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Toxic Effects of Cadmium on the Garden Snail (*Helix aspersa*)

L. K. Russell[1], J. I. DeHaven[1], and R. P. Botts[2]

[1]*Northrop Services Inc., 200 S.W. 35th Street, Corvallis, OR 97330;*
[2]*Environmental Protection Agency, 200 S.W. 35th Street, Corvallis, OR 97330*

Spreading treated municipal wastes on agricultural and forest lands is becoming an established method of disposal (LOEHR 1977). However, there is concern about the deleterious effects of toxicants, particularly cadmium, in the sludges. Cadmium concentrations in sewage sludge have been reported as high as 1500 ppm (BERROW & WEBBER 1972).

The work reported here is a part of a larger project to investigate the ecological effects of municipal wastes on forest lands. Snails, Helix aspersa, were chosen to examine the entrance of cadmium into terrestrial food chains. This experiment was designed to determine cadmium accumulation, acute toxicity, and behavioral, reproductive and growth responses with increasing levels of cadmium.

MATERIALS AND METHODS

Snails obtained from the Department of Entomology, Oregon State University were bred in EPA's research laboratory in Corvallis, Oregon. Cultures were maintained according to the procedure developed at Oregon State University (H.H. Crowell, unpublished notes).

The test snails were held in sealable, polyethylene boxes. The lid of each container was fitted with ventilation holes covered with nylon screen. A substrate of moist quartz sand covered by a piece of woven glass towel was placed in the bottom of each box.

Food in plastic Petri plates was changed at 2-3 day intervals. Unconsumed food was dried and weighed. Each time the food was replaced, the lid and exposed sides of each container were cleansed of feces and mucus and the glass towel was removed, washed in hot water, and returned to the box. At one-week intervals, the sand was washed in hot water and the glass towel was replaced. The snails were rinsed in cold water and weighed before being returned to the box. All boxes of snails were kept on shelves shaded with black plastic. There was constant, dim illumination, and ambient temperatures averaged 20-22°C.

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The diet for the snails was ground Purina Lab-Chow (formulation for rats, mice, and hamsters) supplemented with calcium carbonate, (10% of total ration) and fed ad libitum.

Cadmium (as $CdCl_2$) was added in powder mixture to the $CaCO_3$. A single batch of amended $CaCO_3$ was prepared for each planned concentration level for the entire experiment. The efficiency of $CdCl_2$ dispersal had been evaluated by mixing fluorescent dust with $CdCl_2$. These were ground together and mixed with $CaCO_3$. When thin layers were examined under a microscope with UV illumination the fluorescent particles were well dispersed at dilutions as great as 1:10,000.

A total of 350 snails were assigned in lots of 25 to 14 containers. Subadult (approximately 4-month-old) snails were used; those which had ceased shell growth were rejected. Each lot was assigned to one of the treatments. Two replicate containers of snails were exposed to cadmium at each of seven treatment levels (0, 10, 25, 50, 100, 300, 1,000 ppm Cd). All snails were maintained for 3 days on an unamended diet before the 30-day exposure to cadmium. Each container was checked daily during the same time. All snails were examined; inactive individuals were dislodged from the substrate to prevent extended dormancy.

In addition to checking for mortality the following characteristics were observed:

1. Reproductive behavior. Individuals observed during mating, or bearing spermatophores inserted in the body wall at the time of mating, were recorded. Because of uncertainty in the retention time, the spermatophores were tallied weekly.

2. Dormant state. Temporary dormancy is a general response to adverse conditions in Helix. Dormancy is initiated by formation of mucus seal of the shell aperture. Dormant individuals were removed from the substrate and the number showing this behavior was tallied.

3. New shell growth. At weekly intervals the snails were examined for new shell growth.

After 30 days representative snails were randomly selected for histological examination and chemical analyses. Two snails per treatment were sampled for histological study. Snails were denied food for 24 hours to clear the gut. Each snail was removed from its shell, decapitated and rapidly dissected. Duplicate samples of the following tissues were frozen in liquid N_2 or fixed in 10% formaldehyde: foot, intestine, digestive gland, ovotestis, kidney, mantle edge, and lips. The formalin-fixed tissues were dehydrated through an ethanol series, cleared in xylene, and imbedded in paraplast (M.P. 60°C). Frozen tissues were lyophilized, and after 2 changes of paraffin, were imbedded directly in paraplast in a vacuum oven. Serial sections were cut at 10 μ, duplicate slides were stained as follows: Heidenheins hematoxylin/eosin Y (GRAY 1973), sodium rhodizonate (THOMPSON

1966), Von Kossa silver nitrate (GURR 1958), and alizarin red (COHN 1960).

Pooled soft tissues and shell fragments, 4 snails per treatment, were placed in paper bags. The soft tissue samples were frozen at -15°C for 24 hours, freeze-dried for 3 days and weighed. The dry samples were stored in a desiccator. Both shell and tissue samples were ground and transferred to clean, sealed vials for chemical analysis. Tissues were digested in a 3:1 nitric: perchloric acid solution and analyzed by flame atomic absorption. All glassware was boiled in 25% HNO_3 before use and rinsed with distilled water. This process was repeated. Chemicals were reagent grade and all water used was double distilled.

RESULTS

Two of the 350 snails used in this exposure died: one each in the 50 ppm and 1,000 ppm exposure. In contrast to the lack of mortality response, there were marked effects on feeding and growth in the cadmium-treated snails (Figures 1 and 2). Food consumption declined with each increase in cadmium concentration from 0 ppm to 1,000 ppm. This separation is complete from 16 days

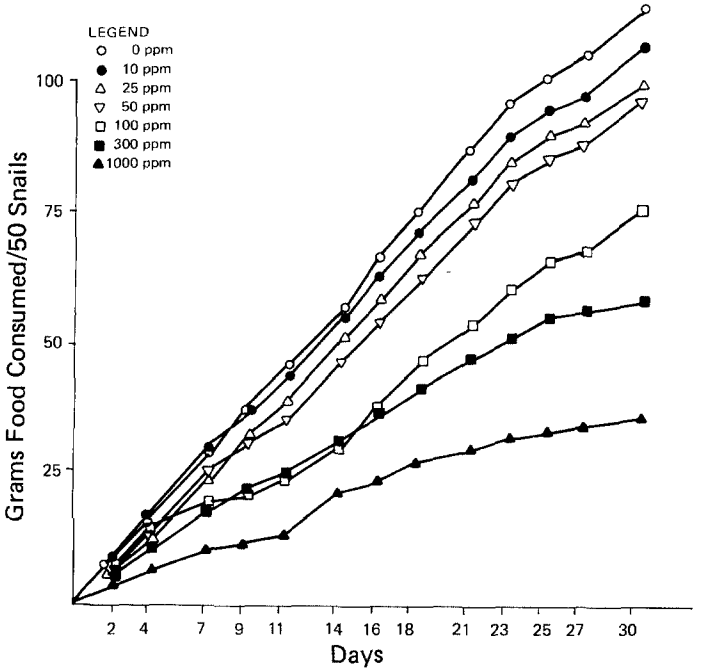


Figure 1. Accumulated food consumption by Cd-treated and control Helix.

onward. Feeding was strongly depressed at 100 ppm Cd and above. The slope indicating total food consumption (30 days) on ln Cd dosage is significantly negative ($t = 11.94$, 10 d.f., $p<0.001$).

Weight gain or loss for each lot of snails over the observation period is closely correlated with the observed feeding rates

(r = 0.98). The most obvious effect of the cadmium treatment, relative weight loss, can be explained by changes in feeding rate, with efficiency of assimilation of the food remaining constant over a three-fold variation in food intake. As found for feeding rates, the regression of growth rates on ln dosage (Figure 2) is linear over tested dosage levels.

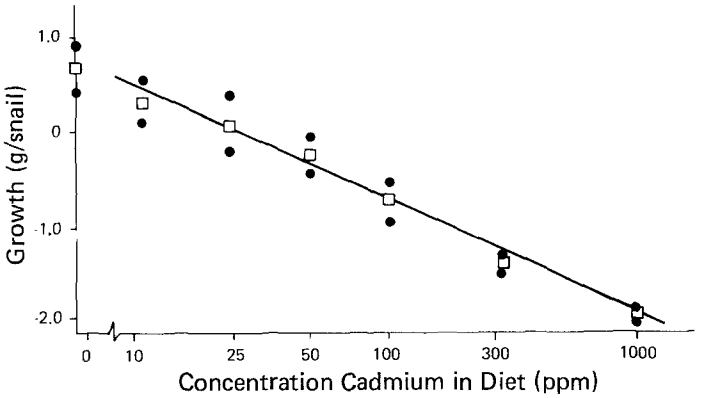


Figure 2. Regression of growth of Helix vs. ln[Cd]. Two lots of 25 snails (●) and mean (□) shown for each treatment. Growth (g) = 1.346 − 0.315 ln [Cd]; r = .955.

Data on the total observed reproductive activity, dormancy, and shell growth are presented in Table 1. There is no indication of effect on these parameters at 10 ppm Cd. At 25 ppm and above, shell growth and reproductive activity declined, while the incidence of the sealing response (dormancy) increased rapidly.

TABLE 1.
Effects of varying levels of ingested cadmium on snail behavior.

	Behavior Pattern (#Observed)					
	Sealing Shell (Days)				Shell	Reproductive
ppm Cd	0-10	11-20	21-30	Total	Growth[1]	Activity[2]
0	0	2	4	6	20	32
10	0	2	3	5	24	31
25	0	0	7	7	17	23
50	6	4	14	24	11	10
100	8	12	26	46	16	5
300	5	13	64	82	1	5
1000	27	66	163	256	0	9

[1] Total individuals with fresh growth on 5 observation dates.
[2] Total individuals mating, or with spermatophores in place.

Within each treatment, dormancy increased from the first to the last 10-day period of observation. The correlation of shell growth ($r = -0.79$) and reproductive activity ($r = -0.72$) with Cd dosage were significant at the 95% level; the correlation ($r=0.99$) of sealing response with dosage was significant at the 99% level.

Helix are able to assimilate significant amounts of cadmium without lethal effects (Table 2). Since the levels of Cd in the diet and feeding rates are known for the experimental snails, it is possible to compute the amount of Cd consumed by each group of snails as well as the amount present in their tissues. For snails fed 0 to 50 ppm Cd, tissue Cd levels approximate the levels in the diet. The higher body load at 300 ppm than at 1,000 ppm suggests a plateau effect at a tissue level around 100 ppm Cd.

TABLE 2.
Cadmium levels in soft tissues, and the estimated efficiency of Cd uptake in snails (values for treatment lots of 50 snails).

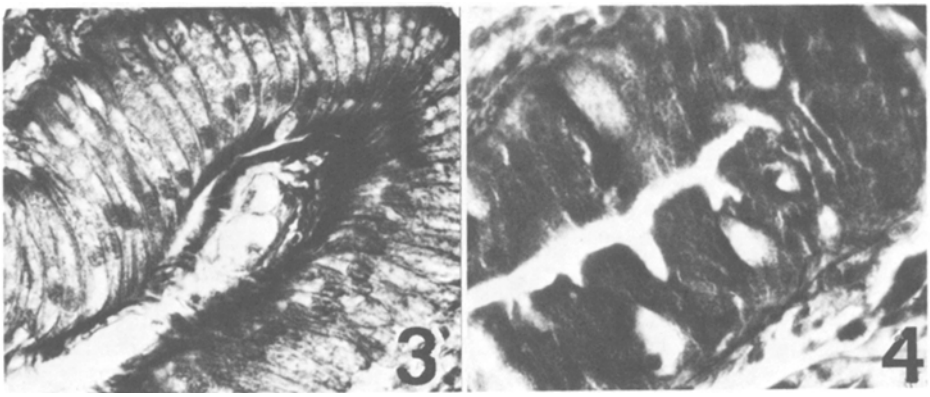
ppm Cd in Diet	Tissue Dry Weight[1] (g)	Cd in Tissues ppm	Cd in Tissues mg	Cd in Food Total Exposure (mg)	Percent Retained
0	49.7	<0.5	<0.02	<0.5	
10	48.2	8.4	0.40	1.1	37.5
25	44.6	33.3	1.49	2.5	59.0
50	43.4	38.9	1.69	4.9	34.5
100	38.7	N.D.[2]	N.D.[2]	7.7	N.D.[2]
300	32.89	137	4.50	17.9	25.2
1000	26.6	98.0	2.60	37.3	7.0

[1] Estimated from 4 or 5 snails per treatment level
[2] N.D., no data.

The nearly linear increase of Cd in tissues with Cd in the diet cannot be a simple function since the relative tissue exposure is also affected by difference in feeding rate and weight change of the snails. The assimilation efficiency for Cd in this system is estimated to range from 7.0% at 1,000 ppm to 59% at 25 ppm. There is a negative correlation between the Cd level and the efficiency of its assimilation in the snail tissues ($r = -0.82$).

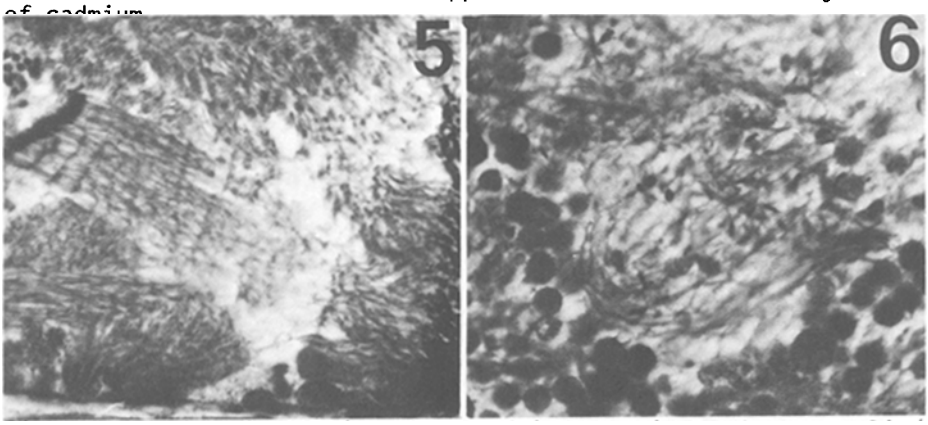
Histopathological responses were observed in most tissues examined including intestine, digestive gland, kidney, ovotestis, and glandular dermal tissues. General reactions involved hyperplasia of epithelia at lower cadmium levels, proceeding to extensive cell disruption and necrosis and connective tissue proliferation at the highest dosages.

The intestinal epithelium of control snails (Figure 3) was characterized by a monolayer of columnar absorbtive cells of equal height with cell division limited to the crypts at the base of the villi. Low levels of cadmium (10, 25 ppm) produced edematous epithelia with cells of varying height and local areas of cell stratification and sloughing of the surface. At levels of 100, 300, and 1000 ppm (Figure 4) enlarged goblet cells appeared with local areas of necrosis and degeneration of villi.



Figures 3, 4. Intestine, freeze-dried sections, haematoxlin/ eosin, 320x. Figure 3, 0 ppm Cd; Figure 4, 1000 ppm Cd.

Gonadal tissue of Helix is unusual in the association of sperm and ova undergoing simultaneous development. Ovotestis follicles of mature untreated Helix (Figure 5) contain bundles of sperm with tightly aligned heads and flagella. In contrast, treated snails showed increasing disorganization at dosages of 100 ppm Cd and above. Effects included disruption of the bundles of sperm, and fragmentation of flagella (Figure 6). Subjectively, fewer mature sperm were observed in the follicles at the highest dose. At lower levels (50 ppm) spermatogonia and spermatocytes appeared to adhere together in masses which were not observed in control tissues. Ova did not appear to be affected at any level of cadmium.



Figures 5, 6. Ovotestis, freeze-dried sections, haematoxylin/ eosin 320x. Figure 5, 0 ppm Cd; Figure 6, 300 ppm Cd.

DISCUSSION

The garden snail is tolerant of high levels of Cd via ingestion and accumulates significant levels in its body tissues. Histochemical tests confirm that the hepatopancreas is the major deposition site for cadmium (COUGHTRY & MARTIN 1976).

Reported environmental levels of cadmium would not cause significant mortality in H. aspersa populations. However, the feeding and growth performance and reproduction behavior of Helix appear to be sensitive to levels of Cd which can be found in vegetation at disposal sites (1-12 ppm; SOPPER & KERR 1979). This study restricted Cd intake to the food $CaCO_3$ ration. The feeding behavior of H. aspersa make it likely that some minerals may be absorbed by contact or through ingestion from the soil. If this is the case, there may be assimilation of heavy metals into the food chain outside the soil-to-plant route.

Terrestrial molluscs, including Helix aspersa, are subject to predation by a variety of vertebrate animals. If the body loads observed in short term feeding trials are approached in field populations, there could be a significant toxic transfer of the heavy metals up the food chain. Further studies are being planned to observe the dynamics of heavy metal uptake and deposition under more natural conditions.

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