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Accumulation and Effects of Cadmium on Guppy (*Poecilia reticulata*) Fed Cadmium-dosed Cladocera (*Moina macrocopa*)

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Cadmium is one of the most toxic heavy metals and great number of reports have been published on the acute and chronic toxicity to various aquatic organisms. In most studies, Cd, usally as $CdCl_2$, has been dissolved directly into water. However, it is well known that aquatic organisms accumulate Cd through food as well as from ambient water. Nevertheless, studies on accumulation and effects of Cd through food are very limited. KOYAMA and ITAZAWA(1977) reported the effects of Cd-contaminated fish food on the Carp and Progy with special reference to physiology and histology. KUMADA *et al.*(1980) reported accumulation, excretion and effects of Cd in the rainbow trout exposed to Cd via Cd-contaminated fish food in comparison with those exposed to Cd in water. WILLIAMS and GIESY(1978) studied the relative importance of food and water sources by *Gambusia affinis*(Poecillidae) and reported that the uptake of Cd from the food contaminated with 1.13 μg/g(dry wt) was not significant. In all of these studies, commercial fish food(flakes or granules) were used for preparing the Cd-contaminated foods.

Zooplankton is one of the most important food for fish and it is known that heavy metals are bound to metal-binding proteins such as metallothionein, even in invertebrate(NOEL-LAMBOT 1976; TALBOT and MAGEE 1978; SUZUKI *et al.* 1980). It has been assumed that Cd accumulated by zooplankton is not released to water compared to Cd soaked in commercial fish food. Therefore, the present study was intended to examine the rate of Cd-uptake and body burdens which cause adverse effects on the survival and growth of guppies when they are exposed to Cd dosed in zooplankton(*Moina macrocopa*) and Cd dissolved in water.

MATERIALS AND METHODS

Preparation of Cd-Dosed *Moina macrocopa*

About six hundred *Moina macrocopa*(12 ± 12 hrs old) were placed daily in a 7-L aquarium(22x16x20 cm^3) containing five liter of dechlorinated tap water. Each cohort of different age was maintained for 13 to 14 days with *Chlorella ellipsoidea* as food. *C.ellipsoidea* was cultured in the medium developed for this algae(WATANABE 1960) and washed three times with artificial soft water; $CaCl_2$ 26.1, $MgSO_4 7H_2O$ 17.1 and $NaHCO_3$ 25.0 mg/L. *M. macrocopa* released their first brood approximately four days after birth and continued to release offsprings at approximately two days intervals.

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Young *M.macrocopa*(12 ± 12 hrs old) were gathered daily from 9 or 10 aquaria through a nylon netting to separate them from adults. The water of aquaria was renewed daily. The young *M.macrocopa* were devided into four groups and placed in a aquarium containing 4 liter of ground-water respectively. The four aquaria contained Cd of 0 (back ground; not detected), 5, 10 and 20 μg/liter respectively. *M. macrocopa* were exposed to Cd for three days. The nominal concentrations in each aquarium was renewed daily and *C. ellipsoidea*(about 1.5×10^6 cells/ml) was added twice a day. *M. macrocopa* were collected on netting and equal wet weight fed to the experimental guppies(TABLE 2).

Cd Accumulation by Guppies from *Moina macrocopa*

Fifty guppies(*Poecilia reticulata*) of yellow strain were placed in a 10-L(30x16x22 cm^3) glass tank containing 7 liter of ground water. The total hardness of the water was approximately 80 mg/L as $CaCO_3$, pH was 7.4 to 7.6 and electroconductivity was 190 to 200 μσ/cm. The water temperature was controled to 23 ± 1° C. Age of guppies was 19 ± 2 days and body length was 6.98 ± 0.30 mm(distance between tips of mouth and terminal vertebra, $\bar{X}$ ± S.D.). All of the Cd dosed *M. macrocopa* fed to the experimental guppies were consumed within 30 minutes. Feces of guppies were removed twice a day and the water in the glass tank was renewed every three days. Fifteen guppies were sampled every 10 days and transferred into clean water for one day. After measurement of body length, pooled samples of 4 to 8 individuals were placed in glass vials(ø 25 mm, length 75 mm). Two replicates samples were taken on 10th day and three replicates samples were taken on the 20th and 30th day.

Cd Accumulation by Guppies from Water

One hundred and seventy guppies(15 ± 3 days old) were placed in a 60-L aquarium(30x59x34 cm^3) containing 40 liter of ground water. Ground water flowed continously to the aquarium at a rate of 200 ml/min after mixing with $CdCl_2$ stock solution. Cd concentrations were adjusted to be 0, 5 and 10 μg/L with peristaltic pumps. Actual Cd concentrations were maintained within 90 to 120% of nominal concentrations. Guppies were fed commercial fish food(granules) of about 3% of total body weight once a day. Thirty guppies were sampled every five days from each tank and placed in clean water for one day. Body length and weight were measured after rinsing with distilled water. Pooled samples of five guppies were placed in glass vials and dried at 100 °C.

Fifty guppies were placed in a 10-L aquaria containing 7 liter of ground water dissolved 0, 45, 90 and 180 μg Cd/L. Cd-containing water was renewed and fish food, 3% of total body weight, was fed daily. Dead guppies were removed once a day. Three to fifteen guppies were sampled on 5th, 10th, 20th and 30th day for Cd-analysis.

Cd-Concentrations

Dried samples in glass vials were digested with HNO_3 and $HClO_3$ on a hot plate until clear and diluted with distilled water of about half volume of the digested samples. Cd concentrations were measured

by an atomic spectrophotometer equiped D_2 lamp(SHIMAZU AA 640 Model). National Bureau of Standards, U.S.A. reference materials were analyzed concurrently with experiments to check the accuracy of the analysis. The data on the Cd concentrations in the guppies exposed to different Cd-concnetrations were statisticaly tested(t-test).

RESULTS

Moina macrocopa reared for three days in water containing 0, 5, 10 and 20 ug Cd/L will be represented as [Cont-Moina], [5 μg Cd/L-Moina], [10 μg/L-Moina] and [20 μg Cd/L-Moina] respectively(TABLE 1). The quantity of Cd-accumulated *M. macrocopa* varied among the four concentrations day by day and was adjusted to that of the least(TABLE 2).

TABLE 1

Cd-Concentrations of *Moina macrocopa* Fed to Guppies

Abridged Expression of Cd-dosed *M. macrocopa*	Cd-concentrations in Water (μg Cd/L)	Cd-concentrations in *M. macrocopa* (μg Cd/g, dry wt)
[Control-Moina]	0	0.56 ± 0.10
[5 μg Cd/L-Moina]	5	69.51 ± 5.02
[10 μg Cd/L-Moina]	10	125.90 ± 6.92
[20 μg Cd/L-Moina]	20	170.61 ± 8.52

X ± S.D., N= 4

Six guppies fed [20 μg Cd/L-Moina] died before 10th day of the experiment and consequently the number of guppies in each group was adjusted to 45 after 7 days. The total number of guppies which died during the experiment were 0, 3, 1 and 8 for [Cont-Moina], [5 μg Cd/L-Moina], [10 μg Cd/L-Moina] and [20 μg Cd/L-Moina] respectively.

Figure 1 represents Cd-concentrations of the guppies fed Cd-dosed *M. macrocopa* on the 10th, 20th and 30th day. When guppies were fed [5 μg Cd/L-Moina] or [20 μg Cd/L-Moina] they attained a a steady state Cd concentration of 3 μg/g and 6 μg/g(dry wt) respectively, while that of the guppies fed [10 μg Cd/L-Moina] went to increase slightly after 20th day.

On the other hand, Cd concentrations of the guppies exposed to 5 and 10 μg Cd/L did not reach a steady state even after 25th day (Figure 1). Much more Cd was accumulated from water than that from Cd-dosed *M. macrocopa*.

Average amount of cumulative Cd(μg) uptaken by single guppy from Cd-dosed *M. macrocopa* was calculated from the data in TABLE 1, TABLE 2 and the rate of dry weight of wet *M. macrocopa*(4.56 ± 0.13 %, N=28). The transfer rate(%) of Cd from Cd-dosed *M. macrocopa* to guppies was calculated by dividing the amount of Cd in a single guppy by the cumulative Cd-amount uptaken by single guppy(TABLE 3)

TABLE 2

Number of Guppies During the Experiment and the Amount of Cd-dosed *M. macrocopa* Given as Food (mg, wet weight)

Day	1	2	3	4	5	6	7	8	9	10
No. of guppies	50	50	50	50	50	50	45[a]	45	45	30[b]
M. macrocopa (mg, wet wt)	200	230	100	120	180	110	110	100	90	120
Day	11	12	13	14	15	16	17	18	19	20
No. of guppies	30	30	30	30	30	30	30	30	30	15[b]
M. macrocopa	130	130	80	90	170	190	140	140	170	150
Day	21	22	23	24	25	26	27	28	29	30
No. of guppies	15	15	15	15	15	15	15	15	15	0[b]
M. macrocopa	150	90	70	140	130	120	120	100	100	100

a) No. of guppies was adjusted to 45
b) Fifteen guppies were removed for Cd-analysis.

TABLE 3

Cumulative Amount of Cd Uptaken by Single Guppy(Average) Through the Cd-dosed *M. macrocopa*(a) and Transfer Rate of Cd from Cd-dosed *M. macrocopa* to Guppy(b)

Abridged expression of Cd-dosed *M. macrocopa*		Days after Cd-exposure		
		10	20	30
[5 μg Cd/L-Moina]	(a)	90	255	390
	(b)	4.09	3.13 ± 0.82	2.54 ± 0.20
[10 μg Cd/L-Moina]	(a)	165	430	855
	(b)	2.08	1.80 ± 0.59	1.71 ± 0.16
[20 μg Cd/L-Moina]	(a)	225	590	1175
	(b)	3.40	2.16 ± 0.10	1.62 ± 0.27

(a) nano-gram (b) Mean ± S.D. (N=3)

$$\text{Transfer rate}_{(\%)} = \frac{\text{Cd in guppy(average)}}{\text{Cd uptaken by single guppy(a)}} \times 100$$

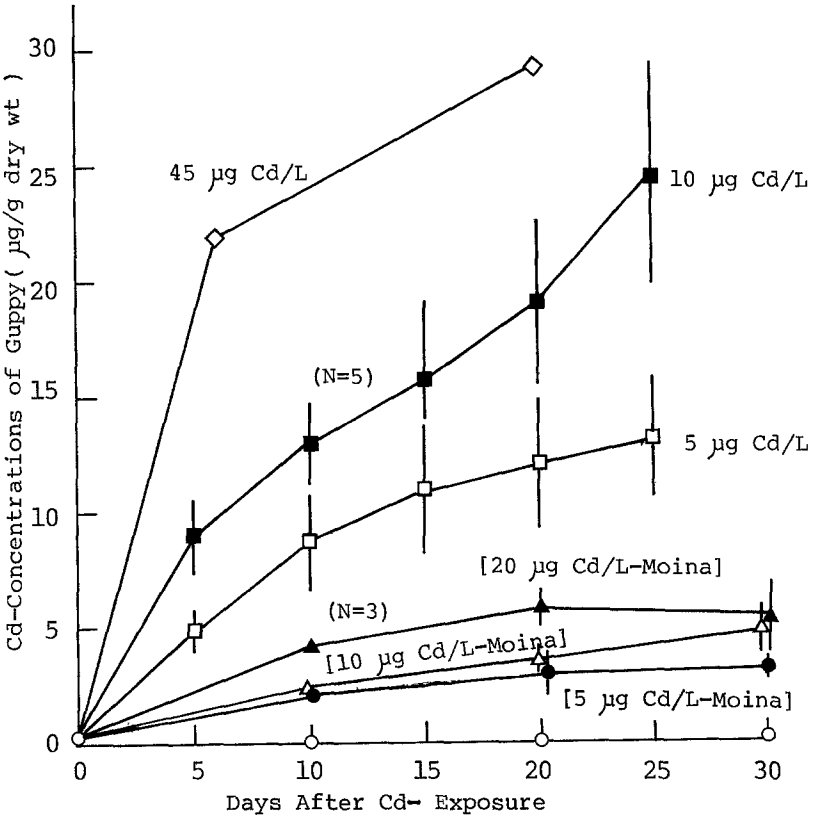

Figure 1. Cd-concentrations of the guppies fed Cd-dosed *Moina macrocopa* as shown in TABLE 1 and those exposed to the flowing water containing 5 or 10 μg Cd/L and static water dissolved 45 μg Cd/L respectively. Vertical bars indicate standard deviation.

on 10th, 20th and 30th day. Significant difference in the transfer rate were observed between the guppies fed [5 μg Cd/L-Moina] and those fed [10 μg Cd/L-Moina] on 30th day($P < 0.01$, t-test) and these values were 2.5%, 1.7% and 1.6% on 30th day respectively(TABLE 3).

The survival rate of guppies exposed to Cd in the static water system went to decrease in inverse propotion to Cd-concentrations of fish when those exceeded approximately 30 μg/g(dry wt)(Figure 2). On the other hand, the survival rate of guppies fed Cd-dosed *M. macrocopa* decreased to 86% with their body burden approximately 4 μg/g. It must be noted that no guppies died at this level when they were exposed to Cd dissolved in water.

Figure 3 shows mean body length of guppies fed Cd-dosed *M. macrocopa*. Slope functions of growth were tested statistically(t-test). Growth of guppies fed [10 μg Cd/L-Moina] or [20 μg Cd/L-Moina] was impaired before around 10th day but restored to the control level before 20th day.

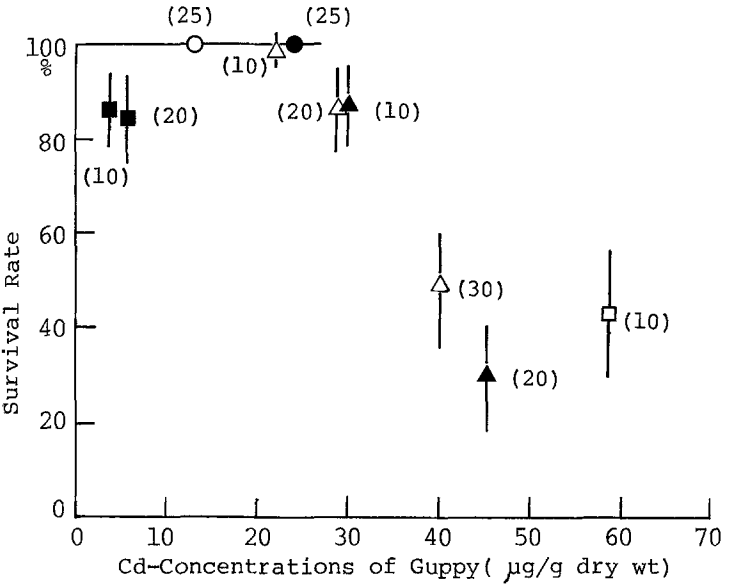

Figure 2. Relationships between Cd-concentrations and the survival rate of the guppy exposed to Cd through different path ways.: Flowing water(○, 5 μg Cd/L; ●, 10 μg Cd/L), Static water (△, 45 μg Cd/L; ▲, 90 μg Cd/L; □, 180 μg Cd/L), Cd-dosed *M. macrocopa*(■, [20 μg Cd/L-Moina]). Days exposed are shown in parenthesis. Vertical bars indicate 95% confidence interval.

TABLE 4

Body Length of Guppies Exposed to 5 and 10 μg Cd/L

Days after Cd-Exposure	Control	5 μg Cd/L	10 μg Cd/L
0	7.39 ± 0.28		
5	8.03 ± 0.20	7.77 ± 0.28	7.54 ± 0.25**
10	8.29 ± 0.41	8.17 ± 0.34	7.79 ± 2.27
20	8.94 ± 0.52	9.10 ± 0.50	8.85 ± 0.37

Body length, mm ($\bar{X}$ ± 95% confidence interval, N=30)
**P < 0.001(t-test)

Body length of the guppies exposed to the flowing water 5 or 10 μg Cd/L are shown in TABLE 4. Significant differece from control was observed only on the fifth day in the guppies exposed to 10 μg Cd/L($P < 0.001$). However, from fifth day, the growth rate was resumed to the control level. There was no dead guppy through the experiment even in the guppies exposed to 10 μg Cd/L.

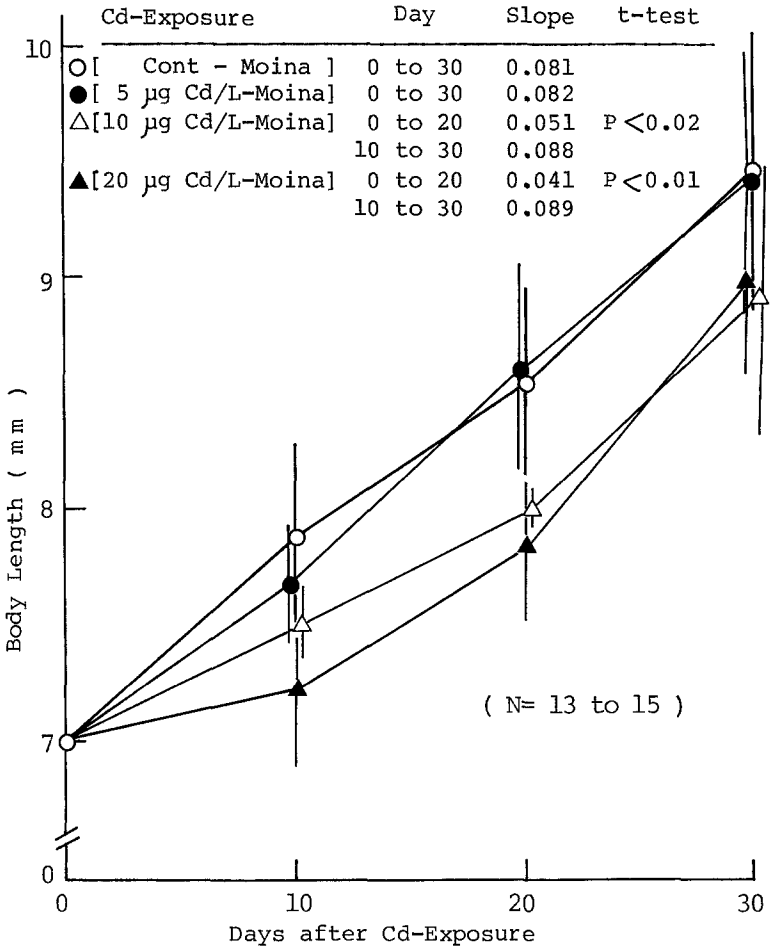

Figure 3. Body length of guppies fed Cd-dosed *M. macrocopa* as shown in TABLE 1. Vertical bars indicate 95% confidence interval.

DISCUSSION

The concentrations of Cd in the guppy reached an equilibrium at relatively low levels when Cd was uptaken through Cd-dosed *M. macrocopa*. However, capacity of Cd-accumulation was much higher than this level as found when exposed to 5, 10 or 45 μg Cd/L. When guppies were fed *M. macrocopa* dosed 90 μg Cd/g(dry wt), Cd concentration of fecal pellets were about 30 μg/g(dry wt). Therefore significant amount of Cd might be excreted with fecal pellets. The transfer rate of Cd from *M. macrocopa* to guppy was similar to the results of KUMADA *et al.*(1980); that is, rainbow trout fed the fish food mixed with 10 μg Cd/g accumulated 1.9% of Cd in the food during 4 weeks. Thus the transfer rate of Cd is relatively low notwithstanding of the nature of food.

Growth of guppy fed [20 μg Cd/L-Moina] was impaired at the initial stage of the experiment but recovered to control level from around 10th day of the experiment. Similar results were also obser-

ved in the guppies and the silver Crucian carps exposed to 10 μg Cd/L(TABLE 4 and unpublished data). These results suggest a detoxication of Cd possibly by inducing the metal-binding protein while they are exposed to sublethal concentrations of heavy metals(MARAFANTE 1976; YAMAMOTO *et al.* 1978; BOUQUEGNEAU 1979). Growth of guppies was impaired with much lesser concentrations of Cd in fish body when Cd was uptaken through Cd-dosed zooplankton than through water. Moreover, eight of fifty guppies fed Cd-dosed *M. macrocopa* died in the initial stage of the experiment when Cd concentration was about 4 μg/g(dry wt), while guppies exposed to 10 μg Cd/L did not die even Cd concentration reached to 25 μg/g. KUMADA *et al.*(1980) reported that the Cd accumulated to various organs via oral route decreased prominently during 10 weeks after cease of Cd-exposure, whereas Cd concentrations in the liver, kidney and muscle of the fish exposed to Cd dissolved in water rather increased after termination of Cd exposure.

Cd concentrations of zooplankton sampled from the natural aquatic environments are mostly less than 10 μg/g(dry wt) even in the polluted environemnt. Present study was performed from the viewpoint similar to ordinary acute toxicity test in which toxicants are dissolved in water with lethal concentrations. Therefore, Cd was accumulated in *M. macrocopa* nearly to the extent of the maximum level torelable to them. However, neither fifty per cent mortality nor remakable growth inhibition were observed during the thirty days experiment. KOYAMA and ITAZAWA(1977) reported that 25% and 80% of Carps died when they were fed the fish food contaminated with Cd of 1700 and 5700 μg/g(dry wt) respectively for more than 15 days.

On the other hand, there was no detectable Cd-accumulation in the Mosquitofish fed the food contaminated with 1.13 μg Cd/g(dry wt) for eight weeks(WILLIAMS and GIESY 1980) which may be rather high Cd-concentration level in unpolluted environment.

Long term experiments will be necessary in future experiments to examine the effects of Cd to fish by feeding the prey with metal concentrations which are observed in the polluted aquatic environments.

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