2003

Influence of Suspended Solids on Acute Toxicity of Carbofuran to Daphnia Magna: I. Interactive Effects

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

ELSEVIER

Aquatic Toxicology 63 (2003) 333–342

AQUATIC TOXICOLOGY

www.elsevier.com/locate/aquatox

Influence of suspended solids on acute toxicity of carbofuran to *Daphnia magna*: I. Interactive effects

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

[a] *National Biological Service, Minnesota Cooperative Fish and Wildlife Research Unit, University of Minnesota, 200 Hodson Hall, 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*

Received 22 January 2002; received in revised form 9 October 2002; accepted 13 November 2002

Abstract

This study explored the effects on *Daphnia magna* from exposure to the pesticide carbofuran in combination with stress from suspended solids exposure. Our objective was to assess whether suspended solids affects the toxicodynamic response of *D. magna* to carbofuran. A series of laboratory experiments was performed where animals were exposed to carbofuran concentrations ranging from 0 to 160 μg/l in combination with suspended solids concentrations ranging from 0 to 10 000 mg/l. In the absence of suspended solids, effects of carbofuran were dose dependent and resulted in an EC_{50} of 92 μg/l. Exposure to suspended solids, up to extreme levels that may be encountered in the environment and in the absence of carbofuran, showed no measurable toxicity. When *D. magna* were exposed to a constant carbofuran concentration, the numbers of affected organisms increased with increasing suspended solids concentrations. At a suspended solids concentration of 1000 mg/l, the EC_{50} for carbofuran was reduced by half to 45 μg/l. The relationship between the toxicity of carbofuran (μg/l) and the concentration of suspended solids (mg/l) can be described with the following equation: carbofuran $EC_{50} = 72\exp(-0.00014\,[\text{suspended solids}])$. An analysis of the data indicates that this relationship is consistent with a potentiated toxicity mechanism rather than an additive model.

Keywords: Multiple stressors; Carbofuran; Suspended solids; *Daphnia magna*

1. Introduction

Aquatic organisms in a natural environment are exposed to mixtures of chemicals and physical stressors which when combined can cause beha-

* 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.

0166-445X/02/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0166-445X(02)00206-0

vioral, physiological, or biochemical changes, death, or other adverse effects. When an organism is exposed to chemical mixtures, toxic responses can become difficult to interpret. The results may reflect effects that are additive, less than additive or antagonistic, or more than additive or synergistic (Hermens et al., 1984a,b; Broderius, 1991). When chemicals with similar modes of action are combined, it is generally assumed that their combined effects are additive (Broderius, 1991). For example, results from experiments with 1-octanol and 2-octanone have shown that in fathead minnows (*Pimephales promelas*), the lethality of these compounds combine additively (Broderius, 1991); however, when medaka (*Oryzias latipes*) larvae were exposed to methyl parathion (*O*,-dimethyl-*O*-(4-nitrophenyl)phosphoro-thiolate), molinate (*S*-ethyl hexahydro-1 *H*-azepine-1-carbothiolate), and carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl *N*-methylcarbamate), a less than additive response was reported (Heath et al., 1993).

The toxicity of chemicals to aquatic organisms can also be modified by altering environmental conditions. For example, increasing salinity has been reported to have varying effects on the toxicity of chemical compounds to aquatic biota (Hall and Anderson, 1995), while oxygen stress and carbaryl (1-napthyl methylcarbamate) have been reported to act in a more than additive fashion in *Daphnia pulex* (Hanazato and Dodson, 1995). Hardness, pH, dissolved organic carbon (OC), and other water quality characteristics have been shown to modulate the toxicity of metals (Erickson et al., 1996). While feeding effects in acute studies with *Daphnia magna* using organic toxicants are not predictable (Adema, 1973), food availability has been shown to decrease some of the toxic effects of lead (Enserink et al., 1995) and cadmium (Kluttgen and Ratte, 1994) to chronically exposed *D. magna*.

Issues concerning the interactions of suspended solids with toxicants are not completely resolved. Prior studies on the combined effects of suspended solids and organic toxicants have focused on changes in chemical bioavailability across respiratory membranes and illustrate that OC in suspended solids will sorb hydrophobic organic compounds (Chiou et al., 1979). Sorbed chemicals have less bioavailability, leaving less toxicant accessible to aquatic organisms, which decreases the overall observed toxicity (Muir et al., 1983; McCarthy, 1983; Schrap and Opperhuizen, 1990). Suspended solids can be lethal to daphnids and in chronic studies 50 and 100 mg/l of clay have been shown to significantly affect the survival of *D. ambigua* and *D. pulex*, respectively (Kirk and Gilbert, 1990). Suspended solids in acute exposures could also increase stress on aquatic organisms under concurrent exposure to a chemical toxicant. The added physical stress could affect the susceptibility of the organism to chemical insult by changing the toxicodynamics of the chemical/organism interaction. Studies addressing these types of interactions have not been reported. These interactions must be studied under conditions where chemical bioavailability can be held constant, thereby isolating toxicodynamic relationships, as opposed to toxicokinetic interactions, between the two stressors. As part of an effort to identify toxicodynamic interactions, the research described herein investigates the interactions of the pesticide carbofuran and suspended solids on *D. magna*.

Carbofuran is a carbamate nematicide and insecticide whose mode of action, like other carbamate and organophosphate pesticides, is via acetylcholinesterase (AChE) inhibition (US EPA, 1988; Kuhr and Dorough, 1976). In invertebrates, AChE inhibitors are known to exert their toxicity on the central nervous system (Eldefrawi, 1985). AChE inhibition in insects causes hyperactivity, loss of coordination, convulsions, paralysis, and death (Kuhr and Dorough, 1976). Consequently, intoxicated organisms experiencing a loss of coordination or orientation may be additionally compromised while coping with environmental stresses, such as suspended solids, which require changes in physical activity. Increased metabolic activity (e.g. Samson et al., 1984) coupled with increased physical stress could also deplete energy stores in an organism (e.g. Rambabu and Rao, 1994). If energy stores and/or physical activity are affected by both carbofuran and suspended solids exposure, and energy availability affects the toxic response of *D. magna*, both stressors could affect similar physiological processes. Therefore, it is reasonable to hypothesize that the toxic effects of carbofuran and suspended solids could combine in an additive manner.

The objective of the experimental series described in this paper is to assess whether or not suspended solids affect the toxicodynamic response of *D. magna* to carbofuran. We previously reported a specialized apparatus that permits exposures to equivalent freely dissolved chemical toxicant, while varying the amount of suspended solids exposure across a series of replicate chambers (Herbrandson et al., 1999). Any difference in toxicity among chambers is, therefore, a result of different suspended solids exposures. Because carbofuran has a relatively low hydrophobicity ($\log K_{ow} = 1.63–2.32$; Trotter et al., 1991; Howard, 1991), minimal sorption to suspended solids and reduction in bioavailability would be expected. This was shown in previous work (Herbrandson et al., 1999). For the current study, toxicity data across a range of carbofuran and suspended solids concentrations were analyzed to evaluate the relationships between the acute toxic effects of carbofuran and suspended solids. The data were used to assess whether these stressors interact in an additive manner.

2. Materials and methods

The Suspended Solids Testing Apparatus (SSTA) is a 37.75 l tank containing 10 rotating 1 l exposure chambers and one non-rotating chamber. Chambers are separated from the main tank by 0.1 μm nylon membrane (MSI, Westboro, MA). This design allows movement of only freely-dissolved chemical between chambers and confines suspended solids, thus preventing exposure to any sorbed chemical. The SSTA has been shown to equilibrate freely-dissolved concentrations of carbofuran between all chambers and the tank over 15–20 h and to maintain constant concentrations for the duration of a 48 h acute experiment (Herbrandson et al., 1999). Experiments were conducted with a constant carbofuran concentration across all chambers, with each chamber containing differing concentrations of suspended solids.

The experiments used suspended solids that were a combination of decomposed peat and subsoil. The subsoil is 0.46% OC and from an uncultivated site in Roseville, MN. The decomposed peat was 34.9% OC and was collected from the Carlos Avery Wildlife Refuge, Forest Lake, MN. 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.

Technical grade carbofuran (99.2%) was provided by FMC (Philadelphia, PA). Aliquots of a carbofuran stock solution in acetone were added to the SSTA tank 20 h prior to the addition of *D. magna* to exposure chambers. Twenty hours prior to the addition of test organisms, the maximum concentration of acetone in the SSTA was less than 15 μl/l. Previous experiments with acetone at the maximum concentration used in these experiments indicated no increased mortality (Herbrandson, 1996), and small concentrations of acetone are not expected to affect carbofuran sorption to suspended solids. A sample of the tank water, taken at the end of each experiment and stored at −20 °C, was used to determine carbofuran concentrations. A previous study established that concentrations did not vary during the length of the experiments (Herbrandson et al., 1999). Thawed samples were centrifuged at $10\,000 \times g$ for 30 min (Eppendorf 5415 C, Brinkman Instruments, Inc., Westbury, NY) and then assayed in triplicate with an enzyme linked immunosorbent assay (ELISA; RaPID Assay, Ohmicron, Newton, PA) to determine an operationally defined freely-dissolved carbofuran concentration. The ELISA is reported by the manufacturer to be 21 times more sensitive to carbofuran than to any other carbofuran degradation product. The manufacturer's reported coefficient of variability (CV) for 2.0 μg/l carbofuran is $\leq 3\%$ between assays and $< 9\%$ within assays. In this laboratory, the CV was 8.2% between assays and 6.1% within assays. All values in tables and figures are measured rather than nominal concentrations.

D. magna used in bioassays were 72–96-h-old and from 17- to 22-day-old parentage. The consistency of *D. magna* cultures was monitored with weekly reference toxicity tests to carbofuran (Herbrandson, 1996). These weekly EC_{50}s averaged 80 μg/l $\pm$ 13, indicating that cultures were stable during the course of the experiments. *D. magna* cultures were maintained in small aquaria, at room temperature (19–21 °C), on a 16:8 h light:dark cycle. Culture and test water was reconstituted NANOpure II (Barnstead Co., Newton, MA) water and prepared following United States Environmental Protection Agency (US EPA) methods for moderately hard water (Weber, 1993). The pH was adjusted daily to 7.15–7.35 with 99.999% HNO_3 (Aldrich Chemical Co., Milwaukee, WI).

D. magna in culture tanks were fed 4.8 mg/l/day of 75:25 yeast-alfalfa-trout chow:Tetra Conditioning Food. Tetra Conditioning Food was obtained from TetraWerke (Melle, Germany). The 48-h experiments were conducted with 2.5 mg/l food to minimize potential bias in suspended solids treatments due to any intrinsic food value. Feeding during the test was also instituted to minimize any effects caused by short-term food availability differences in culture tanks immediately prior to testing.

A battery of toxicity experiments covering a large range of suspended solids and carbofuran concentrations were conducted to investigate the toxic interaction between carbofuran and suspended solids. Carbofuran concentrations ranged from 0 μg/l to a concentration expected to be lethal to all test organisms (160 μg/l). Suspended solids concentrations ranged from 0 mg/l to a maximum that would be considered extreme in the field (10 000 mg/l).

Experiments were performed following standard protocols as described by Herbrandson (1996) and briefly summarized below. Suspended solids concentrations (all containing 3% OC) for each experiment were 0, 10, 50, 100, 500, 1000, 5000 and 10 000 mg/l, with duplicate chambers of 100 and 1000 mg/l. Twenty-four hours prior to the addition of organisms, suspended solids and water were added to the apparatus and rotation of the chambers at 5 rpm was initiated. At 20 h prior to the addition of *D. magna*, aliquots of the carbofuran stock solution were added to the tank. At 0 h 50 *D. magna* were added to test chambers, in random order, as well as culture controls. Rotation of the test chambers was stopped after 24 h of exposure to simulate settling solids conditions (i.e. resuspension events) (Herbrandson et al., 1999). After 48 h, the *D. magna* were removed from the chambers, scored and counted. Surviving *D. magna* were scored into one of three possible categories. Organisms on the bottom of the counting pan, quivering or occasionally, but rarely, moving, were scored as ‘quivering’. *D. magna* that moved along the bottom of the pan, always touching the pan and failing to move vertically, were scored as ‘moving’. All surviving *D. magna* that moved vertically in the counting pan water were considered ‘normal’ even though there were occasional subjective behavioral responses, such as lethargy or ‘pinwheeling’. Only *D. magna* which were rated ‘normal’ were considered to be unaffected by the experimental treatments. Toxicity data were collected from all chambers for all 14 experiments. Carbofuran target concentrations were tested in random order and in duplicate.

Data analysis was performed on concentration–response data from each experiment using ToxCalc 5.0 (Tidepool Scientific Software, McKinleyville, CA). All calculated statistics are a function of the number of *D. magna* exposed per tank (50) and the number of test organisms scored ‘normal’. Suspended solids-dependent EC_{50}s were calculated for each experiment (i.e. each carbofuran concentration) using the maximum likelihood-probit method. Since each experiment tested the same suspended solids concentrations, but different carbofuran concentrations, carbofuran EC_{50}s could also be calculated at each suspended solids concentration. An isobole diagram (Fraser, 1872; Loewe, 1953; Broderius, 1991) was prepared from the suspended solids and carbofuran EC_{50}s and examined to determine the toxic interaction between carbofuran and suspended solids.

3. Results and discussion

3.1. SSTA experiments

The purpose of this experimental series was to determine the toxicodynamic interaction of carbofuran and suspended solids. Carbofuran is not hydrophobic and, therefore, the presence of suspended solids should not affect its bioavailability or toxicokinetics. This assumption was tested and found to be valid (Herbrandson, 1996). Given a carbofuran log K_{ow} of between 1.63 and 2.32 (Trotter et al., 1991; Howard, 1991), less than 0.2% of all carbofuran in the SSTA and less than 3.5% of the carbofuran in the chamber with the highest concentration of suspended solids is expected to sorb to suspended solids (using equation from Karickoff, 1981). Therefore, these experiments should show concentration–response effects that are a result of the toxicodynamic interaction of carbofuran and suspended solids.

Table 1 summarizes the results of all experiments with varying exposures to carbofuran and suspended solids. Experiments with suspended solids at the concentrations tested showed essentially no toxicity in all experiments having no carbofuran. On the other end of the experimental range, greater than 95% of the *D. magna* in all chambers were affected by carbofuran concentrations of 159 μg/l and greater. In the range between these extremes, numbers of affected organisms generally increased as suspended solids concentrations increased within individual experiments having constant carbofuran concentrations (see rows in Table 1). Fig. 1 demonstrates this dependence with representative concentration–response plots of data acquired during experiments with 23, 55, 75, and 87 μg/l freely-dissolved carbofuran. Inspection of suspended solids EC_{50}s in Table 1 indicates that as the concentration of carbofuran is increased, less suspended solids are needed to achieve a similar adverse effect. Note that as the freely-dissolved carbofuran exposure increases, the concentration–response curves in Fig. 1 shift to the left, indicating a decreasing suspended solids EC_{50}. These experimental results clearly show a dependence of suspended solids EC_{50}s (in mg/l) on carbofuran (in μg/l), as indicated in Eq. (1) for suspended solids EC_{50}s between 50 and 10 000 mg/l:

$$\text{Suspended solids } EC_{50} = 3.11 \times 10^4 e^{-0.066[\text{carbofuran}]} \quad (R^2 = 0.92) \qquad (1)$$

In a consistent manner, toxicity to *D. magna* increased with increasing carbofuran concentrations, at constant suspended solids concentrations (see columns in Table 1). Fig. 2 demonstrates this dependence with carbofuran concentration–response curves from *inter*-experimental data at 10, 100, 1000, and 10 000 mg/l suspended solids. As noted in Table 1, carbofuran EC_{50}s are approximately the same for suspended solids concentrations of 0, 10, and 50 mg/l, but they decrease for all suspended solids concentrations greater than 50 mg/l. This finding indicates that below a concentration of approximately 50 mg/l suspended solids, there is no apparent suspended solids effect on *D. magna*'s toxic response to carbofuran. At all concentrations above 50 mg/l, there is an inverse relationship between the amount of suspended solids and the amount of carbofuran necessary to achieve the same effective response (i.e. an EC_{50}). Note in Fig. 2 that as suspended solids concentrations are increased, the carbofuran-dependent concentration–response curves shift to the left, indicating a decreasing EC_{50}. An analysis of these data for suspended solids concentrations between 50 and 10 000 mg/l yields Eq. (2):

$$\text{Carbofuran } EC_{50} = 71.7 e^{-0.000142[\text{suspended solids}]} \quad (R^2 = 0.88) \qquad (2)$$

where carbofuran and suspended solids concentrations are in microgram per liter and milligram per liter, respectively.

3.2. Isobole analysis

To evaluate the relationship between the two stressors, an isobole diagram was constructed (Fig. 3). In Fig. 3, EC_{50}s from individual experiments are plotted, with carbofuran as the independent variable and the suspended solids as the dependent variable. If the interaction between these two stressors is additive, EC_{50}s should fall along a straight line connecting the suspended solids EC_{50} (no carbofuran) and the carbofuran EC_{50} (no

Table 1
Fraction of *D. magna* affected by exposure to combinations of freely-dissolved carbofuran (0–206 µg/l) and suspended solids (0–10 000 mg/l)

Carbofuran concentration (µg/l)	Fraction *D. magna* affected								Suspended solids EC_{50}	95% C. I.
	Suspended solids concentrations (mg/l)									
	0	10	50	100 ($n=2$)	500	1000 ($n=2$)	5000	10 000	mg/l	
0 ($n=3$)	0.03	0.01	0.00	0.01	0.02	0.01	0.00	0.03	> 10 000	
9.5	0.00	0.00	0.00	0.03	0.02	0.04	0.08	0.22	> 10 000	
20	0.00	0.00	0.00	0.05	0.02	0.02	0.00	0.32	> 10 000	
23	0.04	0.00	0.00	0.01	0.30	0.25	0.42	0.66	4880	3260–8350
36	0.00	0.00	0.06	0.10	0.02	0.05	0.70	0.88	3470	775–4.9E+08
55	0.00	0.02	0.00	0.07	0.04	0.52	0.70	0.86	1570	678–5450
70	0.00	0.06	0.06	0.06	0.78	0.99	1.00	1.00	264	216–323
75	0.22	0.14	0.10	0.42	0.76	0.79	0.98	1.00	181	134–238
87 ($n=2$)	0.55	0.52	0.62	0.76	0.95	0.92	1.00	1.00	16	1–34
159	0.98	0.98	1.00	0.96	1.00	0.99	1.00	1.00		
206	0.98	1.00	0.98	1.00	1.00	1.00	1.00	1.00		
Carbofuran EC_{50} (µg/l)	92	90	89	82	67	45	30	19		
95% C.I.	81–116	86–95	67–366	64–150	58–72	30–60	22–38	15–24		

The carbofuran EC_{50}s and 95% confidence intervals are in the bottom 3 rows. The suspended solids EC_{50}s and 95% confidence intervals are in the 3 columns at far right. EC_{50}s are calculated using probit or trimmed (0.05) Spearman–Karber methods.

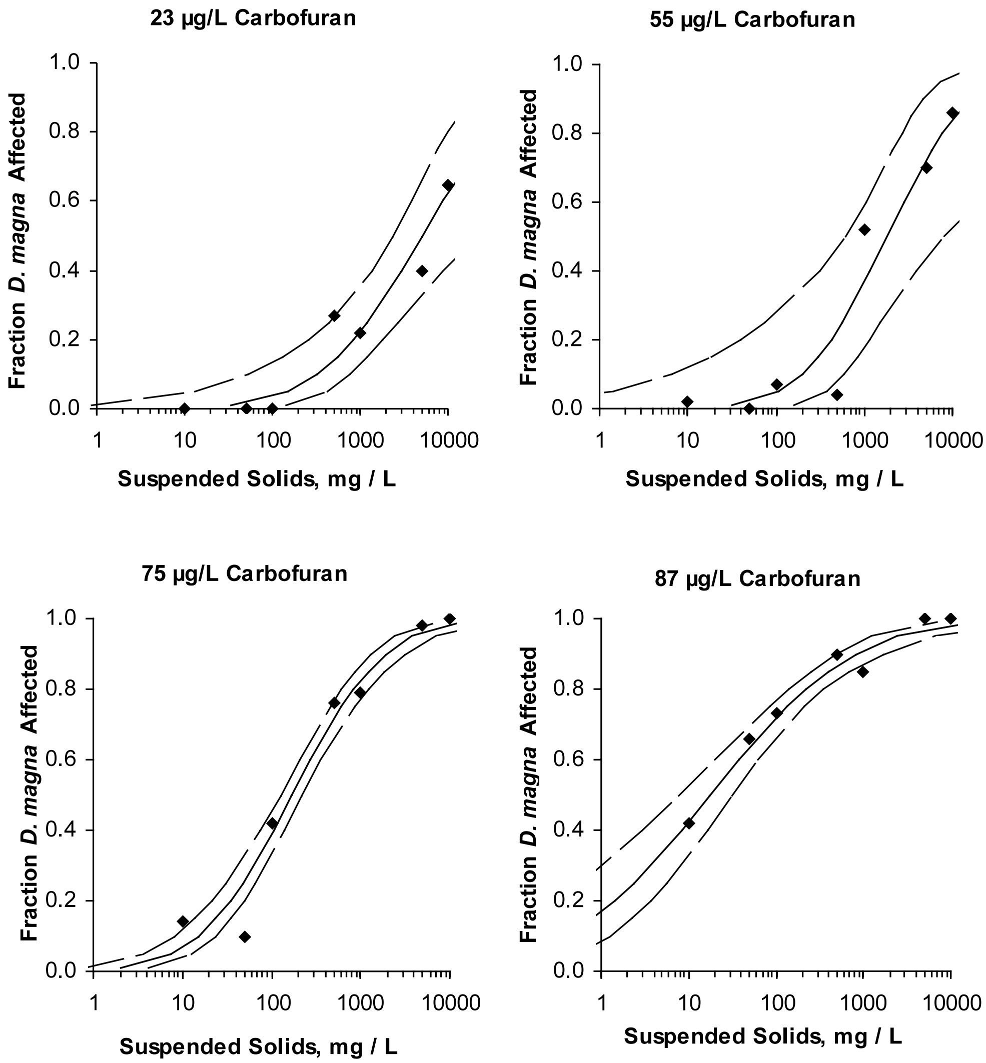

Fig. 1. Representative suspended solids concentration–response graphs from experiments at four different carbofuran concentrations. Each graph shows experimental data across 7 suspended solids concentrations (mean responses from replicate 100 and 1000 mg/l chambers). Maximum-likelihood probit line and 95% confidence limits are presented.

suspended solids) (Altenberger et al., 1990). Therefore, any point along this line is a hypothetical EC_{50} for simultaneous exposure to the two stressors. Such a line illustrating the theoretical additive response for carbofuran and suspended solids is shown in Fig. 3. Since the EC_{50} for suspended solids (without carbofuran) is greater than any suspended solids concentration used in these experiments, it has been estimated to be between 10 000 and 400 000 mg/l (Table 1). We chose an EC_{50} of 35 000 mg/l for illustration purposes, and because it is consistent with the data (see below). Using the additive-response line in Fig. 3, a carbofuran concentration of 75 µg/l would result in a suspended solids EC_{50} of 5500 mg/l if the effects were additive. However, the measured data indicate that the EC_{50} was 181 mg/l (Table 1). This suggests that the toxicity of carbofuran is potentiated by suspended solids, and that the relationship is not additive.

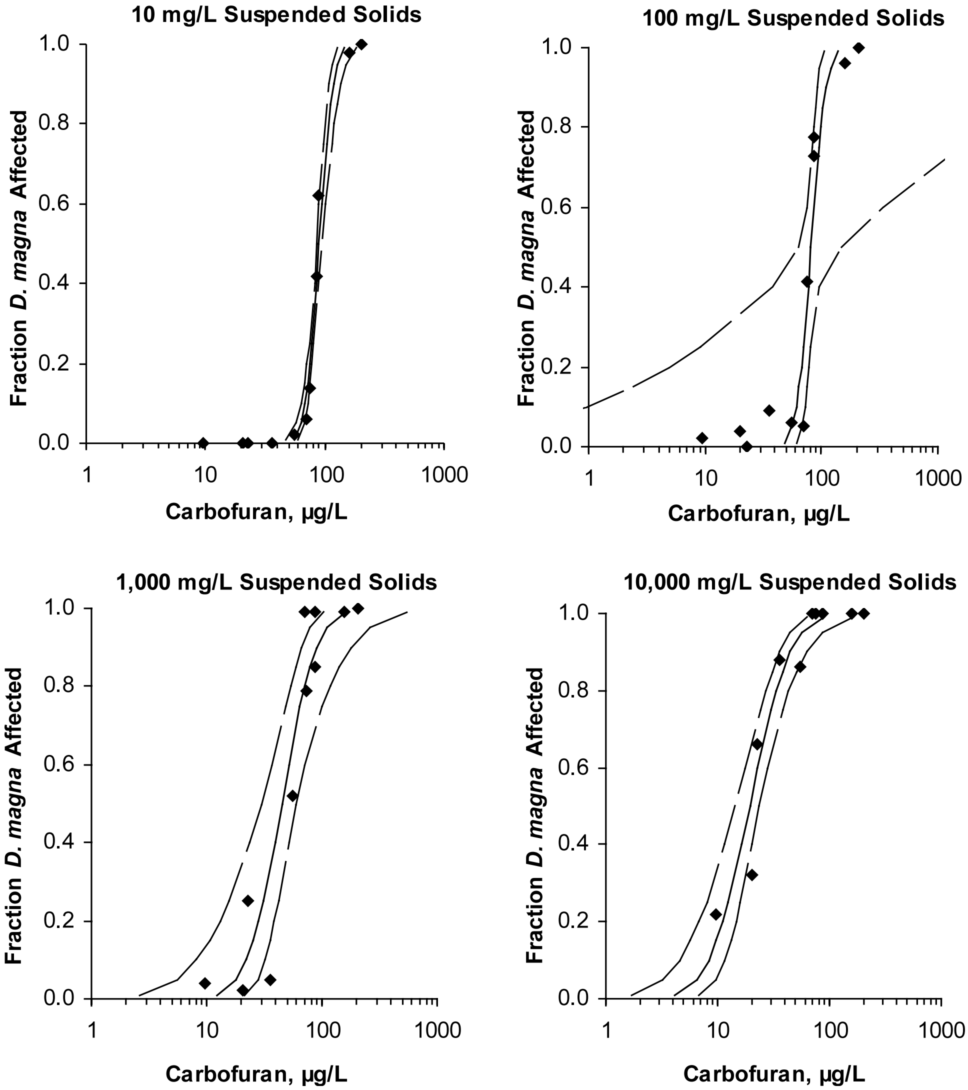

Fig. 2. Representative carbofuran concentration–response graphs from experiments at four different concentrations of suspended solids. Data are derived from 11 experiments (replicate experiments at 0 and 87 µg/l carbofuran). Maximum-likelihood probit line and 95% confidence limits are presented.

When all the experimental data are plotted in Fig. 3, the resulting relationship is hyperbolic rather than linear. An exponential regression of these data gives an estimate of the suspended solids EC_{50} (no carbofuran present) of approximately 35 000 mg/l. Note that the data all lie to the left of the theoretical additive-response line. These results further support the conclusion that the response of carbofuran and suspended solids is potentiated and not additive (Altenberger et al., 1990).

Since carbofuran's mode of toxic action is through AChE inhibition, it would be expected that other AChE inhibitors with a similar K_{ow} might also demonstrate a similar relationship with suspended solids. However, with more hydropho-

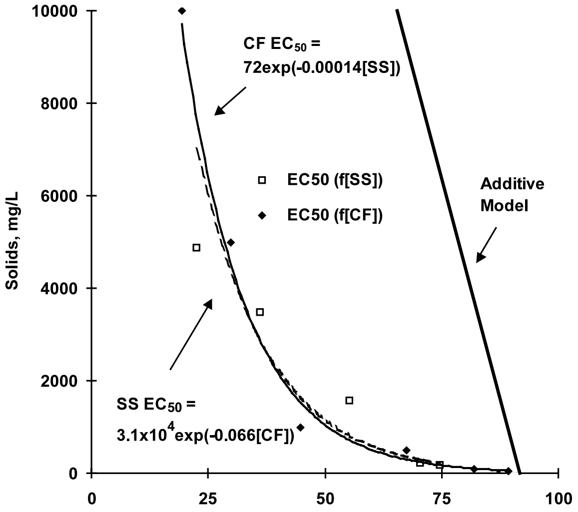

Fig. 3. Suspended solids and carbofuran EC_{50}s plotted against their independent variable. Regression analysis and exponential curve fit includes data for all points between 50 and 10 000 mg/l suspended solids inclusive. The dashed line fits suspended solids EC_{50}s (SS EC_{50}s) and the heavy solid line fits carbofuran EC_{50}s (CF EC_{50}s). Included is a line representing a hypothetical, strictly additive toxicity model that assumes a suspended solids EC_{50} of 35 000 mg/l at 0 mg/l carbofuran.

bic AChE inhibitors, bioavailability and toxicokinetic effects could become more important and confound the observations reported here.

There is reason to believe that reduced food availability in the presence of suspended solids could affect carbofuran toxicity and contribute to the synergistic response with suspended solids (Samson et al., 1984; Rambabu and Rao, 1994) . Reference toxicity tests consistently showed an increase in EC_{50} when test organisms were fed (Herbrandson, 1996). Suspended solids could also elicit a need for increased energy stores or uptake. Additionally, it has been hypothesized that ingested solids cause decreased buoyancy in daphnids, creating a need for additional effort and higher metabolism to maintain a desired depth in the water column (Zurek, 1983). The results of this experimental series establish a suspended solids-dependent increase in carbofuran sensitivity. Studies reported by Herbrandson et al. (2003) suggest this increased sensitivity may be due to a net decrease in the availability of food or energy stores in *D. magna*.

4. Conclusions

In conclusion, the combined toxicodynamic effect of a carbamate pesticide and suspended solids on a water column resident invertebrate was demonstrated. Exposure to suspended solids potentiated the toxicity of carbofuran to *D. magna* in a synergistic manner. An investigation of the mechanisms of this interaction is provided in the following article (Herbrandson et al., 2003).

Acknowledgements

Contributions and assistance from Mary Henry, Naomi Detenbeck, Chip Eulis, David Andersen, Scott Alexander, Calvin Alexander, Betsy Wattenberg, and Donald Jaschke are acknowledged. Helpful reviews were provided by Steven Broderius and Robert Spehar. The research described in this article was funded, in part, by the US Environmental Protection Agency through an interagency agreement (#14-16-0009-1566) with the Northern Prairie Research Station, Minnesota Cooperative Fish and Wildlife Research Unit of the National Biological Division of the US Geological Survey. This article has been reviewed by the US EPA National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division and approved for publication. Approval does not signify that the contents reflect the views of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

References

Adema, D.M.M., 1973. *Dapnia magna* as a test animal in acute and chronic toxicity tests. Hydrobiologia 59, 125–134.

Altenberger, R., Bodeker, W., Faust, M., Grimme, L.H., 1990. Evaluation of the isobologram method for the assessment of mixtures of chemicals. Ecotoxicol. Environ. Safe 20, 98–114.

Broderius, S.J., 1991. Modeling the joint toxicity of xenobiotics to aquatic organisms: basic concepts and approaches. In: Mayes, M.A., Barron, M.G. (Eds.), Aquatic Toxicology and Risk Assessment (ASTM 1124). American Society for Testing and Materials, Philadelphia, pp. 107–127.

Chiou, C.T., Peters, L.J., Freed, V.H., 1979. A physical concept of soil–water equilibria for non-ionic organic compounds. Science 206, 831–832.

Eldefrawi, A.T., 1985. Acetylcholinesterases and anticholinesterases. In: Kerhut, G.A., Glibert, L.I. (Eds.), Comparative Insect Physiology, Biochemistry, and Pharmacology, vol. 12. Pergamon Press, Oxford, pp. 115–130.

Enserink, E.L., Kerkhofs, M., Baltus, C., Koeman, J.H., 1995. Influence of food quantity and lead exposure on maturation in *Dapnia magna*—evidence for a trade-off mechanism. Funct. Ecol. 9, 175–185.

Erickson, R.J., Benoit, D.A., Mattson, V.R., Nelson, H.R., Leonard, E.N., 1996. The effects of water chemistry on the toxicity of copper to fathead minnows. Environ. Toxicol. Chem. 15, 181–193.

Fraser, T.R., 1872. The antagonism between the actions of active substances. Br. Med. J. 2, 485–487.

Hall, L.W., Anderson, R.D., 1995. The influence of salinity on the toxicity of various classes of chemicals to aquatic biota. Crit. Rev. Toxicol. 25, 281–346 (Review).

Hanazato, T., Dodson, S.I., 1995. Synergistic effects of low oxygen concentration, predator kairomone, and a pesticide on the cladoceran *Daphnia pulex*. Limnol. Oceanogr. 40, 700–709.

Heath, A.G., Cech, J., Jr., Zinkl, J.G., Steele, M.D., 1993. Sublethal effects of three pesticides on Japanese medaka. Arch. Environ. Contam. Toxicol. 25, 485–491.

Herbrandson, C., 1996. Ph.D. Dissertation, Toxicology Program, University of Minnesota, Minneapolis, MN.

Herbrandson, C., Bradbury, S.P., Swackhamer, D.L., 1999. A new testing apparatus for assessing the interactive effects of a physical and chemical stressor. Environ. Toxicol. Chem. 18, 679–684.

Herbrandson, C., Bradbury, S.P., Swackhamer, D.L., 2003. Influence of suspended solids on acute toxicity of carbofuran to *Daphnia magna* II. An evaluation of potential interactive mechanisms. Aquat. Toxicol. 63, 343–355 (this issue)

Hermens, J., Canton, H., Janssen, P., de Jong, R., 1984a. Quantitative structure–activity relationships and toxicity studies of mixtures of chemicals with anesthetic potency: acute lethal and sublethal toxicity to *Dapnia magna*. Aquat. Toxicol. 5, 143–154.

Hermens, J., Canton, H., Steyer, N., Wegman, R., 1984b. Joint effects of a mixture of 14 chemicals on mortality and inhibition of reproduction of *Dapnia magna*. Aquat. Toxicol. 5, 315–322.

Howard, P.H. (Ed.), Handbook of Environmental Fate and Exposure Data—Pesticides, vol. 3. Lewis Publishers, Ann Arbor, MI 1991, pp. 73–83.

Karickoff, S.W., 1981. Semi-emperical estimation of sorption of hydrophobic pollutants on natural sediments and soils. Chemosphere 10, 833–846.

Kirk, K.L., Gilbert, J.J., 1990. Suspended clay and the population dynamics of planktonic rotifers and cladocerans. Ecology 71, 1741–1755.

Kluttgen, B., Ratte, H.T., 1994. Effects of different food doses on cadmium toxicity to *Dapnia magna*. Environ. Toxicol. Chem. 13, 1619–1627.

Kuhr, R.J., Dorough, H.W., 1976. Mode of action. In: Carbamate Insecticides: Chemistry, Biochemistry, and Toxicology. CRC, Cleveland, OH, pp. 41–70.

Loewe, S., 1953. The problem of synergism and antagonism of combined drugs. Arneimittelforsch 3, 285–320.

McCarthy, J.F., 1983. Role of particulate organic matter in decreasing accumulation of polynuclear aromatic hydrocarbons by *Dapnia magna*. Arch. Environ. Contam. Toxicol. 12, 559–568.

Muir, D.C.G., Townsend, B.E., Lockhart, W.L., 1983. Bioavailability of six organic chemicals to *Chironomus tentans* larvae in sediment and water. Environ. Toxicol. Chem. 2, 269–281.

Rambabu, J.P., Rao, M.B., 1994. Effect of an organochlorine and three organophosphate pesticides on glucose, glycogen, lipid, and protein contents in tissues of the freshwater snail *Bellamya dissimillis* (Müller). Bull. Environ. Contam. Toxicol. 53, 142–148.

Samson, F.E., Pazdernik, T.L., Cross, R.S., Giesler, M.P., Mewes, K., Nelson, S.R., McDonough, J.H., 1984. Soman induced changes in brain regional glucose use. Fund. Appl. Toxicol. 4, S173–S183.

Schrap, S.M., Opperhuizen, A., 1990. Relationship between bioavailability and hydrophobicity: reduction of the uptake of organic chemicals by fish due to the sorption on particles. Environ. Toxicol. Chem. 9, 715–724.

Trotter, D.M., Kent, R.A., Wong, M.P., 1991. Aquatic fate and effect of carbofuran. Crit. Rev. Environ. Control. 21, 137–176.

US Environmental Protection Agency, 1988. Pesticide Fact Handbook. Carbofuran, Fact Sheet Number 24, Washington, D.C., pp. 140–142.

Weber, C.I., (Ed.), 1993. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. EPA/600/4-90/027F, fourth edition. United States Environmental Protection Agency, Cincinnati, OH, pp. 293.

Zurek, R., 1983. Effect of suspended materials on zooplankton. 2. Laboratory investigations of *Daphnia hyalina* Leydig. Acta Hydrobiol. 24, 233–251.