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Effects of Sodium Selenite on Methylmercury Embryotoxicity and Teratogenicity in Mice[1]

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Effects of Sodium Selenite on Methylmercury Embryotoxicity and Teratogenicity in Mice. NOBUNAGA, T., SATOH, H., AND SUZUKI, T. (1979). *Toxicol. Appl. Pharmacol.* **47**, 79–88. Female IVCS mice were fed food containing methylmercury (MeHg) at 0, 15.9, or 31.9 nmol/g food and drinking water containing sodium selenite at 0, 11.4, or 22.8 nmol/ml for 30 days before gestation and thereafter up to Day 18 of gestation. Groups receiving MeHg had (1) affected estrus cycles, (2) decreased litter size accompanied by increased implantation sites, resorptions, dead embryos, and dead fetuses, (3) increased incidence of cleft palate, and (4) retarded fetal growth. Selenite was not teratogenic and even at the high dose, generally only retarded fetal growth. The low dose of selenite when combined with the high dose of MeHg, increased the incidence of resorptions and implantation sites. Embryolethality was decreased and the incidence of cleft palate was increased in the group receiving the high dose of selenite combined with the high dose of MeHg.

Experimental studies have been reported on the embryotoxic and teratogenic effects of methylmercury (MeHg) in rats (Ramel, 1967; Matsumoto *et al.*, 1967; Moriyama, 1967; Nonaka, 1969; Inoue *et al.*, 1972; Casterline and Williams, 1972; Nolen *et al.*, 1972a,b; Khera and Tabacova, 1973; Scharpf *et al.*, 1973; Ware *et al.*, 1974; Mottet, 1974; Chang and Sprecher, 1976), mice (Spyker and Smithberg, 1972; Spyker *et al.*, 1972; Khera and Tabacova, 1973; Su and Okita, 1976a,b), cats (Moriyama, 1967; Khera, 1973), and hamsters (Harris *et al.*, 1972), and the protective effects of selenium compounds on the toxicity of MeHg have been recognized in rats (Iwata *et al.*, 1973; Johnson and Pond, 1974; Potter and Matrone, 1974; Stilling *et al.*, 1974; Ueda *et al.*, 1975; Ohi *et al.*, 1975a, b, 1976) and in Japanese quail (Ganther *et al.*, 1972; Stoewsand *et al.*, 1974). Excess selenium has an adverse effect on reproduction in some experimental animals (Rosenfeld and Beath, 1954; Schroeder and Mitchner, 1971a). However, the effects of selenium on the embryotoxicity and teratogenicity of MeHg have not been investigated.

The present experiments were undertaken to examine the interaction of MeHg and selenium as well as the independent effect when administered orally before and during gestation of mice.

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METHODS

Animals. The animals used were IVCS mice, which were bred and maintained to the age of 60 days in our laboratory. They were housed four or five per cage during the precoital period and one to two per cage during gestation, in plastic cages with wood chips for bedding. Their food was a dough made from a commercial-chow[2] for mice. The chow used did not contain any measurable amount of mercury and selenium when measured by Magos' method for mercury (1971) (sensitivity: 2.5 pmol/g) and by Watkinson's method for selenium (1966) (sensitivity: 0.13 nmol/g). Food and water were available *ad libitum*. The room temperature was maintained at 23 to 26°C, and the room had a 14-hr light and 10-hr dark cycle. One hundred and four female mice 60 days old were divided into eight groups according to the dose levels of MeHg and selenite (Table 1).

TABLE 1

NUMBER OF FEMALE IVCS MICE ASSIGNED TO EACH COMBINATION OF DOSES OF METHYLMERCURY AND SELENITE

Dose level of selenite in drinking water		Dose level of MeHgCl in food: 0	Low (15.9 nmol/g) (4 ppm)	High (31.9 nmol/g) (8 ppm)
	0	14	14	14
Low	11.4 nmol/ml (3 ppm)[a]	14	14	14
High	22.8 nmol/ml (6 ppm)	10	—	10

[a] Numbers in parentheses are concentrations of mercury as MeHgCl or selenium as $Na_2SeO_3 \cdot 5H_2O$.

Chemicals. Methylmercury chloride was purchased from Wako Pure Chemicals (Tokyo). It was dissolved in distilled water to 0.02% (7.97×10^{-4} M) solution which was refrigerated until the preparation of dosed feed. Sodium selenite ($Na_2SeO_3 \cdot 5H_2O$) was purchased from Nakarai Chemicals (Tokyo), and dissolved in distilled water to 0.03% (1.14×10^{-3} M) solution which was also refrigerated until used.

Dosage. Dosages of MeHg were selected to avoid suppression of reproductive function in mice. In the previous experiments, mice did not show either a decrease of body weight or neurological signs after 40 days of feeding 50 nmol of MeHg/g of feed (Suzuki and Miyama, 1971) or after 60 days of feeding 75 nmol of MeHg/g of feed (Suzuki and Shishido, 1976). After preliminary experiments demonstrated successful conception of mice after 30 days feeding of MeHg-containing food at 31.9 nmol/g, two dosages of MeHg, 31.9 and 15.9 nmol g food (hereafter called high and low, respectively), were selected.

The drinking water was used as the vehicle for administration of selenite in these experiments. In previous studies concentrations of selenium around 25 to 38 nmol/ml (2–3 ppm as selenium) induced mild or severe toxic effects in long-term experiments (Shroeder and Mitchener, 1971a,b; Palmer and Olson, 1974). Two levels in drinking water, 22.8 and 11.4 nmol/ml (hereafter called high and low, respectively), were thus selected.

Procedures. The precoital experimental period was generally 30 days, during which body weights were measured every third day. The amount of food consumed in each cage was determined by measuring the decrease in weight of preweighed feed. The loss of weight of feed due to water evaporation was calculated as 8% per day. The water consumption was determined by measuring the decrease in volume of premeasured water in feeding bottles. To determine estrus cycles of mice, vaginal smears which were sampled with physiological saline, dried on glass slides, and stained with Giemsa solution were examined microscopically every morning.

After 30 days, as mice came into proestrus, they were individually mated overnight with a healthy nontreated male mouse of the same strain. The following morning when vaginal plugs or sperms were found was designated as Day 0 of gestation. Later, gestation was confirmed by increase of body weight, abdominal enlargement, and bleeding into the vagina. If female mice did not mate during the first overnight trial, they were separated from the males and maintained on experimental feed until the next proestrus. Mating trials were terminated on Day 56 after the start of the experiment.

On Day 18 of gestation, the maternal mice were anesthetized by ether, the uterus was exposed, and the number of total implants consisting of surviving fetuses, dead fetuses, dead embryos, resorptions, and implantation sites were recorded. Embryos which had lived until the head, forelegs, and hindlegs were formed (usually after Day 11–12 of gestation) were called fetuses (Rugh, 1968). When the head, forelegs, and hindlegs could not be identified they were called fetuses. Embryos included those autolyzed to the point at which the head and legs were beyond recognition. Placenta sites without embryos were designated resorptions and remnants of implantation without placenta were designated implantation sites. Litter size was defined as the number of surviving fetuses per dam. The surviving fetuses were killed by

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ether or decapitation and examined under a dissecting microscope for gross abnormalities. After dissection of internal organs and tissues, the fetuses were prepared for skeletal examinations followed the method of Dawson (1926) and examined using a dissecting microscope.

RESULTS

The mean daily intakes of food and water are shown in Table 2. The consumption of both food and water per mouse was greater during gestation than in the precoital period for all the experimental groups, but the daily dosages per kilogram of body weight became smaller in gestation than in the precoital period because the increase of body weight of pregnant mice exceeded the increase of feed consumption. Thus, the daily dosages of MeHg were approximately 4 μmol/kg for the low and 8.3 μmol/kg for the high dose group prior to breeding and 3.5 μmol/kg for the low and 7.4 μmol/kg for the high dose group during gestation.

At each corresponding dose level of MeHg, food consumption did not differ significantly between the MeHg group and the MeHg+ selenite group. Also, water consumption was nearly identical in the selenite and MeHg+ selenite groups. In the precoital period, water consumption of all dosed groups except the high MeHg group was decreased below the level of the control group; and in gestation both MeHg groups had elevated water consumption over the control.

Though there were differences in the initial body weights among the various experimental groups (22.6–27.1 g) during the 30 days of the precoital period, their weights became quite similar later on (27.0–28.6 g) with no significant variation among groups.

The length of estrus cycle of IVCS mice in the control group—102 (93.6%) 4-day cycles—was consistent with our laboratory experience. The proportion of estrus cycles with altered lengths was increased in the low MeHg (11.1%), high MeHg (26.9%), and

TABLE 2

FOOD AND WATER INTAKE OF THE PRECOITAL AND PREGNANT MICE ACCORDING TO THE DOSE LEVELS OF METHYLMERCURY AND SELENITE[a]

	Daily intake per mouse during 30 days of precoital period (mean ± SD)		Daily intake per mouse from Day 3 to 18 of gestation (mean ± SD)	
	Water (ml)	Food (g)	Water (ml)	Food (g)
Control	3.9 ± 1.1	7.1 ± 1.7	4.6 ± 1.3	8.3 ± 0.8
Methylmercury (15.9 nmol/g)	3.0 ± 0.4[b]	6.9 ± 1.7	5.5 ± 0.6[b]	7.9 ± 0.9
Methylmercury (31.9 nmol/g)	3.8 ± 1.0	7.1 ± 1.6	5.4 ± 0.9[b]	7.7 ± 1.0
Selenite (11.4 nmol/ml)	3.1 ± 0.9[b]	6.8 ± 1.9	3.9 ± 1.3	8.6 ± 1.2
Selenite (22.8 nmol/ml)	2.8 ± 0.4[b]	7.0 ± 1.9	4.1 ± 0.7	7.9 ± 0.9
Methylmercury (15.9 nmol/g) and selenite (11.4 nmol/ml)	2.8 ± 0.2[b]	6.8 ± 1.0	3.9 ± 1.6	7.8 ± 1.2
Methylmercury (31.9 nmol/g) and selenite (11.4 nmol/ml)	2.8 ± 0.8[b]	6.8 ± 2.0	4.1 ± 0.7	8.0 ± 0.8
Methylmercury (31.9 nmol/g) and selenite (22.8 nmol/ml)	2.7 ± 0.3[b]	6.7 ± 1.7	4.2 ± 1.0	8.1 ± 1.1

[a] Daily intakes of food and water were measured daily for each cage and the amounts consumed were divided by the number of mice in each cage to obtain the value per mouse. The loss of weight of feed due to water evaporation was calculated as 8% per day. When a measurement was lost, the average of two consecutive days was substituted for the actual value to calculate the mean and SD.

[b] A significant difference from the control ($p < 0.05$ by t test).

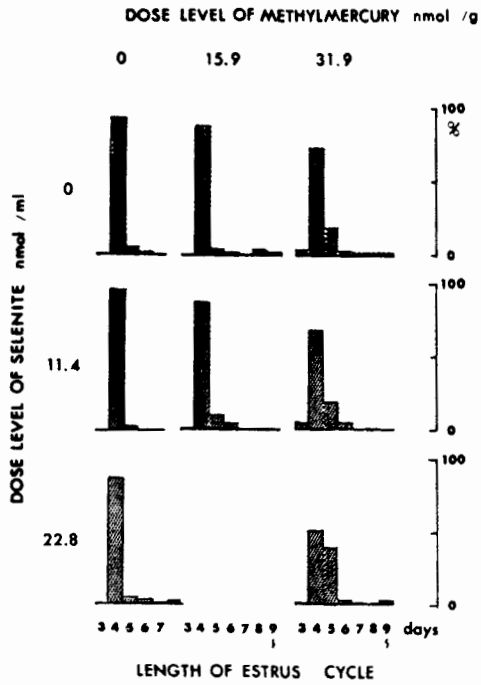

FIG. 1. Frequency distribution of the length of estrus cycles according to the combination of doses of methylmercury and sodium selenite in the precoital period.

high selenite (11.8%) groups and further increased in the group receiving both high MeHg and high selenite (approximately 50%) (Fig. 1).

When the mating trials were terminated at experimental Day 56, the conception rate was 100% except for three groups (90% for the control and high MeHg+high selenite and 86% for the low selenite group). The average length of time until conception ranged from 36.1 to 41.9 days, and no groups showed a significant difference from the control.

Two animals aborted, one in the high MeHg and another in the low MeHg+low selenite group.

Body weight curves from Day 3 of gestation (100%) to Day 18 of gestation are shown in Fig. 2. The two cases of abortion were included in the average in each respective group. Two groups, high MeHg and high MeHg+low selenite, had significantly smaller increases of body weight than the other groups ($p<0.01$, t test).

The number of total implants per dam decreased significantly only in the high MeHg group when compared with the control group ($p<0.05$, t test) (Table 3). Higher incidences of resorptions, dead embryos, and dead fetuses over that of the controls were found in the high MeHg and high MeHg+low selenite groups. When combined with the high dose of MeHg, the low dose of selenite increased, and the high dose of selenite decreased, the incidences of resorptions and implantation sites compared to the high MeHg group ($p<0.05$, χ^2 test). Thus, decreased percentages of surviving fetuses to total implants compared to the control were observed in the high MeHg and high MeHg+low selenite groups. Litter size decreased with the dose of MeHg, while neither of the two selenite dose levels diminished litter size (Table 3). The litter sizes of the groups of

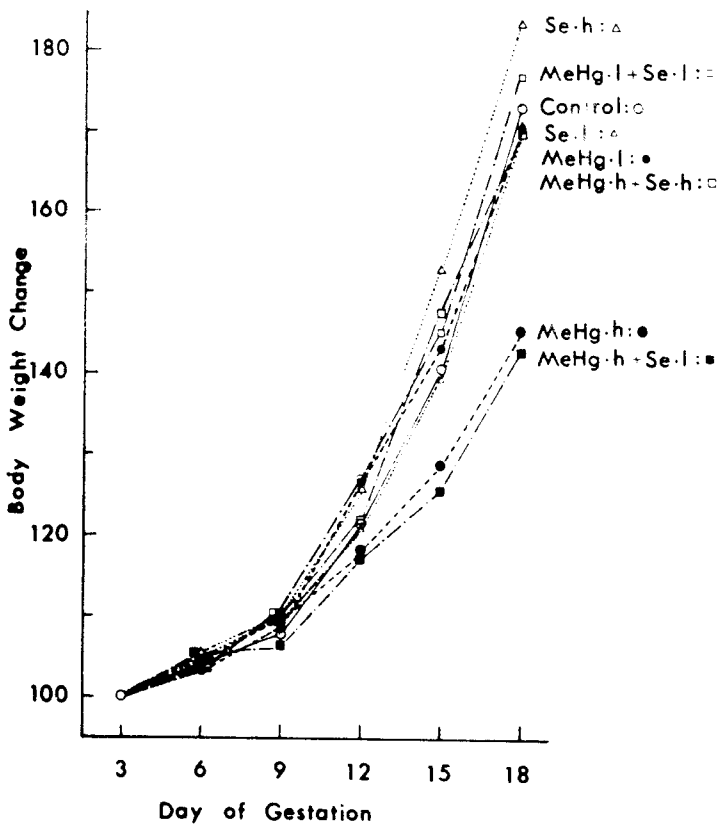

FIG. 2. Body weight curves of pregnant mice from Day 3 (as 100) to Day 18 of gestation. MeHg, methylmercury; Se, sodium selenite; l, the low dose level (15.9 nmol/g food in MeHg groups and 11.4 nmol/g drinking water in Se groups); h, the high dose (31.9 nmol/g in MeHg groups and 22.8 nmol/g in Se groups).

high dose MeHg by itself and that combined with low dose selenite were significantly decreased ($p<0.01$, t test), while litter sizes of the low dose MeHg group by itself and when combined with low dose selenite and the high dose MeHg combined with high dose selenite group were less affected ($p<0.05$, t test).

Both MeHg dose levels and the high dose selenite significantly retarded the growth of surviving fetuses. Fetal growth retardation in the MeHg groups was not prevented by either dose of selenite (Table 3).

Cleft palate was the most frequent gross abnormality observed (Table 4). Both MeHg groups but no selenite groups had significant incidences of fetuses with cleft palate. In the low MeHg+low selenite group, the incidence of cleft palate was lower than in the low MeHg group ($p<0.05$, χ^2 test). When combined with the high dose of MeHg, the high dose but not the low dose of selenite, significantly elevated the incidence of cleft palate compared to the high MeHg group ($p<0.01$, χ^2 test).

The incidence of hare lip and micrognathia was not significant in any group. Also the incidence of retarded mandibular ossification and fusion of ribs was not significantly different between any treated group and controls.

The number of cervical or thoracic ossified vertabrae was quite constant, 7 and 13, respectively, in all the groups, while the total

TABLE 3

EMBRYO AND FETOTOXIC EFFECTS OF METHYLMERCURY AND SELENITE

	Number of litters	Number of total implants and number per mother (Mean ± SD)	Number of dead fetuses	Number of dead embryos	Number of resorptions	Number of implantation sites	Number of surviving fetuses (% to total implants) and litter size (Mean ± SD)	Body weight (g) of surviving fetuses on Day 18 of gestation (Mean ± SD)
Control	9[a]	99 11.0 ± 1.5	0	2	2	3	92(92.9) 10.2 ± 1.6	0.99 ± 0.14
Methylmercury (15.9 nmol/g)	14	130 9.3 ± 2.6	1	3	5	7	114(87.7) 8.1 ± 2.5[b]	0.83 ± 0.08[c]
Methylmercury (31.9 nmol/g)	14	131 9.4 ± 1.4[b]	9[d]	13[d]	24[d]	11[d]	74(56.5) 5.3 ± 3.6[c]	0.81 ± 0.08[c]
Selenite (11.4 nmol/ml)	12	124 10.3 ± 1.7	1	1	1	5	112(90.3) 9.3 ± 1.8	0.96 ± 0.15
Selenite (22.8 nmol/ml)	10	101 10.1 ± 1.3	1	1	3	8	88(87.1) 8.8 ± 1.8	0.92 ± 0.10[c]
Methylmercury (15.9 nmol/g) and selenite (11.4 nmol/ml)	14	139 9.9 ± 1.5	5	4	5	8	117(84.2) 8.4 ± 2.9[b]	0.84 ± 0.06[c]
Methylmercury (31.9 nmol/g) and selenite (11.4 nmol/ml)	14	134 9.6 ± 2.4	7[d]	9[d]	40[d, e]	22[d, e]	56(41.8) 4.0 ± 3.8[c]	0.77 ± 0.07[c]
Methylmercury (31.9 nmol/g) and selenite (22.8 nmol/ml)	9	87 9.7 ± 1.7	2	3	1[e]	5[e]	76(87.4) 8.4 ± 1.4[b]	0.78 ± 0.06[c]

[a] Total number of litters examined was 13, but four of them were incomplete in the description of results.
[b] A significant difference from the control $p < 0.05$, t test).
[c] A significant difference from the control ($p < 0.01$, t test).
[d] A significant difference from the control ($p < 0.05$, χ^2 test).
[e] A significant difference from MeHg(high) group ($p < 0.05$, χ^2 test).

TABLE 4

TERATOGENIC EFFECTS OF METHYLMERCURY AND SELENITE ON DAY 18 OF GESTATION

	Gross malformation					Skeletal anomalies		
	Number of fetuses	Cleft palate	Hare lip	Micrognathia	Number of fetus with gross malformation	Number of fetuses inspected	Mandible	Ribs
Control	92	0	0	0	0	92	0	0
Methylmercury (15.9 nmol/g)	114	19 16.7%[a]	1 1%	1 1%	21 18.4%[a]	85	0	0
Methylmercury (31.9 nmol/g)	74	41 55.4%[a]	0	3 4.0%	41 55.4%[a]	53	1 1.9%	0
Selenite (11.4 nmol/ml)	112	0	0	0	0	73	0	0
Selenite (22.8 nmol/ml)	88	2 2.38	0	1 1%	2 2.3%	88	0	0
Methylmercury (15.9 nmol/g) and selenite (11.4 nmol/ml)	117	8 6.8%[a, b]	0	0	8 6.8%[a, b]	93	0	0
Methylmercury (31.9 nmol/g and selenite (11.4 nmol/ml)	56	34 60.7%[a]	0	2 3.6%	34 60.7%[a]	34	2 5.9%	1 2.9%
Methylmercury (31.9 nmol/g) and selenite (22.8 nmol/ml)	76	65 85.5%[a, c]	0	1 1.3%	65 85.5%[a, c]	76	1 1.3%	0

[a] $p < 0.01$, Fischer's exact method comparing to the control.
[b] A significant difference from MeHg(low) group by χ^2 test, $p < 0.05$.
[c] A significant difference from MeHg(high) group by χ^2 test, $p < 0.01$.

TABLE 5

EFFECTS OF METHYLMERCURY AND SELENITE ON SKELETAL DEVELOPMENT OF SURVIVING FETAL MICE ON DAY 18 OF GESTATION

	Number of fetuses inspected	Number of lumbar, sacral, and coccygeal ossified vertebrae												Mean SD
		6	7	8	9	10	11	12	13	14	15	16	17	
Control	92							2	53	20	16	1		13.6
								2%	58%	22%	17%	1%		0.8
Methylmercury (15.9 nmol/g)	85		2	0	5	21	31	24	1	1				10.9[a]
			2%		9%	25%	36%	28%	1%	1%				1.1
Methylmercury (31.9 nmol/g)	53				2	14	19	16	2					11.0[a]
					4%	26%	36%	30%	4%					0.9
Selenite (11.4 nmol/ml)	73							4	41	21	7			13.4
								5%	56%	29%	10%			0.7
Selenite (22.8 nmol/ml)	88	1	0	0	1	5	3	20	46	10	2	1		12.6[a]
		1%			1%	6%	3%	23%	52%	11%	2%			1.3
Methylmercury (15.9 nmol/g) and selenite (11.4 nmol/ml)	93					4	21	59	9					11.8[a]
						4%	23%	63%	10%					0.7
Methylmercury (31.9 nmol/g) and selenite (11.4 nmol/ml)	34			2	5	10	10	7						10.4[a]
				6%	15%	29%	29%	21%						1.2
Methylmercury (31.9 nmol/g) and selenite (22.8 nmol/ml)	76				6	43	23	4						10.3[a]
					8%	57%	30%	5%						0.7

[a] $p < 0.01$, t test: significantly different from control.

number of lumbar, sacral, and coccygeal vertebrae was diminished in all the treated groups except the low selenite dose group (Table 5).

DISCUSSION

The present results demonstrate that MeHg is embryocidal and teratogenic when administered in food at 15.9 and 31.9 nmol/g. Previous studies (Spyker and Smithberg, 1972; Spyker *et al.*, 1972; Khera and Tabacova, 1973; Su and Okita, 1976a,b) have also demonstrated the embryocidal and teratogenic effects of methylmercury compounds in mice.

In the present experiments, both embryolethality and teratogenicity were increased with the high dose of MeHg. Although survival was increased in the low MeHg compared to the high MeHg group, there were fewer gross malformations in the low MeHg group.

The predominant malformation observed in both the MeHg and MeHg+selenite groups, i.e., cleft palate, was reported previously (Spyker and Smithberg, 1972; Su and Okita, 1976a) and micrognathia, found in fetuses in these groups, was also described in the same previous studies.

Although teratogenic malformations such as deformities of eyes and extremities and hypoplasia of the reproductive organs were reported in lambs born to ewes that grazed on a seleniferous range (Rosenfeld and Beath, 1947), in the present experiment even

the high dose of selenite induced few gross malformations (only two fetuses with cleft palate and/or micrognathia, see Table 4), and only the high dose selenite produced retardation of fetal growth as evidenced by measurements of body weight at Day 18 of gestation.

The simultaneous feeding of selenite with MeHg modified the embryotoxic and teratogenic effects of MeHg differently depending on the dose of selenite. Few gross malformations were observed in the groups receiving the low dose of selenite combined with the low dose of MeHg, but when the low dose of selenite was combined with the high dose of MeHg, increased resorptions and implantation sites were observed. The high dose of selenite when combined with the high dose of MeHg decreased resorptions and implantation sites and increased the incidence of teratogenic malformations when compared to the high dose of MeHg. The selenite at the doses used was not embryolethal or teratogenic.

Our results do not show that selenium uniformly prevents MeHg toxicity as indicated in previous studies where growth retardation and the appearance of neurological signs and mortality in adult rats was prevented by selenium (Iwata *et al.*, 1973; Johnson and Pond, 1974; Potter and Matrone, 1974; Stilling *et al.*, 1974; Ueda *et al.*, 1975; Ohi *et al.*, 1975a,b, 1976). The present results demonstrate that toxicity from MeHg may be either increased or decreased by selenite depending on the combination of doses of MeHg and selenium and the parameters observed.

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