* in half of these reference toxicity tests were unfed, while *D. magna* in the other half were provided 2.5 mg/l food. The mean EC_{50} for five reference toxicity tests with 0 mg/l food was 59 μg/l (±4.3, standard deviation), while the mean EC_{50} for the five reference toxicity tests with feeding was 80 μg/l (±12.8).

When the crustacean freshwater prawn *M. Iamarrei* was exposed to the organophosphate dichlorvos (2,2-dichlorovinyl dimethyl phosphate), hepatic glycogen concentrations decreased, while blood glucose increased (Omkar and Shukla, 1985). Omkar and Shukla suggested that hyperglycemia might be in response to increased energy needs of the brain. Studies have shown soman (methylphosphono fluoridic acid 1,2,2-trimethylpropyl ester) to increase glucose usage in the brain of rats (Samson et al., 1984). If glucose is reduced in the central nervous system of invertebrates by carbofuran, the organism's behavior may change,

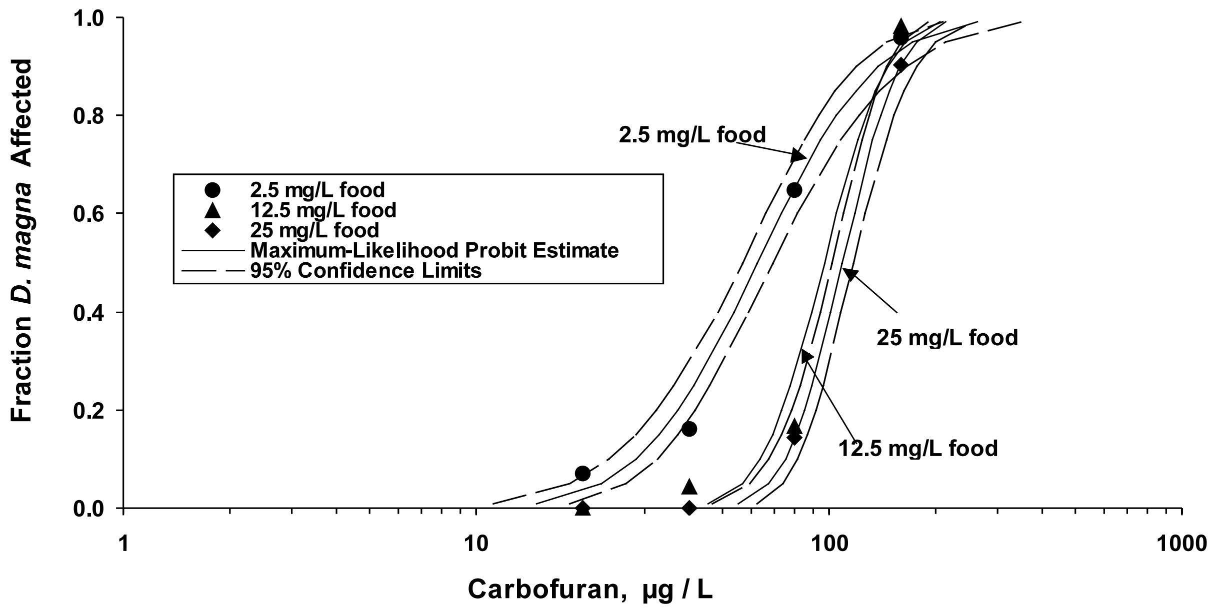

Fig. 1. Carbofuran dose–response relationships for *D. magna* provided 2.5, 12.5, or 25 mg/l food (Experiment 1; see Table 1). Data from duplicate exposures were combined for Maximum-likelihood Probit analysis. Confidence limits could not be established for the 12.5 mg/l treatment group.

affecting its survival or post-test toxicity rating. Increased food availability could increase glucose stores and enhance an organism's tolerance to AChE inhibitors.

4.2. Experimental series 2: food availability significantly reduces combined carbofuran and suspended solids toxicity

The second hypothesis proposed that food stress significantly increases the combined toxicity of carbofuran and suspended solids to *D. magna*. Experimental conditions and results from all experiments are summarized in Table 1 and plotted in Fig. 2. These experiments provide a comparison of results with those from previous SSTA experiments (Herbrandson et al., 1999, 2003).

Test organisms in experiment 2A were provided 25 mg/l food and exposed to 80 μg/l carbofuran (Fig. 2). The calculated EC_{50} from experiment 2A was 1030 (95% confidence limits of 810–1290) mg/l suspended solids (Table 1). *D. magna* provided 2.5 mg/l food and exposed to 70 and 75 μg/l carbofuran, resulted in EC_{50}s of 264 (216–323) and 181 (134–238) mg/l suspended solids, respectively (Herbrandson et al., 2003). Thus, test organisms provided 25 mg/l food (experiment 2A) appeared to have an approximately 5-fold greater tolerance for suspended solids than *D. magna* provided 2.5 mg/l food under similar test conditions.

Data from previous experiments with *D. magna* provided 2.5 mg/l food suggested that suspended solids EC_{50}s were greater than 10 000 mg/l for carbofuran concentrations of 9.5 and 20 μg/l (Herbrandson et al., 2003). The calculated suspended solids EC_{50} from experiment 2B, conducted at 9.4 mg/l carbofuran without food, was 6600 (5200–8000) mg/l of suspended solids. This EC_{50} was considerably less than has been shown with fed organisms and indicate that unfed *D. magna* were less tolerant of suspended solids at equivalent concentrations of carbofuran (Fig. 2).

Experiment 2C was designed to assess the relative effects of food in the presence of a combination of suspended solids (1000 mg/l) and carbofuran (65 μg/l) concentrations expected to elicit toxicity in 50% of the organisms. All results are summarized in Table 1.

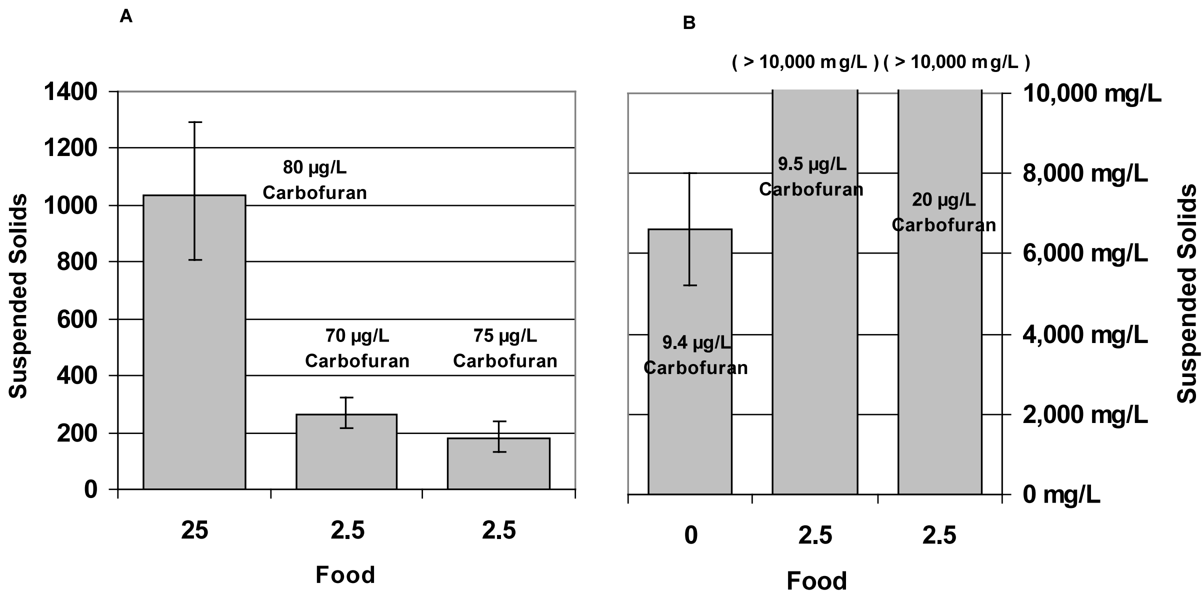

Fig. 2. Suspended solids EC_{50} for *D. magna* fed 25 mg/l and exposed to 80 μg/l carbofuran (Experiment 2A), and unfed *D. magna* exposed to 9.4 μg/l carbofuran (Experiment 2B). Comparable experiments conducted with 2.5 mg/l food at 9, 20, 70, and 75 μg/l (Herbrandson et al., 2003) also shown. Error bars denote standard deviations around mean responses for duplicate exposures.

Six chambers were loaded with 1000 mg/l, 3% f_{oc} suspended solids. Ninety-four, 75 and 24% of the organisms were affected in treatment chambers (2 per treatment) with 0, 2.5, or 25 mg/l food, respectively (Fig. 3). Suspended solids (1000 mg/l) with 0.5% f_{oc} were tested in the remaining (duplicate) chambers with 2.5 and 25 mg/l food. The carbofuran and suspended solids affected 84 and 30% of the *D. magna* in chambers containing 2.5 and 25 mg/l food, respectively (Fig. 3). A statistically significant difference in effects was found between all food treatment groups ($P < 0.05$, 2-tailed t-tests), but not between f_{oc} groups ($P > 0.05$, 2-tailed t-tests). The results of these experiments indicate that increased food availability reduces the combined toxicity of carbofuran and suspended solids.

Zurek (1983) demonstrated that *D. hyalina* exposed to 100–1000 mg/l suspended solids increased their metabolism 10.6–32.4%. It was also shown that the sinking velocity of organisms exposed to suspended solids was increased, leading to a suggestion that the energy needs of exposed daphnia are increased due to the increased specific gravity of organisms that ingested suspended solids (Zurek, 1983). Increased grooming or other protective activity might also be required of organisms exposed to suspended solids. Inspection of affected *D. magna* from chambers with suspended solids, across all carbofuran concentrations tested, indicated that most carapaces contained silt particles or rejected food boluses. ‘Normal’ organisms, generally, did not have these particles present and presumably removed this material with their large post-abdominal claw. Decreased buoyancy and increased grooming needs could both play a role in the toxicodynamics of carbofuran toxicity to *D. magna* by increasing the energy needs of the organism. Also, carbofuran intoxication could decrease the organisms' ability to groom, thereby exacerbating the adverse effects of suspended solids.

Experiments 2A, 2B and 2C also demonstrate that increased food availability decreased the combined toxicity of carbofuran and suspended solids to *D. magna*. In addition, results from Experiment 1 indicated that increased food availability can modulate the toxicity of carbofuran in the absence of suspended solids. Although food availability has been shown to affect carbofuran toxicity, it is not possible with the available data to assess the direct role of food availability on suspended solids toxicity since, in the absence of carbofuran, suspended solids failed to elicit mor-

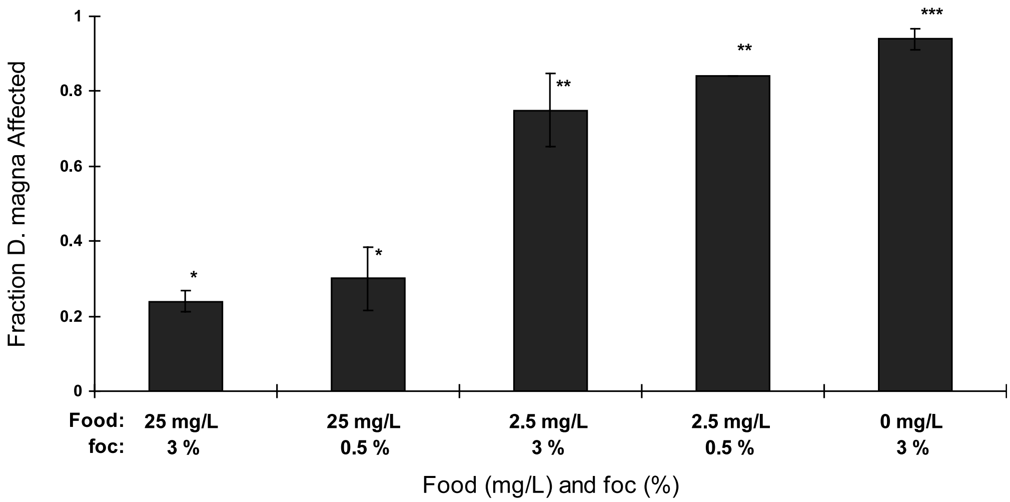

Fig. 3. Effect of varying food concentrations and fraction organic carbon content of suspended solids on carbofuran (65 μg/l) toxicity to *D. magna* (Experiment 2C; see Table 1). Columns represent the average fraction of test organisms affected in each treatment group; error bars are the standard deviations between replicate chambers. Similar numbers of asterisks identify results that were not significantly different ($P > 0.05$).

tality (Herbrandson et al., 2003). As summarized above, it has been proposed that exposure to suspended solids requires the utilization of excessive amounts of energy by daphnia. Thus, observations from Experiments 2A, 2B and 2C are likely a result of independent toxicodynamic dependence of carbofuran toxicity and stress from exposure to suspended solids on food availability, or an increased need for food caused by a toxicodynamic interaction between carbofuran and suspended solids.

4.3. Experimental series 3: suspended subsoil is more toxic than suspended decomposed peat to carbofuran-stressed *D. magna*

Decomposed peat used in all experiments had an f_{oc} of 34.9% and a density of 1.5 kg/l, while subsoil had an f_{oc} of 0.46% and a density of 2.1 kg/l. If *D. magna* ingest decomposed peat, the maintenance of buoyancy should require less energy than if subsoil is ingested. Consequently, it would be expected that the toxicity of carbofuran-stressed *D. magna* exposed to peat would be less than that observed for organisms exposed to similar concentrations of subsoil and carbofuran. Consistent with this assumption the results of experiment 3A (Fig. 4) showed that 65 ± 1.4% of *D. magna* were affected at 0.5% f_{oc} (2.1 kg/l density) suspended solids, while 13 ± 1.4% were affected at 20% f_{oc} (1.8 kg/l density) suspended solids. Thus, within the range of treatments tested, as the f_{oc} of the suspended solids increased, and density decreased, the toxic response of the carbofuran-exposed *D. magna* decreased.

If decomposed peat provides some benefit to *D. magna*, then decreased toxicity of higher f_{oc} suspended solids may be possible. Experiment 3B-1 demonstrates, however, that increasing decomposed peat exposure does not increase the survivability of *D. magna* exposed to 44 μg/l carbofuran. As illustrated in Fig. 5, the fraction of affected *D. magna* was lowest in the rotating 0 mg/l suspended solids chamber and, as decomposed peat concentration increased to 739 mg/l, the percentage of affected organisms increased from 40–78%. Since no food was provided in this experiment, the results suggest decomposed peat has no caloric value and that decomposed peat is a stressor when coupled with carbofuran exposure. The results of experiment 3B-2 (Fig. 5) at 102 μg/l carbofuran, 1.3–1308 mg/l decomposed peat, and 25 mg/l food, also confirms that simultaneous exposure to decomposed peat, in

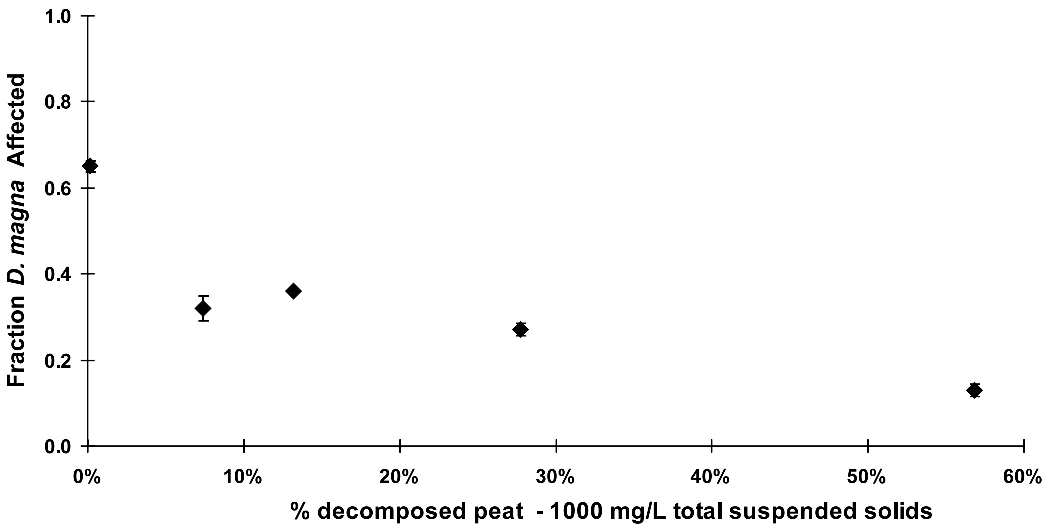

Fig. 4. Effect of varying ratios of suspended solids and decomposed peat (at a constant concentration of suspended solids, 1000 mg/l) on *D. magna* exposed to carbofuran (47 μg/l) (Experiment 3A; see Table 1). Error bars denote standard deviations around the mean responses for duplicate exposures.

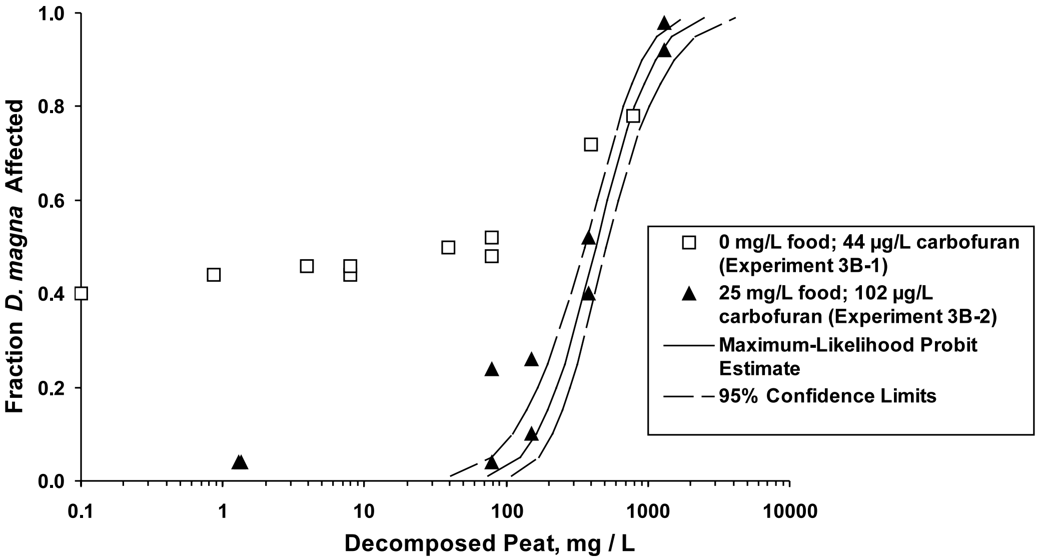

Fig. 5. Decomposed peat concentration dose–response relationships for carbofuran-exposed *D. magna* provided 25 or 0 mg/l food (Experiments 3B-1 and 3B-2; see Table 1).

the absence of subsoil, can increase toxicity in carbofuran-exposed *D. magna*.

Though decomposed peat is a stressor to *D. magna*, the results from experiment 3A clearly show that it is less toxic than subsoil. If carbofuran and subsoil concentrations are held constant, however, it would be expected that the addition of decomposed peat would increase the toxic response of *D. magna*. Interestingly, results from experiments 3C-1 and 3C-2 (Fig. 6), conducted with 999 mg/l subsoil in all chambers, but different total suspended solids concentrations, failed to show any such dependence. The results from experiment 3C-1 with organisms provided 2.5 mg/l food, may indicate a slight decrease in toxicity as decomposed peat concentrations increase to 383 mg/l (1383 mg/l total suspended solids; 10% f_{oc}, density of 1.9 kg/l), but these results are not reflected in experiment 3C-2 with *D. magna* provided 25 mg/l food. Overall, there appears to be no definite relationship between decomposed peat concentrations and the response of organisms under concurrent exposure to decomposed peat, 1000 mg/l subsoil, and carbofuran. The data from these experiments, however, are consistent with a presumption that particle density affects the combined toxicity of suspended solids and carbofuran. As decomposed peat concentration is increased, the total suspended solids exposure increases, but the average density of the particles decreases. This decrease in particle density could decrease the energy required to maintain buoyancy in the suspended solids environment.

Evaluating the results across the three experimental series indicates that both decomposed peat and subsoil are stressors. However, the results support the hypothesis that suspended subsoil is more harmful to carbofuran-exposed *D. magna* than decomposed peat. In addition, it appears that the greater density of subsoil may contribute to its higher toxicity. In addition, increased food availability decreases the toxic response of *D. magna* when exposed to carbofuran and suspended solids.

Daphnids, in general, have been shown to be non-selective in their choice of ingested particles (DeMott, 1982; Lampert, 1974; Kerfoot and Kirk, 1991). As non-selective filter or suspension feeders, *D. magna* cannot effectively concentrate food resources (Gerritsen et al., 1988). Therefore, even when a large amount of particles is being ingested by the organisms, food intake can decrease. In a system where the majority of particles are not food, daphnids can only maintain food uptake by ingesting extremely large quantities of other sus-

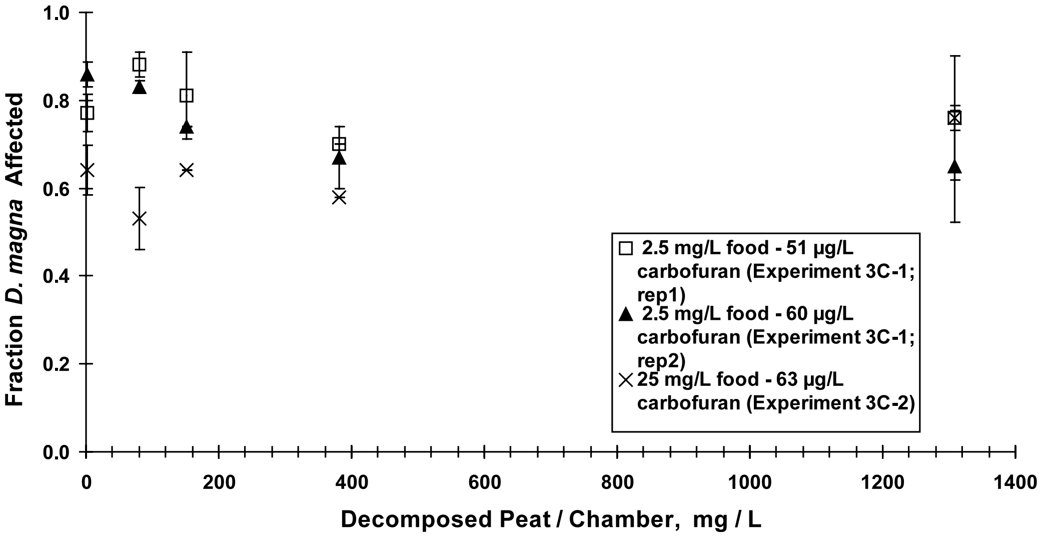

Fig. 6. Effect of decomposed peat on *D. magna* exposed to suspended subsoil (1000 mg/l) and 2.5 or 25 mg/l food (Experiments 3C-1 and 3C-2; see Table 1). Symbols represent mean fraction affected; error bars denote standard deviations.

pended solids. Based on relationships developed by Porter et al. (1983), Christensen (1973), *D. magna* in experiments reported here would be expected to filter about 1.9–3.2 ml/h. For an aqueous food concentration of 2.5 mg/l, food would be ingested at a rate of 4.8–8.0 µg/h. In a chamber with 1000 mg/l suspended solids, a *D. magna* must ingest particles at 1.9–3.2 mg/h to consume 4.8–8.0 µg/h food, a 400-fold increase over the food-only ingestion rate. Presumably, there exists a threshold concentration of suspended solids at which *D. magna* ability to ingest food begins to decrease. Since the toxicity of carbofuran-exposed *D. magna* has been shown to be food dependent, the role of suspended solids in reducing food availability alone could have a large impact on carbofuran toxicity.

While decreasing food availability may be responsible for some of the interaction between carbofuran and suspended solids, it apparently is not the only interactive effect that can occur with these stressors. Inter-experimental data from Herbrandson et al. (2003) show no change in carbofuran EC_{50}s from 0–100 mg/l suspended solids (EC_{50}s of 90–80 µg/l), while at 500, 1000, 5000 and 10 000 mg/l suspended solids carbofuran EC_{50} values (95% C. I.) of 67 (58–72), 45 (30–60), 30 (22–38), and 19 (15–24) µg/l were recorded (note that the mean carbofuran EC_{50} for unfed *D. magna* in reference toxicity tests was 35 µg/l). Assuming food limitation is linear and saturation of ingestion begins at 50 mg/l suspended solids, food dilution at 500, 1000, 5000, and 10 000 mg/l suspended solids ranges from approximately 90 to greater than 99%. If food dilution was solely responsible for the interactive effect between carbofuran and suspended solids toxicity, significantly decreasing carbofuran EC_{50}s between 500 and 10 000 mg/l suspended solids would not be expected. Further, in experiment 2B and 3B-2, food was withheld; however, suspended solids concentration-dependent dose–response curves were obtained at fixed carbofuran concentrations of 9.4 and 44 µg/l respectively. If food availability were solely responsible for the potentiated effect of suspended solids and carbofuran, a constant level of mortality should also have been observed in these experiments.

Experiments designed to assess the relative toxic effects of decomposed peat and subsoil provide suggestive insights as to additional mechanisms of carbofuran and suspended solids interactions. For example, the results from experiment 3A, demonstrating decreased toxicity at increasing suspended solid f_{oc}, are presumed to be caused by physical differences between the high f_{oc} decomposed peat and the low f_{oc} of predominately inorganic subsoil. A possible mechanism whereby f_{oc} and suspended solids density could affect energy usage was discussed previously. Observed differences in toxicity between decomposed peat and suspended solids could also be a function of particle shapes or edge characteristics. For example, sharp edges of inorganic particles could irritate the gut or externally-exposed tissues and cause changes in the allocation of energy resources or reductions in ingestion or respiration rates. These biochemical or behavioral responses could conceivably exacerbate the effects of carbofuran toxicity. It is possible that at very high suspended solids f_{oc}, exposure to some organic compounds could be decreased by sorption or biodegradation, and thereby contribute to reduced toxicity; however, it has been shown that concentrations of freely-dissolved carbofuran remain equivalent in 0 and 5000 mg/l, 3% f_{oc}, suspended solids chambers and the tank throughout a 48 h experiment (Herbrandson et al., 2003).

5. Conclusions

These experiments have shown that both carbofuran toxicity and the joint toxicity of carbofuran and suspended solids to *D. magna* can be modulated by food availability. While it is clear that food dilution could contribute to energy stress, it appears likely that additional interactive processes also contribute to the observed synergism between carbofuran and suspended solids. Subsoil has been shown to adversely affect test organisms more than decomposed peat and the ingestion of subsoil is expected to require greater energy expenditures from *D. magna*. These results are consistent with a presumption that the toxicodynamics of carbofuran toxicity in *D. magna* is directly affected by energy availability and that the interaction between carbofuran and suspended solids is caused, at least in part, by energy stress on the organism.

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