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Effects of Dietary Cd and Cu on Feeding and Growth Rates of the Landsnail *Helix engaddensis*

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Effects of dietary cadmium and copper on feeding and growth rates of adult *Helix engaddensis* snails were studied for a 1-month period. The concentrations of cadmium were 0, 50, 100, 200, 400, and 800 μg Cd/g dry food, while those of copper were 0, 4, 20, 100, 500, and 2500 μg Cu/g dry food. Both metals inhibited feeding and growth rates significantly after 1 week and 3 weeks of exposure to Cd and Cu, respectively. Inhibition caused by Cd was found to be irreversible, which indicates toxicity, while that of Cu was reversible, indicating starvation because snails identified and refused to consume contaminated food. After 4 weeks of exposure, the NOEC of Cd was 50 μg/g and the LOEC was 100 μg/g, while those for Cu were 20 and 100 μg/g, respectively. $EC_{50,75,90,100}$ (growth and feeding) values were calculated, and indicated that *H. engaddensis* snails are suitable for short-term toxicity bioassays. © 2000 Academic Press

Key Words: **landsnail; *Helix engaddensis*; copper; cadmium; feeding; growth.**

INTRODUCTION

Snails and slugs are among the most important terrestrial bioindicators of metal pollution because they are able to accumulate large quantities of metals in their tissues (Berger and Dallinger, 1989). Therefore, terrestrial molluscs may play a major role in food chain transport of metals (Van Straalen and Ernst, 1991). The high capacity of snails to accumulate metals has been attributed to the synthesis of metal-binding proteins (metallothioneins) and to the deposition of some metals in insoluble intracellular granules. The kinetics of metal accumulation and detoxification is still the subject of discussion. Berger and Dallinger (1989) found that terrestrial snails might regulate some metals assimilated from food. Van Straalen *et al.* (1987) suggested that nutritional metals might be regulated, while xenobiotic metals are accumulated. This might not be applicable to animals with hemocyanin, such as isopods and gastropods. These two groups accumulate Cu over a wide range of environmental concentrations (Hopkin, 1990). In addition, Laskowski and Hopkin (1996a) found that snails are more important pathways for transfer along food chains of Cu and Cd than Zn and Pb.

Using snails in toxicity bioassays is an attractive method since snails are easy to culture in the laboratory and can be fed artificial diets with the desired amounts of metals, and they respond quickly to metal contamination in the range of sublethal doses. However, this might be complicated by the fact that snails fed on diets supplemented with metals may decrease food consumption or even estivate and stop feeding and, hence, decrease growth rates (Simkiss and Watkins, 1990; Laskowski and Hopkin, 1996b). This means that high metal concentrations in food affect population growth rates (Laskowski and Hopkin, 1996b). This effect could be due to direct poisoning, starvation, or both. Laskowski and Hopkin (1996a) suggested that the absence of *Helix aspersa* from the immediate vicinity of a factory was due to a combination of metal toxicity and prolonged estivation due to rejection of aerially contaminated food by snails. According to Gomot (1997), the mechanism involved in the inhibition of growth of snails fed on metal-supplemented food is still unknown.

The purposes of the present study were (1) to evaluate the dose-dependent effects of dietary Cd (nonessential metal) and Cu (essential metal) on growth and feeding rates of the snail *Helix engaddensis*; (2) to examine the suitability of this snail to be used in laboratory short-term toxicity bioassays; (3) to estimate the concentrations of Cd and Cu (EC) that reduce growth and feeding rates by 50, 75, 90, and 100% compared to the control; (4) to estimate the no-observed-effect concentration (NOEC) and the lowest-observed-effect concentration (LOEC) for both metals; and (5) to compare the sensitivity of the snail to both metals and to check whether feeding and growth inhibition caused by Cu and Cd is reversible when the metals are no longer added to the diet of the snail.

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MATERIALS AND METHODS

Collection and Culture of Snails

H. engaddensis adult snails were collected from a house garden in Qalqilia and cultured in the laboratory in large glass aquaria. The snails originate from a population that is genetically isolated for more than 15 years. Snails were fed on carrots and lettuce and kept at room temperature. Before starting the experiment, snails of similar weight were selected, cleaned, and kept in transparent plastic boxes (size $17 \times 13 \times 7$ cm). Each box contained 10 snails (Table 1). Boxes were perforated at their sides to allow proper aeration. The bottom of each box was covered by a thin sponge soaked with deionized water to keep 100% humidity. The experiments were run in a growth chamber at 15°C and 16/8 h light/dark period. Before starting the experiments, snails were offered an artificial control food for 3 days in order to acclimatize.

Preparation of Food

Metal stock solutions (1 g/L) were prepared using cupric chloride dihydrate ($CuCl_2 \cdot 2H_2O$, Sigma) and cadmium nitrate tetrahydrate ($Cd(NO_3)_2 \cdot 4H_2O$, Hopkin & Williams). An artificial food containing ca. 5% dry mass was prepared by mixing 4 g Cerelac—with vegetables—baby food (Nestlé, Belgium) and 1 g Agar (Sigma, St. Louis, MD) with solutions containing the required concentrations of metals to give 100 ml of agar medium. A fungicide (*p*-hydroxy bezoic acid methyl ester = methyl paraben, Sigma) was added to the solutions as 0.3 ml/100 ml food. Each 100 ml medium was divided equally among four Petri dishes (25 ml/dish). After cooling, Petri dishes were kept in the refrigerator. Foods containing required concentrations of each metal were prepared (Table 1). Control food was prepared the same way except using distilled water instead of the above-described metal solutions.

TABLE 1
Concentrations of Cadmium and Copper Added to the Diet of Each Group of *Helix engaddensis* Snails and the Average Weight of Snails in Each Group at the Start of the Experiment

Group and metal	Metal concentration in the diet ($\mu g \cdot g^{-1}$)	Average weight ± SE (g)
G1-Cd	0 (control)	7.02 ± 0.16
G2-Cd	50	7.02 ± 0.20
G3-Cd	100	7.02 ± 0.22
G4-Cd	200	6.99 ± 0.24
G5-Cd	400	7.00 ± 0.25
G6-Cd	800	7.07 ± 0.21
G1-Cu	0 (control)	7.01 ± 0.17
G2-Cu	4	6.85 ± 0.12
G3-Cu	20	7.05 ± 0.16
G4-Cu	100	6.91 ± 0.16
G5-Cu	500	6.88 ± 0.15
G6-Cu	2500	6.99 ± 0.15

Feeding of Snails

Snails were offered food *ad libitum*. Boxes were examined daily and food was offered as required. Once every 7 days, the snails were weighed and the boxes were cleaned. Unconsumed food that remained in the Petri dishes was freed of feces and dried in an oven at 60°C until a constant weight was obtained. Knowing that the dry mass of food in each dish was about 1.25 g, the dry weight of consumed food was calculated for each group on a weekly basis. During the first 4 weeks of the experiment, snails were fed metal-contaminated food. Thereafter, all groups of snails were fed food with no metals added (control food) for another 2 weeks.

Statistical Analysis

All statistical tests were performed using SYSTAT for Windows 5.02 (SYSTAT, Inc., Evanston, IL, 1993). $P < 0.01$ was used in all tests to determine statistical significance. Each week, average weights of groups were tested for differences in their mean weights using ANOVA tests. Thereafter, Tukey tests were performed for pairwise comparisons between groups. The growth coefficient for each group was calculated weekly as mean weight of that group × 100/mean weight at the start of the experiment (Gomot, 1997). The values of the growth coefficient obtained each week were plotted against log values of the concentrations of metals used in the experiment. The straight-line equation obtained enabled the calculations of the EC that reduce growth rate by 50, 75, 90, and 100% compared to the control (EC_G). Feeding rates were calculated as the average dry food (mg) consumed by every snail each week. Food consumption values were transformed into percentages of food consumption compared to the control group, which was considered 100%. Percentages were plotted against log the concentrations of each metal studied. This semi-log regression enabled the calculations of the EC that reduce food consumption by 50, 75, 90, and 100% (EC_C).

RESULTS

Mortality

During the 6 weeks of the experiment, 11 of 120 snails died. From the snails fed on Cd-contaminated diet, 10 deaths were reported. These were distributed as 2 from group 4, 4 from group 5, and 6 from group 6. The death case observed among snails fed on Cu-contaminated food was from group 6. All death cases occurred between the third and the sixth week of the experiment.

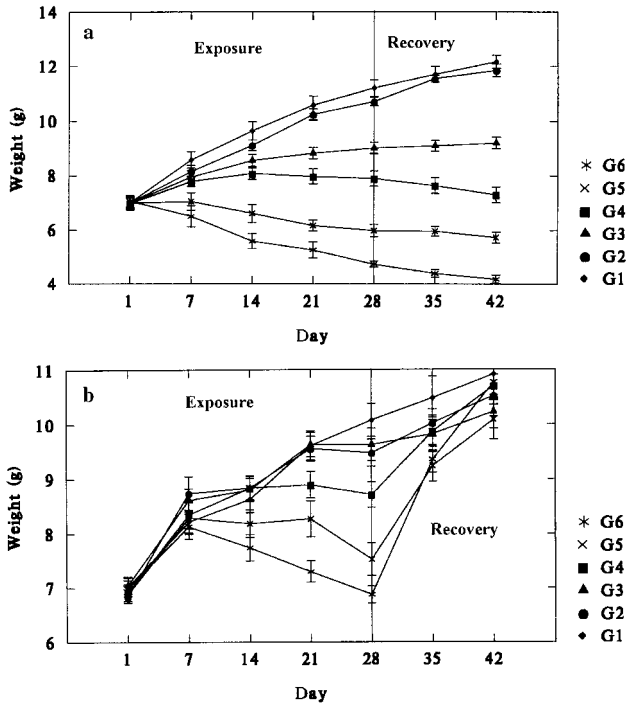

FIG. 1. Growth curves of *Helix engaddensis* snails exposed to cadmium-contaminated food (a) and copper-contaminated food (b) for 28 days (Exposure) and offered control food for another 14 days (Recovery). Cadmium concentrations were G1 = 0, G2 = 50, G3 = 100, G4 = 200, G5 = 400, and G6 = 800 μg/g dry food. Copper concentrations were G1 = 0, G2 = 4, G3 = 20, G4 = 100, G5 = 500, and G6 = 2500 μg/g dry food. Values represent means ± standard errors.

Growth

Effect of dietary cadmium on the growth of *H. engaddensis* was obviously dose-dependent (Fig. 1a). The inhibitory effect of Cd on growth of snails was distinct after 1 week of exposure to dietary concentrations of 400 and 800 $\mu g \cdot g^{-1}$. These concentrations inhibited growth completely. Snails in the two groups estivated most of the time during the experiment and started a negative growth period that continued over the whole period of the experiment (Table 2). Snails exposed to Cd concentrations less than 400 $\mu g \cdot g^{-1}$ continued to grow at slower rates than the control. At the end of the 4 weeks of exposure, average weights of all groups were significantly less than the average weights of G1 (control) and G2 (50 $\mu g \cdot g^{-1}$) (Table 2). Therefore, the highest dietary concentration of Cd that did not indicate any inhibitory effect on growth (NOEC) after 4 weeks of exposure was 50 $\mu g \cdot g^{-1}$ and the lowest concentration that slowed growth rate (LOEC) was 100 $\mu g \cdot g^{-1}$. From the first week of the experiment, a statistically significant relationship continued to exist between growth coefficients and concentrations of cadmium in the diet. This enabled the calculations of $EC_{G50,75,90,100}$ for each of the 4 weeks of exposure (Table 3). All EC_G values decreased from Week 1 on. For example, the EC_{100G} decreased from 430 $\mu g \cdot g^{-1}$ after Week 1 to 270 $\mu g \cdot g^{-1}$ after Week 4. At the end of the 4 weeks of exposure, all snail groups were offered control food (no Cd was added) for 2 weeks, during which snails did not exhibit significant signs of recovery. Average weights of all groups (except 1 and 2) either remained constant or continued to decrease (Fig. 1a). Significant differences in average weight between the control group and groups 3 (100 $\mu g \cdot g^{-1}$), 4 (200 $\mu g \cdot g^{-1}$), 5 (400 $\mu g \cdot g^{-1}$), and 6 (800 $\mu g \cdot g^{-1}$) continued to exist until the end of the experiment (Table 2).

Effect of dietary copper on growth of snails was different from that of Cd. No inhibitory effect of copper was clear during the first 2 weeks of the experiment (Fig. 1b). By the

TABLE 2
Effect of Dietary Cadmium and Copper on Average Weight of *Helix engaddensis* Snails

	Mean weight (g) (± SE)					
Group	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
G1-Cd	8.58 ± 0.31	9.64 ± 0.33	10.58 ± 0.32	11.21 ± 0.30	11.70 ± 0.29	12.16 ± 0.24
G2-Cd	8.16 ± 0.21	9.11 ± 0.19	10.24 ± 0.20	10.70 ± 0.15	11.54 ± 0.13	11.85 ± 0.23
G3-Cd	7.95 ± 0.22	8.55 ± 0.22	8.82 ± 0.20*	9.01 ± 0.20*	9.10 ± 0.19*	9.20 ± 0.21*
G4-Cd	7.79 ± 0.19	8.07 ± 0.21*	7.98 ± 0.27*	7.90 ± 0.28*	7.65 ± 0.29*	7.31 ± 0.28*
G5-Cd	7.06 ± 0.32*	6.62 ± 0.33*	6.17 ± 0.20*	5.98 ± 0.23*	5.90 ± 0.18*	5.73 ± 0.21*
G6-Cd	6.51 ± 0.40*	5.58 ± 0.28*	5.26 ± 0.30*	4.74 ± 0.12*	5.73 ± 0.14*	4.17 ± 0.15*
G1-Cu	8.22 ± 0.20	8.64 ± 0.25	9.61 ± 0.27	10.07 ± 0.30	10.48 ± 0.40	10.91 ± 0.43
G2-Cu	8.75 ± 0.30	8.84 ± 0.21	9.55 ± 0.23	9.48 ± 0.25	10.02 ± 0.25	10.52 ± 0.38
G3-Cu	8.62 ± 0.21	8.80 ± 0.21	9.63 ± 0.22	9.63 ± 0.30	9.82 ± 0.33	10.23 ± 0.30
G4-Cu	8.35 ± 0.25	8.84 ± 0.22	8.90 ± 0.24	8.72 ± 0.23*	9.87 ± 0.25	10.70 ± 0.34
G5-Cu	8.29 ± 0.29	8.18 ± 0.26	8.27 ± 0.33*	7.53 ± 0.30*	9.24 ± 0.28	10.09 ± 0.36
G6-Cu	8.13 ± 0.23	7.74 ± 0.25	7.30 ± 0.20*	6.87 ± 0.16*	9.36 ± 0.24	10.77 ± 0.33

Note. From Weeks 1 to 4 snails were offered metal-contaminated diets. During Weeks 5 and 6 all snail groups were offered control food.
* Mean weight is significantly different from that of the control ($P < 0.01$).

TABLE 3
Weekly Estimated Effects of Dietary Cadmium and Copper on Growth of the Snail *Helix engaddensis* Exposed to Cadmium and Copper for 4 Weeks

Week	Cd (50–800 $\mu g \cdot g^{-1}$ food)	Cu (4–2500 $\mu g \cdot g^{-1}$ food)
1	Growth inhibition according to $Y = 53.28 - 20.20 \log(X)$, $r = 0.96$, $P = 0.010$ $EC_{50G} = 120\ \mu g \cdot g^{-1}$; $EC_{75G} = 230\ \mu g \cdot g^{-1}$; $EC_{90G} = 340\ \mu g \cdot g^{-1}$; $EC_{100G} = 430\ \mu g \cdot g^{-1}$; $NOEC = 200\ \mu g \cdot g^{-1}$; $LOEC = 400\ \mu g \cdot g^{-1}$	Relationship was not significant
2	Growth inhibition according to $Y = 106.81 - 42.89 \log(X)$, $r = 0.98$, $P = 0.004$ $EC_{50G} = 110\ \mu g \cdot g^{-1}$; $EC_{75G} = 190\ \mu g \cdot g^{-1}$; $EC_{90G} = 250\ \mu g \cdot g^{-1}$; $EC_{100G} = 310\ \mu g \cdot g^{-1}$; $NOEC = 100\ \mu g \cdot g^{-1}$; $LOEC = 200\ \mu g \cdot g^{-1}$	Relationship was not significant
3	Growth inhibition according to $Y = 147.66 - 59.97 \log(X)$, $r = 0.99$, $P = 0.001$ $EC_{50G} = 120\ \mu g \cdot g^{-1}$; $EC_{75G} = 190\ \mu g \cdot g^{-1}$; $EC_{90G} = 240\ \mu g \cdot g^{-1}$; $EC_{100G} = 290\ \mu g \cdot g^{-1}$; $NOEC = 50\ \mu g \cdot g^{-1}$; $LOEC = 100\ \mu g \cdot g^{-1}$	Growth inhibition according to $Y = 50.68 - 12.39 \log(X)$, $r = 0.97$, $P = 0.007$ $EC_{50G} = 400\ \mu g \cdot g^{-1}$; $EC_{75G} = 2210\ \mu g \cdot g^{-1}$; $EC_{90G} = 6190\ \mu g \cdot g^{-1}$; $EC_{100G} = 12320\ \mu g \cdot g^{-1}$; $NOEC = 100\ \mu g \cdot g^{-1}$; $LOEC = 500\ \mu g \cdot g^{-1}$;
4	Growth inhibition according to $Y = 172.99 - 71.16 \log(X)$ $r = 0.99$, $P < 0.000$ $EC_{50G} = 100\ \mu g \cdot g^{-1}$; $EC_{75G} = 170\ \mu g \cdot g^{-1}$; $EC_{90G} = 220\ \mu g \cdot g^{-1}$; $EC_{100G} = 270\ \mu g \cdot g^{-1}$; $NOEC = 50\ \mu g \cdot g^{-1}$; $LOEC = 100\ \mu g \cdot g^{-1}$	Growth inhibition according to $Y = 52.51 - 15.36 \log(X)$ $r = 0.97$, $P = 0.005$ $EC_{50G} = 100\ \mu g \cdot g^{-1}$; $EC_{75G} = 520\ \mu g \cdot g^{-1}$; $EC_{90G} = 1370\ \mu g \cdot g^{-1}$; $EC_{100G} = 2620\ \mu g \cdot g^{-1}$; $NOEC = 20\ \mu g \cdot g^{-1}$; $LOEC = 100\ \mu g \cdot g^{-1}$

Note. EC_{50G}, estimated concentration that reduces growth rate to 50% compared to the control; NOEC, no-observed-effect concentration; LOEC, lowest-observed-effect concentration.

end of the third week, average weights of groups 5 and 6 were significantly less than that of the control group (Table 2). After 4 weeks of exposure, average weights of groups, 4, 5, and 6 (Cu concentrations: 100, 500, and 2500 $\mu g \cdot g^{-1}$, respectively) were significantly less than the average weight of the control group. Groups 2 and 3 (Cu concentrations: 4 and 20 $\mu g \cdot g^{-1}$ respectively) did not reveal any significant inhibitory effect during the 4 weeks of exposure (Fig. 1b and Table 2). When all snail groups were offered control food by the end of the fourth week, snails of groups 4, 5, and 6 stopped estivation and started to recover and gain weight quickly (Fig. 1b). At the end of the sixth week, average weights of all snail groups were similar and all statistical differences between groups in average weight disappeared (Table 2). The highest concentration of dietary Cu that did not have any inhibitory effect (NOEC) after 4 weeks of exposure was 20 $\mu g \cdot g^{-1}$, while the lowest concentration that slowed growth rate (LOEC) was 100 $\mu g \cdot g^{-1}$. A significant relationship between Cu concentration in the diet and growth coefficients of groups was first established after the third week of exposure. $EC_{C50,75,90,100}$ decreased rapidly from the third to the fourth week (Table 3). For example, the EC_{100C} decreased from 12,320 $\mu g \cdot g^{-1}$ at the end of the third week to 2620 $\mu g \cdot g^{-1}$ at the end of the fourth week.

Feeding

Feeding rates of snails expressed as milligrams food per snail are presented in Fig. 2. Feeding rate of snails offered Cd-contaminated diet was clearly dose-dependent. Effect of dietary Cd was obvious from the first week of the experiment. This was clear by the decrease in feeding rate from 350 mg/snail (control group) to 70 mg/snail (G6 = 800 μg Cd/g food). Feeding rates continued to decrease with time of the experiment in a manner similar to that of growth rates. During the fourth week of exposure, snails of group 6 refused to consume food and remained in estivation. Even after control food was offered, feeding rates of snails of groups 4, 5, and 6 remained much lower than those of the control group. Snails of G3 started to improve food consumption during the fifth and sixth weeks of the experiment until food consumption reached 43% of the control (Fig. 2a). By the end of the experiment, food consumption of G2 was about 88% of the control. Values of $EC_{C50,75,90,100}$ were estimated on a weekly basis (Table 4).

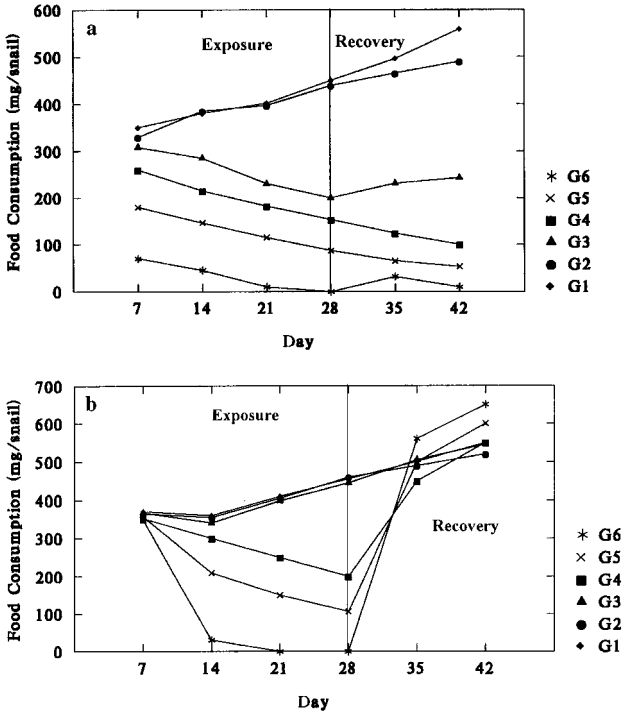

FIG. 2. Food consumption of *Helix engaddensis* snails exposed to cadmium-contaminated food (a) and copper-contaminated food (b) for 28 days (Exposure) and offered control food for another 14 days (Recovery). Cadmium concentrations were G1 = 0, G2 = 50, G3 = 100, G4 = 200, G5 = 400, and G6 = 800 μg/g dry food. Copper concentrations were G1 = 0, G2 = 4, G3 = 20, G4 = 100, G5 = 500, and G6 = 2500 μg/g dry food.

Effect of dietary copper on feeding rates of snails started to be noticeable by the end of the second week (Fig. 2b). A clear decrease in food consumption of snails receiving more than 200 $\mu g \cdot g^{-1}$ occurred during the third and fourth weeks. During this period, snails of group 6 (2500 $\mu g \cdot g^{-1}$) refused to consume food completely and spent these 2 weeks in estivation. Concentration of Cu less than 100 $\mu g \cdot g^{-1}$ indicated no obvious inhibitory effects on food consumption. During the 2 weeks of recovery, snails of groups 4, 5, and 6 ended their estivation period and started to consume food at exceptionally high rates (higher than the control in some cases). Equations for the inhibitory effect of dietary Cu on food consumption were first established after the third week of exposure. Values of $EC_{C50,75,90,100}$ are shown in Table 4.

DISCUSSION

In this experiment, the mortality rate among *H. engaddensis* snails fed Cd-contaminated diet was about 16.7% for doses between 50 and 800 μg Cd/g food. This figure looks higher than the 11–13% noted by Berger *et al.* (1993) in *H. pomatia* adults for doses between 10 and 100 μg Cd/g food. Gomot (1997) observed much lower death rates among *H. asperas aspersa* and *H. aspersa maxima* juveniles fed Cd-contaminated diet (range 0–800 μg Cd/g food). During the 4 weeks of exposure, 5% (3/60) of *H. aspersa aspersa* and 6.7% (4/60) of *H. aspersa maxima* died. Deaths were distributed among all groups, including the control. Laskowski and Hopkin (1996b) studied the effect of Zn, Cu, Pb, and Cd on fitness of *H. aspersa*. They found no relationship between mortality of juveniles or adults and concentration of any metal or combination of metals. Mortality rate was 6.7% among juveniles and 1.9% among adults during the 4 months of the experiment. In this experiment, mortality rate among snails fed on Cu-contaminated diet was 1.7%, a figure very close to that obtained by the last authors for adult snails. According to Gomot (1997), juvenile snails are not sensitive for short-term environmental pollution since they are more homogeneous and resistant than adults. This could explain the high mortality rate among our snails fed Cd-contaminated diets. Snails are known to be less sensitive to Cu than Cd and are able to accumulate large quantities of it (Hopkin, 1990), hence the low mortality rate among snails fed on Cu-contaminated food compared to Cd.

Both Cu and Cd in the diet were found to inhibit growth of *H. engaddensis* in a dose-dependent manner (Fig. 3). The inhibitory effect of the two metals is variable. The inhibitory effect of cadmium in the diet at sublethal doses was significant after the first week of exposure. Copper did not inhibit growth significantly during the first 2 weeks of exposure. Snails fed Cu-contaminated diet continued to feed and grow during this period. According to Hopkin (1993), snails assimilate and accumulate Cu efficiently from food may be because in natural environments Cu always occurs in concentrations near the minimum nutritional requirements of these animals. Therefore, it appears that snails spent the first 2 weeks of the experiment accumulating Cu from the diet. As a result of that, Cu reached high concentrations inside the snail, causing them to reduce or stop feeding and, hence, growing. This indicates that snails are able to detect high concentrations of Cu, and may be other essential metals, in the diet and can respond to that by reducing or stopping contaminated food consumption before toxicity takes place. This is supported by the fact that snails started feeding and growth immediately after being offered control food during the fifth and sixth weeks. This is in accordance with the suggestion of Simkiss and Watkins (1990), that *H. aspersa* is able to detect high concentration of zinc in the diet and reduce its feeding rate if contamination is too high. Other test organisms, such as the isopod *Porcellio scaber*, indicated significant reduction in feeding rate when fed zinc-contaminated plant leaves (Drobne and Hopkin, 1995). Metal-polluted river sediments were found to reduce feeding of the midge larvae *Chironomus riparius* (Lepänen *et al.*, 1998). With regard to cadmium, the story seems to be totally different. Snails fed on Cd-contaminated diets reduced or

TABLE 4
Weekly Estimated Effects of Dietary Cadmium and Copper on Food Consumption of the Snail *Helix engaddensis* Exposed to Metals for 4 Weeks

Week	Cd (50–800 $\mu g \cdot g^{-1}$ food)	Cu (4–2500 $\mu g \cdot g^{-1}$ food)
1	Food consumption inhibition according to $Y = 207.42–61.7 \log(X)$, $r = 0.96$, $P = 0.004$ $EC_{50C} = 360\ \mu g \cdot g^{-1}$; $EC_{75C} = 900\ \mu g \cdot g^{-1}$; $EC_{90C} = 1580\ \mu g \cdot g^{-1}$; $EC_{100C} = 2300\ \mu g \cdot g^{-1}$	Relationship was not significant
2	Food consumption inhibition according to $Y = 244.08–73.15 \log(X)$, $r = 0.99$, $P = 0.000$ $EC_{50C} = 240\ \mu g \cdot g^{-1}$; $EC_{75C} = 530\ \mu g \cdot g^{-1}$; $EC_{90C} = 840\ \mu g \cdot g^{-1}$; $EC_{100C} = 1160\ \mu g \cdot g^{-1}$	Relationship was not significant
3	Food consumption inhibition according to $Y = 215.4–73.42 \log(X)$, $r = 0.98$, $P = 0.004$ $EC_{50C} = 180\ \mu g \cdot g^{-1}$; $EC_{75C} = 390\ \mu g \cdot g^{-1}$; $EC_{90C} = 630\ \mu g \cdot g^{-1}$; $EC_{100C} = 860\ \mu g \cdot g^{-1}$	Food consumption inhibition according to $Y = 132.56–36.94 \log(X)$, $r = 0.97$, $P = 0.005$ $EC_{50C} = 170\ \mu g \cdot g^{-1}$; $EC_{75C} = 820\ \mu g \cdot g^{-1}$; $EC_{90C} = 2080\ \mu g \cdot g^{-1}$; $EC_{100C} = 3880\ \mu g \cdot g^{-1}$
4	Food consumption inhibition according to $Y = 207.3–73.12 \log(X)$, $r = 0.95$, $P = 0.01$ $EC_{50C} = 140\ \mu g \cdot g^{-1}$; $EC_{75C} = 310\ \mu g \cdot g^{-1}$; $EC_{90C} = 500\ \mu g \cdot g^{-1}$; $EC_{100C} = 680\ \mu g \cdot g^{-1}$	Food consumption inhibition according to $Y = 132.13–39.51 \log(X)$, $r = 0.97$, $P = 0.005$ $EC_{50C} = 120\ \mu g \cdot g^{-1}$; $EC_{75C} = 510\ \mu g \cdot g^{-1}$; $EC_{90C} = 1230\ \mu g \cdot g^{-1}$; $EC_{100C} = 2210\ g \cdot g^{-1}$

Note. EC_{50C}, estimated concentration that reduces food consumption to 50% compared to the control.

stopped food consumption and growth in a progressive manner from Week 1 to Week 4. When offered control food during the fifth and sixth weeks, snails failed to consume food and growth inhibition continued. Thus, it is assumed that toxiciy to snails fed Cd-contaminated diets is irreversible. The ability of snails to distinguish metal-contaminated food is shared by other test organisms such as woodlice. For example, the woodlouse *Porcellio laevis* was found to be able to distinguish and avoid cadmium-contaminated plant leaves (Odendaal and Reinecke, 1999). The mechanism involved in growth inhibition of organisms exposed to elevated metals in the diet is still unknown (Gomot, 1997). In snails of the genus *Helix*, a growth hormone, necessary for growth, is secreted by the neurosecretory cells of the mesocerebrum–supraoesophageal area of the nerve collar (Gomot *et al*., 1992). Cadmium may disturb the function of the neurosecretory cells of the mesocerebrum causing "growth stoppage" (Gomot, 1997). Szücs *et al*. (1994) found that acute exposure to Cd of neurons of the nerve collars of *Lymnaea stagnalis* can reversibly block the Ca channels, whereas chronic exposure can irreversibly modify the structure of the channel. Other studies suggest that Cd may block calcium uptake through the gut, causing calcium deficiency with disturbance of Ca^{2+} homeostasis (Schoenmakers *et al*., 1992) or may alter food intake by an inhibiting action on the nerve centers (Gomot, 1997). Metals were found to reduce growth of other test organisms such as the earthworm *Eisenia fetida* (Spurgeon and Hopkin, 1996). They attributed the effect of metals on growth and maturation time to the direct toxicity of metals and to changes in the "scope of growth" of the exposed worms. Copper-contaminated microalgae were found to affect growth of rotifers, causing a delay of 1 or 2 days in populational development (Moreno-Garrido *et al*., 1999). The present results indicate that the inhibitory effect of Cd on feeding and growth is irreversible. This is not in agreement with Szücs *et al*. (1994). It is thought that growth inhibition is due to starvation and not, at least totally, due to effects on growth hormone production, as suggested by Gomot (1997). If the suggestion of Gomot (1997) were true, then snails should have consumed control food without weight gain. Because they continued to refuse to feed even when control food was offered, the other possibility, suggested by the same author, that cadmium might alter food intake by an inhibiting action on the nerve centers, may be true.

When compared to *H. aspersa*, *H. engaddensis* seems to be more sensitive to Cd. Values of EC_G presented in Table 5 indicate that *H. aspersa maxima* is clearly less sensitive to cadmium than both *H. engaddensis* and *H. aspersa aspersa*. As mentioned earlier, in the current study snails were adults, while the snails used in the experiment of Gomot (1997) were juveniles. This could account, at least partially, for the higher sensitivity exhibited by our snails. Moreover, *H. engaddensis* snails are relatively slow growing compared to *H. aspersa*, which are fast growing (Gomot, 1994). Fast growth dilutes metal content of molluscs, especially in cases

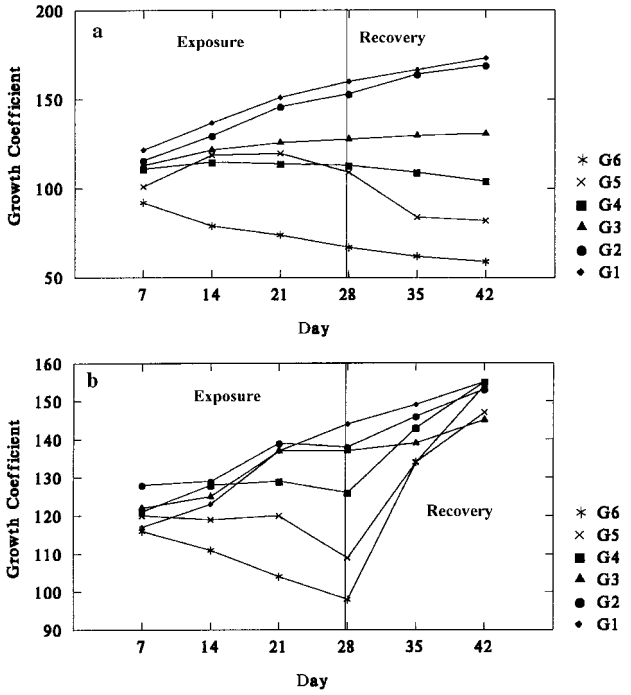

FIG. 3. Growth coefficients of *Helix engaddensis* snails exposed to cadmium-contaminated food (a) and copper-contaminated food (b) for 28 days (Exposure) and offered control food for another 14 days (Recovery). Cadmium concentrations were G1 = 0, G2 = 50, G3 = 100, G4 = 200, G5 = 400, and G6 = 800 μg/g dry food. Copper concentrations were G1 = 0, G2 = 4, G3 = 20, G4 = 100, G5 = 500, and G6 = 2500 μg/g dry food.

where tissue deposition exceeds metal accumulation (Swaileh, 1996). Therefore, fast growth might contribute to the lower sensitivity to Cd exhibited by *H. aspersa*.

TABLE 5

Comparison between *Helix engaddensis*, *Helix aspersa maxima* and *Helix aspersa aspersa* in Sensitivity to Dietary Cadmium When Exposed for 2 and 4 Weeks

	H. a. maxima[a] ($\mu g \cdot g^{-1}$)	*H. a. aspersa*[a] ($\mu g \cdot g^{-1}$)	*H. engaddensis* ($\mu g \cdot g^{-1}$)
Week 2			
EC_{50G}	180	180	110
EC_{75G}	470	370	190
EC_{90G}	850	570	250
EC_{100G}	1235	760	310
NOEC	50	50	100
LOEC	100	100	200
Week 4			
EC_{50G}	120	140	100
EC_{75G}	330	290	170
EC_{90G}	590	460	220
EC_{100G}	870	620	270
NOEC	50	< 50	50
LOEC	100	50	100

[a] Gomot (1997).

CONCLUSIONS

Adult *H. engaddensis* snails are suitable for laboratory toxicity testing of heavy metals. These snails demonstrated fast response to dietary Cd (1 week) and Cu (2 weeks) at sublethal doses. This enables the calculation of EC_G and EC_C, in addition to NOEC and LOEC.

H. engaddensis exhibited higher sensitivity to dietary Cd than Cu when sublethal doses were tested. Both metals inhibited feeding and growth in a dose-dependent manner. However, snails consumed Cu-contaminated food for the first 2 weeks without exhibiting negative effects on both growth and feeding rates. Thereafter, snails slowed down or stopped consumption of Cu-contaminated food. Consequently, growth was slowed down or even completely inhibited. When offered control food for 2 weeks snails were able to identify it and they started feeding and gaining weight at high rates. After 2 weeks, snail weights were similar to those of the control group. Thus, growth inhibition of *H. engaddensis* caused by Cu-contaminated food is reversible.

The inhibitory effect of dietary Cd on growth and feeding rates of *H. engaddensis* was significant from the first week of the experiment. Inhibition of feeding and growth seems to be irreversible, since snails were unable to consume control food, and thus grow, after being fed Cd-contaminated food for 4 weeks. It is assumed that cadmium might irreversibly alter food intake because of an inhibitory action on the nerve centers. Therefore, growth and feeding inhibition and weight loss of snails fed Cd-contaminated diet result, basically, from starvation caused by toxicity.

Compared to literature data available for *H. aspersa*, *H. engaddensis* seems to be more sensitive to dietary Cd (Table 5). The NOEC of cadmium on growth after the 4 weeks of exposure was 50 μg Cd/g food and the LOEC was 100 μg Cd/g food. The NOEC and LOEC for copper were 20 and 100 μg Cu/g food, respectively.

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