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POSTEXPOSURE FEEDING DEPRESSION: A NEW TOXICITY ENDPOINT FOR USE IN LABORATORY STUDIES WITH *DAPHNIA MAGNA*

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Abstract—In situ bioassays with daphnids currently employ lethality as an endpoint, and although sublethal responses (reproduction and feeding rate) can be measured in the field, such endpoints pose major practical challenges. Previous studies have indicated that *Daphnia magna* exposed to toxic substances can exhibit delayed recovery in feeding behavior (postexposure feeding depression). This simple, robust response has the potential to be an ecologically relevant and potentially diagnostic endpoint. This study developed and tested the use of postexposure feeding depression as a toxicity endpoint in the laboratory environment. First, replicate numbers were manipulated to produce statistically reliable results. Second, postexposure feeding depression in *D. magna* was studied under laboratory conditions, by employing toxic substances with differing modes of action. Although most substances caused feeding inhibition during direct exposure, not all substances produced postexposure feeding depression. However, the use of lethality as a supplementary endpoint provided an alternative measure when no feeding depression was apparent after exposure. In combination, these endpoints offer a potentially more sensitive, ecologically relevant alternative to the use of lethality alone for in situ bioassay studies.

Keywords—*Daphnia magna* Postexposure feeding depression Lethality

INTRODUCTION

Feeding behavior in *Daphnia* spp. has long been studied, from the individual to community level, because of its ecological importance. Food (or energy) intake is important at the individual and population level because acquisition and allocation of energy determines developmental rate, growth rate, fecundity, and survival, all of which are important components of fitness and determinants of population structure and dynamics [1]. *Daphnia* spp. are an important component of the zooplankton grazers in lakes because they have high algal grazing rates (because of a relatively large body size) and control phytoplankton biomass and species composition [2,3]. Thus, *Daphnia* spp. provide an important link between different trophic levels in lake communities and are important prey items for predatory zooplankton [4] and fish [5]. A change in *Daphnia* spp. population dynamics due to a reduction in feeding rate would be ecologically important because it has the potential to change community structure and function. Several studies have demonstrated that toxic impairment of feeding rates at the individual level has direct effects on population parameters such as growth and reproduction [6–8] and indirect effects on community structure, leading to great increases in phytoplankton biomass [9,10].

Impairment of feeding rate seems to be a general response to toxicant exposure. By exposing *Daphnia magna* to a range of metals and organic chemicals, Allen et al. [11] demonstrated a significant reduction in feeding rates after exposure to all substances tested. Short-term feeding tests have been developed with *Ceriodaphnia dubia*, and under laboratory conditions have demonstrated contaminant-induced feeding depression after exposure to a range of effluents and toxic substances with different modes of action [12–14]. Reductions in feeding rate in these tests can be detected in less than 90 min, thus showing that feeding depression is a rapid, as well as general, indicator of toxic stress. Because feeding depression is a rapid, sensitive, and ecologically relevant indicator of toxic stress in *Daphnia* spp., it is an ideal candidate for use as a bioassay endpoint.

In situ bioassays previously have been deployed that use cladocerans as test organisms and have successfully detected toxic impacts in rivers [15–19]. Cladocerans adapt well to deployment in the field and are suitable as test organisms because survival rates in uncontaminated conditions are frequently greater than 85% for *C. dubia* [15] and greater than 90% for *D. magna* [16]. However, most in situ bioassays that use cladocerans have used lethality as an endpoint [15,16,18], which may not be the most sensitive endpoint to use. Sublethal, physiological endpoints generally are recognized to be more sensitive than lethal endpoints because physiological endpoints are often the initial reaction of organisms to stress and can be detected before mortality occurs [19]. Therefore, feeding depression could be a useful sublethal endpoint to measure in in situ bioassays with *D. magna* and already has been successfully employed as an in situ endpoint with *Gammarus pulex* [16]. To gain an accurate measure of the toxicity of a water body by a *D. magna* in situ bioassay, animals must be continually exposed, in cages, to the surrounding environment. For a bioassay that uses feeding depression as an endpoint, this would introduce some difficulty into the experimental design. Directly measuring feeding rates of daphnids in the field while simultaneously exposing them to the surrounding environment is extremely difficult because the microparticles, such as algae, that constitute the primary food source of *D. magna* would leak from exposure chambers and compromise measurements of feeding rate.

Taylor et al. [20] found that *D. magna* previously exposed to sublethal concentrations of cadmium exhibited a persistent

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feeding depression after transfer to uncontaminated medium. Therefore, this persistent response could provide the basis for a feeding endpoint as part of a *D. magna* in situ bioassay, which could circumvent the difficulties of measuring feeding rates in the field mentioned above. The bioassay could consist of a period where animals would be placed in the field and exposed to contaminants (exposure), then immediately transferred to clean medium where feeding rates could be measured under controlled conditions (after exposure).

To be of use as an endpoint, postexposure feeding depression would have to be demonstrable at concentrations below lethality, after exposure to a wide range of toxic substances with different modes of action. Therefore, the aim of this paper was to investigate the consistency and sensitivity of the postexposure feeding response under laboratory conditions and to develop a standardized method for a *D. magna* postexposure feeding depression bioassay that could be adapted for use in the field at a later stage.

MATERIALS AND METHODS

Culture regime and acclimation to experimental conditions

Daphnia magna (clone F [21]) was maintained as described in Barata and Baird [22]. The *D. magna* used for all experiments were 4- to 5-d-old fourth instars. This life stage was chosen because it permitted the bioassay to be completed within a single molt cycle. This was important because the molting process can interfere with feeding, thus introducing error (R.A. McWilliams, personal observation). To minimize maternal effects, only animals from the third to sixth broods were used in experiments [22]. Cultures were maintained and experiments were conducted in a constant-temperature room (14:10 h light:dark, 20 ± 1°C standard error [SE]). In addition, all feeding experiments were performed in the dark because this has been found to produce uniform feeding rates [23].

All animals were acclimated in the same 6-L tank to minimize environmental variation. The volume of American Society for Testing and Materials (ASTM) hard water [24] that was used was determined by the number of animals to be acclimated: 200 and fewer, 3 L; 200 to 400, 5 L; and 400 to 500, 5.5 L. Seaweed extract (Glenside Organics, Throsk, UK) was added to the culture medium at a concentration of 6 ml/L and food (*Chlorella vulgaris*, Beijerink, strain CCAP C211/12, Institute of Freshwater Ecology, Cumbria, UK) was added daily at a concentration of 5×10^5 cells/ml. Animals were maintained under these conditions until 4 d old.

Calculation of feeding rates

Feeding rates were calculated as in Allen et al. [11] by using an electronic particle counter fitted with a 70-μm orifice tube (Coulter Multisizer, Coulter Electronics, Luton, UK). Algal samples of 2 ml were withdrawn by micropipette from exposure chambers by shaking vigorously to resuspend any settled cells, and dispensed into 18 ml of the electrolyte Isoton II (Coulter Electronics) in an accuvette disposable sample container (Coulter Electronics).

Method development

Preliminary experiments were conducted to establish a method for the bioassay with the aim of minimizing variation in baseline feeding rates. The method developed was as follows. Groups of five animals were randomly assigned to 175-ml glass jars containing 120 ml of ASTM with *C. vulgaris* at a concentration of 5×10^5 cells/ml and allowed to feed for 24 h; this constituted the exposure period. Animals were subsequently transferred to fresh medium containing *C. vulgaris* at a concentration of 5×10^5 cells/ml, and left to feed for a further period of 4 h; this constituted the postexposure feeding period. At the end of each feeding period, animals were removed from the jars by using a 3-ml plastic pipette, 2-ml algal samples were withdrawn, and cell numbers were counted. The preliminary experiments found that groups of animals feeding in the same vessel produced lower variation in baseline feeding rates than single animals. The duration of the feeding periods and the bioassay volume chosen also were found to produce minimal variation in baseline feeding rates. Previous work in our laboratory had demonstrated that a food concentration of 5×10^5 cells/ml was sufficient to prevent complete depletion of the food source during a 24-h feeding period [11,25].

Number of replicates required

Here, the aim was to establish the number of replicates required to detect at least a 20% difference between treatments during a 4-h feeding period, by using an analysis of variance design with a power of at least 80% (at the $\alpha = 0.05$ significance level) [26]. The method for the 4-h feeding period described above was used. Twenty independent replicate groups of animals were used, and a bootstrapping technique [27] was used to randomly assign each feeding vessel to a set of replicates with a sample size ranging from 2 to 10. The average power of each sample size was obtained by resampling each set of replicates 100 times.

Bootstrapping analysis revealed that no number of replicates tested reached the desired power of 0.8. Therefore, further bootstrapping analyses were performed to establish power curves to determine what magnitude of effect could be detected with 80% power by using the number of replicates chosen for the bioassay methodology.

Endpoint consistency and sensitivity

By using the method developed in previous sections, the consistency of the postexposure feeding endpoint was assessed by exposing *D. magna* to a range of chemicals with different modes of action, then measuring the postexposure feeding response over a 4-h period. Lethality tests were conducted to investigate endpoint sensitivity by testing the hypothesis that feeding depression median inhibitory concentration (IC50) occurs below the median lethal concentration (LC50). Experiments were carried out as follows. Stock solutions were prepared in ASTM hard water 2 d before the experiment. Experimental dilutions were made from stock solutions by adding the appropriate subsamples to ASTM hard water containing *C. vulgaris* at a concentration of 5×10^5 cells/ml just before the experiment was started.

Postexposure feeding response

For each concentration, six 175-ml glass jars were filled with 120 ml of the test substance experimental dilution, and five animals were randomly assigned to five of the jars. The remaining jar, with no animals, was used to establish initial algal concentrations. Controls were set up in the same way with ASTM hard water containing *C. vulgaris* at a concentration of 5×10^5 cells/ml only. Animals were left to feed for 24 h. They were then transferred with a 3-ml plastic pipette into 60-ml glass jars containing 60 ml of ASTM hard water with *C. vulgaris* at a concentration of 5×10^5 cells/ml and allowed to feed for 4 h, after which they were removed from

Table 1. Preparation of nominal concentrations of test substances used in lethality and feeding experiments

Test substance	Stock solution (μg/L)	Lethality tests (μg/L)		Feeding test (μg/L)	Comment
		With food	Without food		
Cadmium ($3CdSO_4 \cdot 8H_2O$)	100	—	—	0.2, 0.6, 1.0, 2.5, 5.0	No lethality test conducted
Zinc ($ZnSO_4 \cdot 7H_2O$)	100,000	1,000, 1,470, 2,160, 3,180, 4,670, 6,860, 10,000, 14,810, 21,770, 32,000		100, 240, 570, 1,350, 3,200	—
Copper ($Cu(II)SO_4 \cdot 5H_2O$)	1,000	15, 19, 25, 32, 42, 54, 70, 90, 116, 150	15, 18, 22, 26, 32, 38, 46, 55, 66, 80	5, 7, 10, 14, 20	—
Lead ($Pb(II)(NO_3)_2$)	3,000	100, 180, 310, 550, 970, 1,700, 3,000		550, 730, 970, 1,280, 1,700	Solutions prepared in ASTM[a] soft water [24] because of low bioavailability in hard water
cis-Permethrin (type I pyrethroid)	200	5, 6.7, 9, 12, 16, 22, 29, 39, 52, 70	0.1, 0.2, 0.3, 0.4, 0.6, 0.9, 1.4, 2.1, 3.2, 5.0	0.5, 0.9, 1.6, 2.8, 5.0	—
λ-Cyhalothrin (type II pyrethroid)	3.0	0.075, 0.1, 0.2, 0.3, 0.5, 0.8, 3.0		0.0075, 0.015, 0.03, 0.075, 0.15	—
Pirimiphos-methyl (organophosphate)	500	0.1, 0.2, 0.3, 0.5, 0.8, 1.0, 2.0, 4.0, 6.0, 10.0		0.05, 0.11, 0.22, 0.47, 1.0	—
Lindane (organochlorine)	7,000	350, 400, 470, 530, 610, 700	300, 500, 800, 1,300, 2,000, 3,500	400, 520, 600, 800, 1,000	Stock solution prepared from filtrate of a supersaturated solution of 120 mg/L lindane
Fluoranthene (polycyclic aromatic hydrocarbon)	100	—	—	25, 35, 50, 71, 100	No lethality test conducted

[a] ASTM = American Society for Testing and Materials.

the jars. Three jars also were set up without animals to be used as blanks (to establish initial algal concentrations). Algal samples were then withdrawn from each set of jars, algal clearance rates were measured, and feeding rates were calculated for each feeding period.

Lethality tests

For each concentration, five 175-ml glass jars were filled with 120 ml of the appropriate solution, and five animals were randomly assigned to each jar. Controls were set up in the same manner with ASTM hard water containing *C. vulgaris* at a concentration of 5×10^5 cells/ml only. Food was renewed after 24 h. After 48 h, the number of dead animals in each jar was counted. Death was assessed as immobilization, which was defined as lack of movement after shaking the jar. The experiments were then repeated in the absence of food.

Chemical concentrations and analysis

With the exception of the four metals, the toxic substances used in the study were relatively insoluble. For these latter substances, stock solutions were prepared by a thin-layer evaporation technique [28], and were mixed for 2 d on an orbital shaker. Nominal concentrations were prepared for cadmium, zinc, copper, and lead (AnalaR grade, Merck, Poole, UK); permethrin (98% purity, Supelco, Bellefonte, PA, USA); λ-cyhalothrin (98% purity, Reidel-de-Haën, Seelzel, Germany); pirimiphos-methyl (98% purity, Supelco); lindane (γ-hexachlorocyclohexane, 98% purity, Supelco); and fluoranthene (98% purity, Aldrich, Gillingham, UK), as shown in Table 1.

For metal analysis, duplicate water samples of 3 ml were collected from each concentration and stock solution before algae were added at the beginning of each experiment, and acidified with 1% of 80% (w/v) HNO_3 (Aristar grade, Merck) to preserve for later analysis. Duplicate samples also were collected from the highest concentration in each series after 24 and 48 h to observe whether concentrations decreased during the experiment. Concentrations were analyzed with an Analytical Technology Unicam model 939QZ graphite furnace atomic absorption spectrophotometer (Cambridge, UK), with the exception of zinc concentrations, which were analyzed with a Perkin-Elmer model 2280 flame atomic absorption spectrometer (Ueberlingen, Germany). Minimum detection limits for cadmium, zinc, copper, and lead were 0.2, 0.1, 0.5, and 1.0 μg/L, respectively.

For organic chemical analysis, duplicate water samples from the highest three concentrations in each series and stock solutions were collected before algae were added at the beginning of each experiment. Duplicate samples also were collected from the highest concentration in each series and stock solutions after 24 and 48 h to observe whether concentrations decreased during the experiment. Chemical test solutions were extracted from water samples and preconcentrated on a solid-phase, Bond-Elut C_{18} extraction column (Varian, Phenomenex, Macclesfield, UK). Test substances were eluted from the solid-phase cartridges by using high-performance liquid chromatography (HPLC)-grade solvents (Merck), which were subsequently evaporated in a nitrogen evaporator (model N-vap 112, Organonation Associated, Berlin, MA, USA), and the residue was resuspended in the relevant mobile phase. Solvents used for extraction and resuspension for each test substance are given in Table 2. Actual concentrations were determined with a high-performance liquid chromatograph fitted with an ultraviolet detector (analytical, model spectromonitor 3200

Table 2. Elutents, mobile phase, wavelength, retention time, and recovery for organic chemicals used during Bond-Elut extraction and high-performance liquid chromatography analysis[a]

Test substance	Elutent	Mobile phase	Wavelength (nm)	Retention time (min)	Mean recovery (%) (±SE, $n = 5$)
Permethrin	6 ml ACN:toluene (3:1, v/v)	ACN:H_2O (70:30, v/v)	210	6.5	72.18 (1.0)
λ-Cyhalothrin	6 ml ACN:toluene (3:1, v/v)	ACN:H_2O (80:20, v/v)	210	19	56.22 (5.2)
Pirimiphos-methyl	3 ml methanol, 2 ml toluene	ACN:H_2O (80:20,v/v)	247	6.0	71.4 (5.2)
Lindane	4 ml DCM, 1 ml methanol	ACN:H_2O (80:20, v/v)	205	5.6	68.04 (5.5)

[a] ACN = acetonitrile; DCM = dichloromethanol.

variable wavelength detector, Supelco), by injecting a 100-μl sample onto a Supelcosil 22021 07 LC-ABZ 5-μm, 250 × 4.6-mm column (Supelco), with the exception of lindane, which was injected onto a Phenomenex 412745 Phenosphere Next C_{18} 5-μm 250 × 4.6-mm column (Phenomenex, Macclesfield, UK). Flow rate for all test substances was set at 1 ml/min. Respective mobile phase, wavelengths, and retention times are given in Table 2. Recovery of test substances from water samples was determined by extracting and eluting standard solutions of a known concentration from Bond-Elut cartridges. Mean recoveries of test substances are given in Table 2. Fluoranthene analysis and recovery was as given in Barata and Baird [28]. Only the stock solution could be analyzed for λ-cyhalothrin, because all nominal exposure concentrations were below the 4-μg/L limit of detection. Detection limits for permethrin, pirimiphos-methyl, and lindane were 8, 2, and 15 μg/L, respectively.

Statistical analysis

The feeding IC50 represented the toxicant concentration that reduced feeding rate to 50% of that of the controls. This was calculated from feeding rate data obtained during the 24-h exposure period by fitting data to linear (lindane), *y*-square root (copper and zinc), or allosteric (copper, permethrin, λ-cyhalothrin, and fluoranthene) regression models, by means of SPSS® (Ver 7.5.2, Chicago IL, USA) by the least-squares method. Model accuracy was assessed using the adjusted coefficient of determination (r^2), and the significances of the regressions were determined by *F* tests. From each lethality test, a 48-h LC50 was calculated by the standard probit procedure [29]. Analyses for both tests used actual concentration data.

Absolute feeding rate data obtained during the 24-h exposure and 4-h postexposure period were converted to percentages of the control feeding rate. This allowed relative recovery to different substances to be compared directly, because different feeding period durations produce different mean baseline feeding rates. Post hoc analysis to identify the lowest-observed-effect concentration was carried out with the Williams test [30].

RESULTS AND DISCUSSION

Method development—Number of replicates required

No number of replicates tested reached the desired power of 0.8 when 20% was the specified minimum detectable difference between feeding rate means. Ten replicates, the highest number tested, only gave a power of 0.78. By extending of the number of samples tested, it was found that 11 replicates were required to reach the 0.8 power level. A power of 0.81 was achieved by using 11 replicates.

The number of replicates selected to use was five, which gave a power of 0.55. This number of replicates was chosen because of practical constraints. The numbers of animals required to give higher levels of replication than this become impracticable to produce in terms of time and expense of culturing daphnids and algae. Although a power of 0.55 proved to be suitable to use because subsequent statistical analysis of data consistently detected contaminant-induced effects on feeding rates, a high probability (45%) still existed of committing a type II error. A bioassay that has a high probability of committing type II errors (and thus a failure to detect effects) may not be suitable for use by other investigators. Therefore, to increase statistical power and confidence in the results obtained when employing five replicates, an increase in the minimum detectable difference would be necessary. Figure 1 shows power curves that demonstrated an increase in statistical power with increasing number of replicates used and increasing levels of minimum detectable differences of 20 to 30%. The minimum difference that five replicates could detect with 80% power was found to be 27%. Although a detectable difference of 27% between feeding rate means has the advantage of high statistical power (0.8),

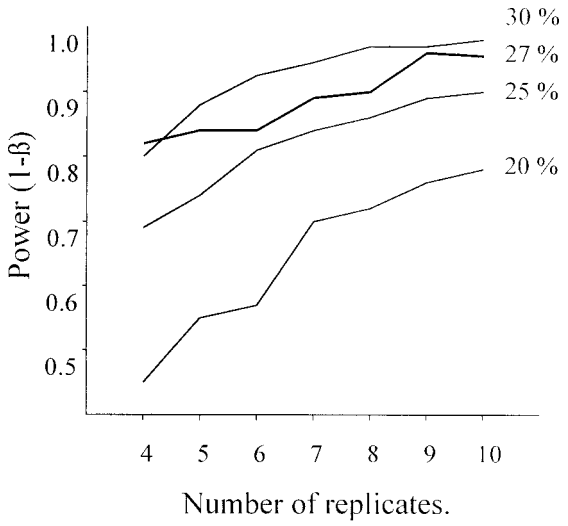

Fig. 1. Statistical power of postexposure feeding depression bioassay when different numbers of replicates are used, where minimum detectable differences of 20, 25, 27, and 30% between feeding rates are specified (when $p = 0.05$).

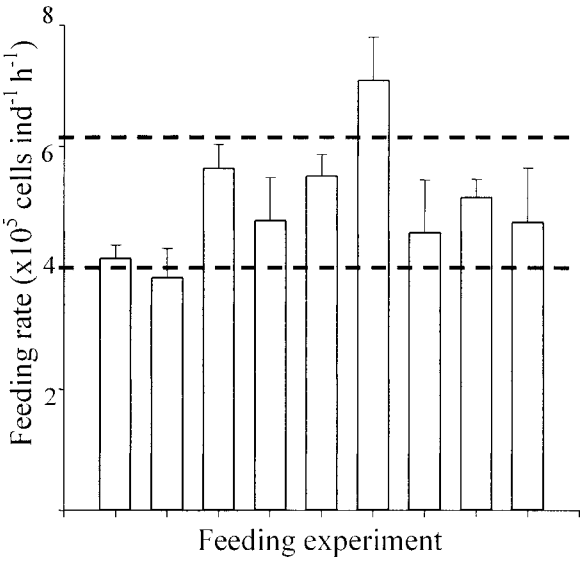

Fig. 2. Postexposure baseline (control) feeding rates obtained from all feeding experiments in this study. Dashed bars indicate the range in which most feeding rates lie. Error bars indicate standard deviations.

it has the disadvantage of not being able to detect smaller differences between feeding rates. The ability of this bioassay to detect small differences in feeding rates will be important in field studies, where contaminant-induced effects on feeding rates may be small because of low levels of contaminants or lower bioavailability of contaminants than in laboratory studies. Therefore, it may be advantageous to give priority to detection of smaller differences between feeding rate means, rather than very high statistical power. In this case, a power of 0.55 with five replicates should be suitable.

In summary, the variables selected and the method developed for the bioassay were as follows. For exposure, five replicates of five animals were placed in 175-ml glass jars containing 120 ml of ASTM hard water with *C. vulgaris* at a concentration of 5×10^5 cells/ml and allowed to feed for 24 h. This part of the bioassay will be adapted for use in the field in future. After exposure, animals were transferred with a 3-ml plastic pipette to 60-ml glass jars containing 60 ml of ASTM hard water with *C. vulgaris* at a concentration of 5×10^5 cells/ml and allowed to feed for 4 h. The mean algal concentration of three blank jars was used to calculate initial algae concentrations. This part of the bioassay would always be performed under the same conditions whether being used in the laboratory or the field.

Variation in baseline feeding rates

Preliminary experiments aimed to develop a method that minimized variation in baseline (control) feeding rates, which would allow the toxic signal to be reliably separated from background noise. Variation in postexposure baseline feeding rate was examined because this was the endpoint under study. The method outlined above produced postexposure baseline feeding rates that had low levels of variation. Control postexposure feeding rates for experiments in this study had coefficients of variation from 5 to 19%. Minimal variation in baseline feeding rates led to the production of a consistent baseline response, where feeding rates mostly were between 4 and 6×10^5 cells/individual/h during the postexposure period (see Fig. 2). Consistency in baseline feeding rates is advantageous because it offers quality assurance advantages, such as checking to determine if the test animals are in good health.

Endpoint consistency and sensitivity

Chemical analysis. Actual concentrations for all chemicals, with the exception of permethrin and lindane, differed by less than 10% from nominal concentrations; thus, nominal concentrations were used for all calculations. Actual permethrin concentrations were 53 ± 2.0% (SE) of nominal concentrations, and actual lindane concentrations were 10 ± 0.2% (SE) of nominal concentrations (because of the filtration step in preparing the stock solution; see Table 1). For subsequent calculations, nominal concentrations for permethrin and lindane were adjusted to actual measured concentrations. With the exception of copper, test substance concentrations did not decrease during the relevant 24- and 48-h experimental periods. Copper concentrations decreased by 48% after 48 h, but did not decrease during the first 24 h. Measurements of any decrease in permethrin or λ-cyhalothrin concentrations over the experimental periods were not obtained. Solomon et al. [31] reported that the time taken for a 50% decrease in permethrin concentrations in limnocorrals was 1.65 and 3.5 d for 0.5 μg/L and 50 μg/L. respectively. Because the concentrations and times used in this study are within this range, some decrease most likely would have occurred. Hill et al. [32], reported a half-life of 1 d for a λ-cyhalothrin formulation applied to mesocosms; thus, a decrease in test concentrations most likely would have occurred throughout the experimental periods.

Endpoint sensitivity (LC50 vs feeding IC50). From Table 3, it can be seen that for most test substances, the LC50 in ASTM hard water medium with food was greater than the LC50 in ASTM hard water medium without food, which was greater than the IC50. Lead or pirimiphos-methyl did not have an effect on exposure feeding rates; thus, an IC50 could not be obtained, but for both these chemicals the LC50 in medium with food was greater than the LC50 in medium without food. Exposure to fluoranthene had no effect on mortality rates below the solubility limit obtained in this study, in the presence or absence of food, so LC50 values could not be obtained. Di Toro et al. [33] reported the LC50 of fluoranthene to *D. magna* as 117 μg/L, although it was not stated if this was in the presence or absence of food. Because it was possible to obtain an IC50 value for fluoranthene below the solubility limit, the assumption also can be made that the LC50 was greater than the IC50. The consistent elevation of LC50 values in the presence of food seems to relate to enhanced resistance of fed individuals, rather than differences in exposure routes of test substances (G. Taylor, personal communication). Further evidence for this hypothesis was demonstrated by exposure of *D. magna* to permethrin during feeding and lethality experiments, where the IC50, tested in the presence of food, was greater than the LC50 without food. Overall, feeding depression could be seen to be a sensitive endpoint, because where feeding depression occurred during exposure, feeding IC50 values obtained were consistently lower than LC50 (with food) values for all chemicals tested.

Postexposure feeding depression

Figure 3 shows, with the exception of lead and pirimiphos-methyl, that substances that produced significant levels of feeding depression during exposure also produced significant levels of feeding depression after exposure (statistical details are given in Table 4) during direct exposure, allowing calculation of

Table 3. Forty-eight–hour median lethal concentration (LC50) values, in the presence and absence of food, and direct exposure feeding median inhibition concentration (IC50) values (both with 95% confidence limits), with adjusted coefficient of determination (r^2), indicating fitted model accuracy

Test substance	48-h LC50 (μg/L)		IC50 (μg/L)	r^2
	With food	Without food		
Cadmium	133[a]	120[a]	1.31 (1.0–1.7)	0.70*
Zinc	6,590 (3,176–16,349)	6,037 (3,567–11,138)	50.36 (22.8–94.5)	0.80*
Copper	58.77 (34.3–124.1)	24.93 (0.9–216)	12.13 (10.4–14.1)	0.84*
Lead	3,415 (3,436–3,586)	967 (109–1,774)	—	—
Permethrin	5.36 (2.5–10.6)	0.54 (0.03–19.3)	1.09 (0.1–1.2)	0.92*
λ-Cyhalothrin	0.23 (0.02–2.0)	0.10 (2×10^{-3}–0.4)	0.001 (5×10^{-4}–2×10^{-4})	0.92*
Pirimiphos-methyl	1.74 (0.9–3.3)	0.40 (0.1–1.9)	—	—
Lindane	218 (157–633)	162 (85–403)	27.82 (21.9–34.2)	0.90*
Fluoranthene	No mortality below solubility limit (100 μg/L)		70.0 (59.0–83.1)	0.64*

an IC50. Results for only four concentrations are shown for zinc (Fig. 3c and d) and pirimiphos-methyl (Fig. 3m and n). Although Figure 3m shows that significant levels of feeding of feeding depression occurred at concentrations of pirimiphos-methyl of 0.22 and 0.47 μg/L, animals were probably very close to death, making lethality the most important effect at that point. These concentrations were very close to the LC50 (with food) value of 1.74 μg/L, and data from the highest concentration (1.0 μg/L) were omitted because of excessive mortalities. No IC50 could be obtained as levels of feeding depression occurring at all concentrations were not substantial enough, indicating that pirimiphos-methyl has no great effect on feeding in *D. magna* under direct exposure over the period studied. Figure 3g clearly shows that lead has no effect on feeding, with no feeding rates at any concentration being significantly different from control feeding rates (statistical details are given in Table 4). Although the LC50 (with food) value for lead was considerably higher than the highest concentration used in the postexposure response experiment (3,414 μg/L and 1,700 μg/L, respectively), the concentration ranges used for each experiment overlapped. In the lethality test (with food), mortalities occurred at the highest concentration also used in the feeding test, indicating that lethality occurs before any effect on sublethal feeding responses. The LC50 (without food) value (967 μg/L) also fell within the concentration range used in the feeding test.

Figure 3 also shows that, with the exception of fluoranthene, all test substances that produced significant levels of feeding depression during exposure also produced significant levels of feeding depression after exposure (statistical details are given in Table 4). These test substances were cadmium (Fig. 3a and b), zinc (Fig. 3c and d), copper (Fig. 3e and f), permethrin (Fig. 3i and j), λ-cyhalothrin (Fig. 3k and l), and lindane (Fig. 3o and p). This indicates that for most test substances, when feeding depression occurred during exposure, this depression persisted in the postexposure period at significant levels. Where substances depressed feeding during exposure, Figure 3 demonstrates evidence of some recovery during the postexposure feeding period. This was most apparent with λ-cyhalothrin, where feeding rates recovered from 0 to 24% during exposure, to 5 to 62% after exposure. With the exception of copper, postexposure feeding rates did not mirror the pattern of feeding rates during exposure. This could indicate that recovery from contaminant exposure is not entirely dependent on exposure concentration. With the exception of copper, substance concentrations producing feeding rates significantly different from controls during exposure also produced significantly different feeding rates after exposure. Therefore, al-

Table 4. Details of Williams test performed on exposure and postexposure feeding rate data at the $\alpha = 0.05$ significance level

Test substance	Critical t value ($t_{k\alpha}$)	Degrees of freedom	Exposure t value	Significant[a]	Postexposure t-value	Significant[a]
Cadmium	1.86	20	12.16	Yes	5.78	Yes
Zinc	1.87	16	8.53	Yes	7.58	Yes
Copper	1.86	20	8.05	Yes	5.16	Yes
Lead	1.86	20	1.81	No	1.85	No
Permethrin	1.86	20	12.16	Yes	7.57	Yes
λ-Cyhalothrin	1.86	20	18.25	Yes	6.76	Yes
Pirimiphos-methyl	1.87	16	2.46	Yes	1.68	No
Lindane	1.86	20	10.49	Yes	3.68	Yes
Fluoranthene	1.86	20	7.65	Yes	1.55	No

[a] Significant at $p < 0.05$.

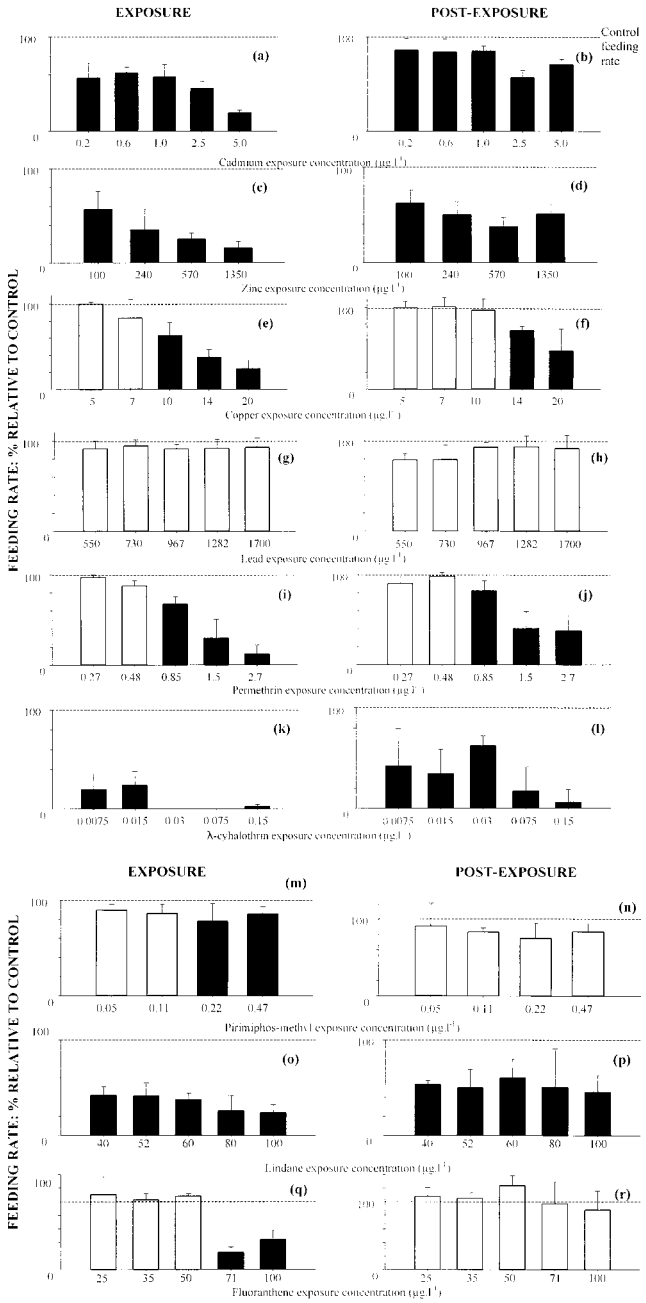

Fig. 3. Feeding rates of *Daphnia magna* as a percentage of control feeding rates during the 24-h exposure and 4-h postexposure periods of the bioassay. Control feeding rates are 100% and are indicated by the dashed bar. Black bars denote feeding rates significantly different from the control ($p < 0.05$). Error bars indicate standard deviations.

though evidence existed of recovery after exposure, feeding depression persisted. Exposure to copper at 10 µg/L produced feeding rates that were 63% of control feeding rates ($t = 3.91$, $t_{3,5} = 1.83$, $p < 0.05$; Fig. 1e), recovering to 96% of control feeding rates ($t = 0.36$, $t_{3,5} = 1.83$, $p > 0.05$; Fig. 1f) after exposure. Concentrations higher than this still produced significantly different feeding rates than in controls both during exposure and after exposure.

Test substances that produced feeding depression during both exposure and postexposure periods were cadmium (nonessential metal), zinc (essential metal), copper (essential metal), permethrin (sodium channel blocker [33]), λ-cyhalothrin (sodium channel blocker and γ-aminobutyric inhibitor [33]), and lindane (γ-aminobutyric inhibitor [33]). Fluoranthene, a polycyclic aromatic hydrocarbon only induced feeding depression during exposure, with feeding rates recovering to levels comparable with controls during the postexposure period. In the absence of photoactivation (which was the case here), fluoranthene has a narcotic mode of action [34]. Thus, these results indicate that *Daphnia* may recover rapidly from narcosis. Test substances that did not induce feeding inhibition during exposure or after exposure were lead (nonessential metal) and pirimiphos-methyl (acetylcholinesterase inhibitor [35]). No reports exist in the literature of either of these chemicals producing effects on feeding, although diazinon [36] and methyl parathion [37] (also acetylcholinesterase inhibitors) have been found to have negative effects on feeding in *D. magna*. Having no effect on feeding (both during exposure and after exposure) may reflect the mode action of these specific chemicals, but whether this occurs with any other organophosphates or polycyclic aromatic hydrocarbons would require further investigation. Recent work in our laboratory found that chlorpyrifos, an organophosphate, had no effect on *C. dubia* feeding rates (A. Treuner, personal communication). When examining chemicals that have no postexposure feeding response, a lethal endpoint could be used as a complement to the postexposure feeding endpoint. As previously demonstrated with lead, pirimiphos-methyl, and fluoranthene (see Table 3 and Fig. 3), lethality occurs at concentrations very close to or before any sublethal response can be measured, making it a valid endpoint to use under these circumstances.

Results demonstrated that a persistent feeding depression response occurred after exposure to most of the test substances, which had different modes of action and toxicities (demonstrated by IC50 values in Table 3). Feeding depression also was shown to occur below lethal levels for most test substances (Table 3). This indicates that postexposure feeding depression is a sensitive, robust response and has potential use as a bioassay endpoint. With use of lethality as a complementary endpoint, a wide range of substances can be detected, further enhancing the ability of a bioassay that uses these dual endpoints to detect toxicity.

Interest has been increasing in the use of single-species in situ bioassays because they can be used to complement more traditional methods of water quality assessment such as whole effluent toxicity tests and sampling of the resident benthic macroinvertebrate community to derive biotic indices. If used together in a weight-of-evidence approach, in situ bioassays offer certain advantages that can be used to overcome weaknesses associated with traditional assessment tools. In situ bioassays can help overcome some disadvantages of laboratory whole effluent toxicity tests because physical, chemical, and biological processes that cannot be reproduced in the laboratory can be integrated into the test; and because artifacts associated with laboratory testing, such as the collection and storage of samples, can be eliminated [38]. In situ bioassays also can give a rapid indication of water quality, because effects measured at the individual level often will be manifested more rapidly (hours to days) than resulting changes in community structure (months to years) measured during macroinvertebrate sampling [1]. Community-level effect measures are insensitive to sublethal levels of stress [39]; therefore, in situ bioassays that employ more sensitive, sublethal endpoints offer the potential for measuring impacts at an earlier stage. Short-term results gained from in situ bioassay deployments therefore could be useful in determining the initial causes of long-term

alterations in macroinvertebrate community structure. An in situ bioassay employing a sublethal endpoint with *D. magna* as test organism therefore should provide a sensitive, ecologically relevant tool for detecting potential toxic impacts. In this respect, the postexposure feeding depression bioassay would be suitable for adaptation for use in the field because minimal variation in baseline feeding rates, known statistical power (0.55), and a sensitive (when used in combination with lethality because some substances have no effect on postexposure feeding rates), robust endpoint should allow successful detection of contaminants. Therefore, future work will include adaptation of the laboratory bioassay for use in the field and deployment at contaminated field sites to assess its ability to detect toxicity in situ.

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