Arch. Environ. Contam. Toxicol. 42, 93–98 (2002)
DOI: 10.1007/s002440010296

ARCHIVES OF
Environmental
Contamination
and Toxicology

Interactions in Developmental Toxicology: Effects of Concurrent Exposure to Lead, Organic Mercury, and Arsenic in Pregnant Mice

M. Bellés,[1] M. L. Albina,[1] D. J. Sánchez,[1] J. Corbella,[2] J. L. Domingo[1]

[1] Laboratory of Toxicology and Environmental Health, School of Medicine, "Rovira i Virgili" University, San Lorenzo 21, 43201-Reus, Spain
[2] Toxicology Unit, School of Medicine, University of Barcelona, 08036-Barcelona, Spain

Received: 26 March 2001/Accepted: 15 July 2001

Abstract. The development toxicity of lead nitrate (25 mg/kg, SC), methylmercury chloride (12.5 mg/kg, PO), and sodium arsenite (6 mg/kg, SC) was assessed in CD1 mice following administration on gestation day 10 of these chemicals separately or in their binary and ternary combinations. Cesarean sections were performed on day 18 of gestation, and fetuses were examined for malformations and variations. Three fetuses from each dam were used for whole-body analyses of Pb, Hg, and As. Maternal toxic effects were more remarkable in the group concurrently exposed to Pb, Hg, and As than in those given binary combinations of the elements. In turn, maternal toxicity was more notable in these groups than in those given separately the test compounds. With regard to developmental toxicity, the most relevant effects (decreased fetal weight, cleft palate) corresponded to the Hg-treated groups. It is in agreement with the finding that in all experimental groups the levels of Pb and As in whole fetuses were under their respective detection limits. In general terms, the present data suggests that at the current doses, the interactive effects of Pb and As on Hg-induced developmental toxicity were not greater than additive. In contrast, exposure of pregnant mice to Pb and As at doses that were practically nontoxic to dams, concurrently with organic Hg at a toxic dose, caused supra-additive interactions in maternal toxicity.

Human exposure to chemicals, either occupational or environmental, is rarely limited to a single chemical, for most chemical mixtures data on exposure and toxicity are rather fragmentary (Yang 1994, 1998; Carpenter *et al.* 1998). Nowadays, approximately 95% of the resources in toxicology are still devoted to studies of single chemicals (Yang 1994; Cassee *et al.* 1998). In relation to this, metals are not an exception (Beyersmann 1994; Madden and Fowler 2000). In recent decades a great deal of research using experimental animals has been carried out to study the toxic effects of metals, but the great majority of these studies have involved exposure to one toxic element. It has been corroborated by means of a recent bibliographical research using Medline as the database. When "toxicity of metal combinations in mammals" or "toxicity of metal mixtures in mammals" were used as base terms for the search, only 184 and 74 references, respectively, were found. In contrast, the phrase "toxicity of metals in mammals" showed 13,627 references (www.ncbi.nlm.nih.gov/entrez/query.fcgi).

In recent years, developmental toxicologists have investigated the interactive effects from concurrent exposure to a variety of chemical and physical agents. However, in a literature review on interactions in developmental toxicology, among approximately 160 reports reviewed (Nelson 1994), only 4 corresponded to studies with binary combinations of metals (Layton and Ferm 1980; Garcia and Lee 1981; Gale 1984; Naruse and Hayashi 1989), and only 1 was designed to assess the teratogenicity of a combination of three metals in mammals (Mason *et al.* 1989).

Arsenic (As) compounds are ubiquitous in the environment. Abundant evidence exists for the teratogenicity of As in the chick, hamster, mouse, and rat when administered intraperitoneally or subcutaneosly (Golub 1994, 1998; DeSesso *et al.* 1998). However, administration of inorganic As by the most relevant routes for risk assessment (oral and inhalation) did not cause malformations in offspring even at near-fatal doses to pregnant females (Holson *et al.* 1999, 2000a, 2000b; Jacobson *et al.* 1999; Stump *et al.* 1999). In turn, it has been shown that lead (Pb) may cross the placental barrier and cause developmental toxicity in hamsters; rats, and mice (Domingo 1994). Moreover, although the placenta may represent a certain barrier to mercury (Hg) in mammals, developmental toxicity of organic compounds of this element has been observed in rats, mice, golden hamsters, and cats (Domingo 1994; Gómez *et al.* 1994).

In spite of the well-established embryo/fetal toxicity of As, Pb and organic Hg, the potential developmental effects derived from exposure to combinations of these elements in mammals are not well known. The present study was undertaken to determine whether concurrent administration of As, Pb and organic Hg to pregnant mice could change the severity and/or types of effects produced by the metals Pb and Hg and the metalloid As on an individual basis. It can be of interest taking into account that As, Pb, and methylmercury-induced develop-

Correspondence to: J. L. Domingo; *email:* jlldr@fmcs.urv.es

mental toxicity can occur in pregnant women after both occupational and environmental exposure to high levels of these elements.

Materials and Methods

Chemicals

Pb, Hg, and As were administered as lead (II) nitrate, methylmercury chloride, and sodium arsenite. These compounds were purchased from E. Merck (Darmstadt, Germany). Solutions of Pb and As were prepared in 0.9% saline, and those of Hg were prepared in deionized water. Solution concentrations were adjusted so that a 30-g mouse would receive a volume of 0.10 ml.

Animals

Virgin male and female Charles River CD1 mice, weighing 28–32 g, were purchased from Criffa (Barcelona, Spain). Animals were housed in a fully air-conditioned facility with a constant day-night cycle (dark period from 10 PM to 10 AM) at a temperature of 22 ± 2°C and a relative humidity of 50 ± 10%. After an acclimation period of 1 week, female mice were mated with males (2:1) overnight and examined the following morning for copulatory plugs. The day on which a vaginal plug was found was designated as day 0 of gestation. Animals were assigned to experimental groups by stratified randomization so that body weights were equivalent across all groups on gestation day 0. The number of pregnant females per group was 10, except in the groups treated with Pb, As, and Hg plus As in which the number of pregnant mice was 11. All animals were allowed free access to tap water and Panlab rodent chow (Panlab, Barcelona).

Treatment

On day 10 of gestation, pregnant mice were divided into eight groups, which consisted of one control group and seven experimental groups to which Pb, Hg, and As were administered. Lead nitrate and sodium arsenite were given by SC injection, while methylmercury chloride was given by gavage. A single dose of each compound was administered to the animals according to the following scheme: group 1, 25 mg/kg of lead nitrate; group II, 12.5 mg/kg of methylmercury chloride; group III, 6 mg/kg of sodium arsenite; group IV, 25 mg/kg of lead nitrate plus 12.5 mg/kg of methylmercury chloride; group V, 25 mg/kg of lead nitrate plus 6 mg/kg of sodium arsenite; group VI, 12.5 mg/kg of methylmercury chloride plus 6 mg/kg of sodium arsenite, and group VII, 25 mg/kg of lead nitrate plus 12.5 mg/kg of methylmercury chloride plus 6 mg/kg of sodium arsenite. Approximately 5 min passed between each compound administration (binary and ternary combinations). Animals in the control group were given an SC injection of 0.9% saline, an oral administration of deionized water, and a second SC injection of 0.9% saline. The choice of the compound doses was based on the results of previous studies showing that 50 mg/kg of lead nitrate (SC), 25 mg/kg of methylmercury chloride (PO), and 12 mg/kg of sodium arsenite (SC) given on gestation day 10 caused maternal and developmental toxicity in mice (Domingo 1994, 1995; Colomina *et al.* 1995; Domingo *et al.* 1995).

Observations

On gestation day 18 all animals were sacrificed under diethyl ether anesthesia, and the pregnant uteri were excised immediately and immersed in a 10% solution of ammonium sulfide for at least 10 min to identify entire litter resorptions. The number of early and late resorptions, as well as the number of dead and live fetuses, was recorded. Each living fetus was dissected free of its placenta and fetal membranes, weighed, and examined grossly for externally visible abnormalities. The placenta and three fetuses selected randomly from each dam were kept for analyses of Pb, Hg, and As concentrations. The remaining fetuses were fixed in 95% ethanol, macerated in 1% KOH, stained with Alizarin red S, and examined for skeletal anomalies (Staples and Schnell 1968), or were fixed in Bouin's fluid, sectioned, and evaluated for internal malformations and variations (Wilson 1965).

For the determination of Pb, Hg, and As concentrations, placenta or the whole fetus were mixed with 65% nitric acid (E. Marck) and heated under pressure at 190°C. The samples were then diluted with deionized water, filtered, and the Pb, Hg, and As concentrations were measured by inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer Elan 6000) as previously described (Llobet *et al.* 1998). Detection limits were 0.005 μg/g for Pb and 0.02 μg/g for Hg and As.

Statistical Analysis

Data were evaluated by means of analysis of variance (ANOVA), followed by a Student-Newman-Keuls multiple range test. Statistical analyses of fetal anomalies were performed on both an individual fetus and a litter basis. Statistical methods included chi-square analyses for comparisons of frequency data and independent sample *t* test for analysis of frequency data between a treatment group and the control group. Significance was set at the 0.05 probability level.

Results

There were no deaths or abortions in the groups exposed to Pb or As only or in the group given a concurrent administration of both elements (Table 1). In contrast, all Hg-exposed groups, either alone or in binary and ternary combinations, showed significant increases in the number of dead females. This number was significantly higher in the group treated with Hg plus As than in that receiving the ternary combination of elements. In addition to deaths and abortions, in the groups exposed to As only, Pb plus Hg, and Pb, Hg, and As, there were some dams carrying completely resorbed litters. However, in relation to food consumption and body weight gain the only significant differences among groups were the decreases seen in the group concurrently exposed to Pb, Hg and As (Table 1).

At termination on day 18 of gestation, significant reductions in maternal body weight and gravid uterine weight were observed in the groups treated with Pb plus As, Hg plus As (only in body weight gain), and Pb, Hg, and As. However, no significant differences between the binary and the ternary combinations were noted. There were no significant differences among groups in corrected body weight, corrected body weight change, absolute and relative liver weight (excepting the group given Pb plus Hg), and absolute and relative kidney weight (with the exceptions of the Pb/Hg, and Hg/As groups) (Table 1).

A summary of the reproductive findings is presented in Table 2. When the experimental groups were compared to the control group, only a few significant differences could be seen in the number of total implants per litter, the number of total nonviable implants (early and late resorptions plus dead fetuses) per

Table 1. Maternal effects of lead nitrate (25 mg/kg SC), methylmercury chloride (12.5 mg/kg PO), and sodium arsenite (6 mg/kg SC) concurrently administered on day 10 of gestation to mice

	Control	Pb	Hg	As	Pb + Hg	Pb + As	Hg + As	Pb + Hg + As
No. of plug-positive females (day 0 of gestation)	10	11	12	12	14	10	16	14
No. of litters with fetuses[1]	10 (100)^{a}	11 (100)^{a}	10 (83.4)^{b}	11 (91.7)^{b}	10 (71.4)^{c}	10 (100)^{a}	11 (68.8)^{c}	10 (71.4)^{c}
No. of deaths[1]	0 (0)^{a}	0 (0)^{a}	1 (8.3)^{b}	0 (0)^{a}	3 (21.5)^{cd}	0 (0)^{a}	5 (31.2)^{d}	2 (14.3)^{bc}
No. of abortions[1]	0 (0)^{a}	0 (0)^{a}	1 (8.3)^{b}	0 (0)^{a}	0 (0)^{a}	0 (0)^{a}	0 (0)^{a}	0 (0)^{a}
No. of completely resorbed litters[1]	0 (0)^{a}	0 (0)^{a}	0 (0)^{a}	1 (8.3)^{b}	1 (7.1)^{b}	0 (0)^{a}	0 (0)^{a}	2 (14.3)^{b}
Food consumption (g/dam) on gestation days 0–18[2]	119.90 ± 4.67	117.69 ± 13.06	109.63 ± 9.45	120.10 ± 8.81	122.61 ± 16.46	117.20 ± 1.28	120.80 ± 12.54	120.51 ± 10.06
Food consumption (g/dam) on gestation days 10–18[2]	59.60 ± 2.72^{a}	51.33 ± 2.33^{ab}	51.55 ± 8.47^{ab}	55.51 ± 2.79^{ab}	56.86 ± 9.68^{ab}	52.01 ± 1.63^{ab}	51.53 ± 5.04^{ab}	43.39 ± 9.91^{b}
Body weight gain (g) on gestation days 0–8[2]	32.32 ± 5.43^{a}	26.47 ± 7.03^{ab}	28.34 ± 9.04^{ab}	25.84 ± 5.93^{ab}	29.46 ± 4.41^{ab}	24.46 ± 5.71^{ab}	25.03 ± 6.01^{ab}	22.68 ± 5.12^{b}
Body weight gain (g) on gestation days 10–18[2]	22.01 ± 5.01^{a}	19.84 ± 6.49^{a}	21.55 ± 3.50^{a}	18.70 ± 4.80^{a,b}	21.20 ± 3.86^{a}	15.81 ± 4.09^{ab}	16.69 ± 4.45^{ab}	13.41 ± 4.59^{b}
No. of dams	10	11	10	11	10	10	11	10
Body weight (g)[2]	60.05 ± 5.76^{a}	54.66 ± 6.89^{ab}	53.04 ± 8.35^{ab}	52.63 ± 5.67^{ab}	57.68 ± 1.98^{ac}	50.27 ± 6.26^{bc}	51.67 ± 4.35^{bc}	47.98 ± 4.88^{b}
Gravid uterine weight (g)[2]	20.77 ± 5.49^{a}	18.92 ± 6.18^{ab}	17.88 ± 7.43^{ab}	17.38 ± 5.66^{ab}	19.09 ± 2.99^{ab}	12.51 ± 5.63^{b}	16.20 ± 4.22^{ab}	12.70 ± 5.69^{b}
Corrected body weight (g)[2,3]	36.65 ± 3.11	35.74 ± 3.04	39.54 ± 5.58	36.46 ± 2.74	39.76 ± 1.41	37.76 ± 2.11	37.04 ± 2.99	36.42 ± 3.94
Corrected body weight change (g)[2,4]	9.99 ± 2.57	7.90 ± 2.31	9.56 ± 2.86	8.72 ± 2.48	9.93 ± 2.27	9.79 ± 1.04	8.98 ± 3.98	8.98 ± 2.59
Liver weight (g)[2]	2.56 ± 0.36^{a}	2.42 ± 0.24^{a}	2.70 ± 0.29^{a}	2.49 ± 0.26^{a}	3.11 ± 0.23^{b}	2.53 ± 0.20^{a}	2.73 ± 0.22^{a}	2.61 ± 0.42^{a}
Relative liver weight (%)[2,5]	6.96 ± 0.68^{a}	6.79 ± 0.52^{a}	6.88 ± 0.68^{a}	6.82 ± 0.32^{a}	7.81 ± 0.49^{b}	6.69 ± 0.39^{a}	7.41 ± 0.61^{ab}	7.17 ± 0.98^{ab}
Kidney weight (g)[2]	0.40 ± 0.04^{a}	0.40 ± 0.03^{a}	0.41 ± 0.03^{a}	0.39 ± 0.04^{a}	0.45 ± 0.03^{b}	0.40 ± 0.02^{a}	0.46 ± 0.04^{b}	0.39 ± 0.04^{a}
Relative kidney weight (%)[2,5]	1.08 ± 0.09^{a}	1.15 ± 0.11^{ab}	1.08 ± 0.10^{ab}	1.08 ± 0.10^{ab}	1.15 ± 0.12^{ab}	1.08 ± 0.09^{a}	1.25 ± 0.17^{b}	1.19 ± 0.18^{ab}

[1] Percentages of dams in parentheses.
[2] Results are expressed as mean values ± SD.
[3] Corrected body weight = (Body weight at sacrifice) − (Gravid uterine weight).
[4] Corrected body weight change = (Corrected body weight) − (Body weight on gestation day 0).
[5] Relative liver and kidney weight are calculated as percentages of corrected body weight.
Statistics: [1] Chi-square test. [2] ANOVA and Student-Newman-Keuls test.
[a,b,c,d] Values in the same row not showing a common superscript are significantly different at $p < 0.05$.

Table 2. Reproductive effects of lead nitrate (25 mg/kg), methylmercury chloride (12.5 mg/kg), and sodium arsenite (6 mg/kg) concurrently administered on day 10 of gestation to mice

	Control	Pb	Hg	As	Pb + Hg	Pb + As	Hg + As	Pb + Hg + As
No. of dams	10	11	10	11	10	10	11	10
No. of implants/litter	13.11 ± 2.85[a]	13.30 ± 1.70[a]	13.50 ± 2.07[a]	12.36 ± 1.80[ab]	12.78 ± 1.85[ab]	9.78 ± 3.63[bc]	11.64 ± 1.75[ab]	11.60 ± 2.27[ab]
No. of viable implants/litter	12.89 ± 3.06[a]	12.70 ± 2.00[a]	12.87 ± 2.29[a]	11.00 ± 3.77[ab]	11.89 ± 2.47[a]	7.89 ± 2.89[b]	10.27 ± 2.76[ab]	8.00 ± 4.05[b]
No. of total nonviable implants/litter	0.22 ± 0.44[a]	0.60 ± 0.84[a]	0.62 ± 1.06[a]	1.36 ± 2.42[a]	0.89 ± 1.05[a]	1.89 ± 2.42[ab]	1.36 ± 2.11[ab]	3.60 ± 3.63[b]
Sex ratio (M/F)	0.45 ± 0.11	0.48 ± 0.20	0.40 ± 0.19	0.47 ± 0.22	0.48 ± 0.15	0.47 ± 0.21	0.48 ± 0.10	0.47 ± 0.14
Average fetal body weight/litter (g)	1.35 ± 0.04[a]	1.30 ± 0.09[a]	1.12 ± 0.13[b]	1.25 ± 0.06[a]	1.10 ± 0.09[b]	1.31 ± 0.11[a]	1.01 ± 0.10[b]	1.11 ± 0.15[b]

Results are expressed as mean values ± SD.
Statistics: ANOVA and Student-Newman-Keuls test.
[a,b,c] Values in the same row not showing a common superscript are significantly different at $p < 0.05$.

litter, or in the sex ratio. The only exceptions corresponded to a decrease in the number of total implants per litter in the group given Pb plus As, and an increase in the number of total nonviable implants per litter in the group exposed to the ternary combination. In turn, the number of viable implants (live fetuses) per litter was significantly reduced in the group concurrently exposed to Pb and As, and in the group treated with Pb, Hg, and As. Fetal body weight was significantly lower in all the Hg-treated groups. However, the differences among these groups did not reach the level of statistical significance.

There were no significant differences between the control group and the experimental groups with respect to the incidence and types of external anomalies (data not shown). The types and frequency of skeletal and internal anomalies observed in the current study are shown in Table 3. As for the average fetal body weight, the incidence of cleft palate was higher in fetuses of all Hg-treated groups than in those of the remaining groups. However, significant differences among these groups again were not noted. Although all groups treated with Hg showed a higher incidence of skeletal defects, once more the differences among these groups did not reach the level of statistical significance.

The concentrations of Pb, Hg, and As in placenta, as well as those in whole fetuses are presented in Table 4. Though Pb could not be detected in the placenta of dams exposed to Pb plus As, As was not detected in any experimental group. By contrast, Hg was found in placenta of all Hg-exposed groups. In turn, Hg was the only metal detected in whole fetuses; Pb and As were under their respective detection limits.

Discussion

It is becoming recognized that human exposure (occupational or environmental) to chemicals does not correspond to a single compound. It has been noted that in a concurrent or sequential exposure to multiple chemicals, each component of the mixture or combination has a unique toxic potential and may influence the toxicity of other mixture or combination components by affecting their toxicokinetics or toxicodynamics (Simmons 1995).

Pb, Hg, and As are well-known environmental pollutants to which humans can result concurrently exposed. Information regarding interactive developmental effects of metals and metalloids in mammals (Layton and Ferm 1980; Garcia and Lee 1981; Gale 1984; Mason *et al.* 1989; Naruse and Hayashi 1989) or other animal species (Hoffman *et al.* 1992; Stanley *et al.* 1994; Trieff *et al.* 1995; Pagano *et al.* 1996) is rather scarce. Taking this into account, the purpose of the current study was to evaluate the potential maternal and developmental toxic interactions of a concurrent exposure to Pb, organic Hg, and As in the pregnant mouse. Toxic interactions refer to the qualitative and/or quantitative modifications of the toxicity of one chemical by another, the process principally occurring within the organism after the exposure phase (Krishnan and Brodeur 1994). Interaction can result in additive, greater-than-additive (potentiation, synergism), or less-than-additive (antagonism) toxic response (Ince *et al.* 1999; Preston *et al.* 2000).

In the current study, a single Pb administration did not cause significant maternal toxicity, and the only effect of As exposure was the presence of one dam with a completely resorbed litter. In contrast, in the group of pregnant mice exposed to organic Hg, 16.6% of dams resulted affected by the treatment (one death, one abortion). On the other hand, while single SC administration of Pb nitrate and sodium arsenite at 25 and 6 mg/kg, respectively, did not cause significant developmental toxicity, oral treatment of pregnant mice with 12.5 mg/kg of methylmercury chloride resulted in significant embryo/fetal toxicity. An increase in the number of nonviable implants as well as a higher incidence of fetuses with cleft palate, were previously reported to occur in pregnant mice as a consequence of exposure to organic Hg (Domingo 1994; Gomez *et al.* 1994; Colomina *et al.* 1995). The differences between the behavior of Hg and that of Pb and As, observed in the present study, are in agreement with the absence of Pb and As found in whole fetuses, which means that at the current doses the placenta could act as a complete barrier for these elements.

With respect to the binary and ternary combinations, the most relevant interactive effects were seen in the group concurrently exposed to Pb, Hg, and As. In this group, maternal toxicity was evidenced by a significant decrease in the number of litters with fetuses, as well as a reduction in food consump-

Table 3. Summary incidence of skeletal and internal defects in mouse fetuses after maternal exposure to lead nitrate (25 mg/kg), methylmercury chloride (12.5 mg/kg), and sodium arsenite (6 mg/kg) on day 10 of gestation to mice

	Control	Pb	Hg	As	Pb + Hg	Pb + As	Hg + As	Pb + Hg + As
No. of fetuses examined skeletally (litters)	49 (10)	54 (11)	49 (10)	46 (11)	49 (10)	49 (10)	40 (11)	34 (10)
Total altered fetuses (litters)	12 (6)^{ab}	10 (4)^{a}	42 (9)^{abc}	13 (7)^{abc}	48 (10)^{bc}	12 (6)^{ab}	39 (11)^{c}	26 (9)^{abc}
Parietal bone, reduced ossification	0 (0)	1 (1)	2 (2)	0 (0)	2 (1)	0 (0)	0 (0)	6 (3)
Occipital bone, reduced ossification	3 (1)^{a}	0 (0)^{a}	1 (1)^{a}	0 (0)^{a}	1 (1)^{a}	3 (1)^{a}	12 (7)^{b}	4 (2)^{a}
Frontal bone, reduced ossification	2 (1)^{a}	0 (0)^{a}	10 (4)^{a}	0 (0)^{a}	10 (3)^{a}	2 (1)^{a}	21 (10)^{b}	8 (3)^{a}
Caudal, delayed ossification	4 (2)	4 (2)	20 (5)	3 (3)	15 (7)	4 (2)	18 (7)	12 (5)
Calcaneous, delayed ossification	4 (2)^{a}	3 (2)^{a}	37 (8)^{b}	10 (6)^{ab}	48 (10)^{bc}	11 (5)^{ad}	40 (11)^{c}	25 (9)^{bcd}
Sternebrae, delayed ossification	1 (1)^{a}	0 (0)^{a}	1 (1)^{a}	0 (0)^{a}	2 (2)^{a}	1 (1)^{a}	21 (10)^{b}	13 (7)^{b}
Assymetrical sternebrae	1 (1)	0 (0)	2 (1)	1 (1)	7 (3)	1 (1)	7 (6)	2 (2)
Xiphoid, delayed ossification	0 (0)^{a}	0 (0)^{a}	0 (0)^{a}	0 (0)^{a}	3 (2)^{a}	0 (0)^{a}	8 (5)^{b}	7 (3)^{ab}
Supernumerary ribs	0 (0)	3 (3)	5 (4)	0 (0)	3 (2)	0 (0)	0 (0)	3 (3)
Asymmetrical ribs	1 (1)	3 (3)	1 (1)	1 (1)	0 (0)	1 (1)	1 (1)	1 (1)
Metacarpians, delayed ossification	0 (0)	0 (0)	5 (3)	3 (1)	2 (1)	3 (1)	4 (2)	1 (1)
Metatarsians, delayed ossification	0 (0)^{a}	0 (0)^{a}	7 (4)^{a}	3 (1)^{a}	8 (2)^{a}	3 (1)^{a}	19 (9)^{b}	9 (4)^{a}
No. of fetuses examined internally (litters)	54 (10)	56 (11)	46 (10)	55 (11)	47 (10)	29 (10)	48 (11)	31 (10)
Total altered fetuses (litters)	0 (0)^{a}	0 (0)^{a}	28 (8)^{b}	0 (0)^{a}	25 (7)^{b}	0 (0)^{a}	38 (11)^{b}	15 (7)^{b}
Cleft palate	0 (0)^{a}	0 (0)^{a}	28 (8)^{b}	0 (0)^{a}	25 (7)^{b}	0 (0)^{a}	34 (10)^{b}	15 (7)^{b}
Dilated bladder	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (4)	1 (1)
Hydrocephalia	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (1)	0 (0)
Renal hypotrophy	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	7 (4)	0 (0)
Pelvis dilatation	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (1)	0 (0)

Statistics: Chi-square test.
[a,b,c,d] Values in the same row not showing a common superscript are significantly different at $p < 0.05$.

Table 4. Lead, mercury, and arsenic concentrations (μg/g tissue) in placenta and fetuses (whole-body) of mice after concurrent exposure to lead nitrate (25 mg/kg), methylmercury chloride (12.5 mg/kg), and sodium arsenite (6 mg/kg) concurrently administered on day 10 of gestation

	Control	Pb	Hg	As	Pb + Hg	Pb + As	Hg + As	Pb + Hg + As
Lead								
Placenta	ND	0.76 ± 0.57	ND	ND	0.60 ± 1.32	ND	ND	1.98 ± 2.19
Fetus	ND	ND	ND	ND	ND	ND	ND	ND
Mercury								
Placenta	ND	ND	6.12 ± 1.77	ND	6.92 ± 2.34	ND	7.94 ± 2.05	7.14 ± 3.43
Fetus	ND	ND	5.41 ± 1.80	ND	6.44 ± 2.53	ND	6.82 ± 2.09	6.73 ± 3.57
Arsenic								
Placenta	ND	ND	ND	ND	ND	ND	ND	ND
Fetus	ND	ND	ND	ND	ND	ND	ND	ND

The number of placenta samples analyzed was 10 except in the groups given Pb, As, and Hg plus As, in which was 11. The number of fetuses analyzed was 30 except in the groups given Pb, As, and Hg plus As, in which was 33.
Results are expressed as mean values ± SD.
ND: not detected; detection limit: 0.005 μg/g for lead and 0.02 μg/g for mercury and arsenic.

tion, body weight gain, body weight at termination, and gravid uterine weight. This toxicity was comparatively higher than that observed in the groups exposed to the binary combinations. In turn, animals in these groups showed some toxic effects that were not noted after a single exposure to the individual elements. Taking into account that maternal toxic effects of a single Pb administration were not observed, the toxicity seen in the binary Pb combinations suggests that a potentiation (the increased effect of a toxicant by the concurrent action of another agent at a dose that is not toxicant) of the Hg- and As-induced effects occurred. The results in the ternary combination would be also a consequence of supra-additive interactions among the three elements.

With respect to the developmental toxicity of Pb, Hg, and As combinations, the most notable effects were found in the groups exposed to Hg, either in the binary or in the ternary combinations. It is in agreement with the finding that in all experimental groups the levels of Pb and As in whole fetuses were under their respective detection limits. Only a few nonsignificant differences between the groups exposed to the binary and ternary combinations of the elements and the group given Hg alone could be noted. It indicates that at the current doses of the test compounds, the interactive effects of Pb and As on Hg-induced developmental toxicity were not greater than additive.

In conclusion, exposure of pregnant mice to Pb and As at doses that were in fact practically nontoxic to dams, concurrently with organic Hg at a relatively low but toxic dose,

caused supra-additive interactions in maternal toxicity. In contrast, significant interactions between these elements and Hg-induced embryo/fetal toxicity were not evident.

References

Beyersmann D (1994) Interactions in metal carcinogenicity. Toxicol Lett 72:333–338

Carpenter DO, Arcaro KF, Bush B, Niemi WD, Pang SK, Vakharia DD (1998) Human health and chemical mixtures: an overview. Environ Health Perspect 106(suppl 6):1263–1270

Cassee FR, Groten JP, van Bladeren PJ, Feron VJ (1998) Toxicological evaluation and risk assessment of chemical mixtures. Crit Rev Toxicol 28:73–101

Colomina MT, Albina ML, Domingo JL, Corbella J (1995) Effects of maternal stress on methylmercury-induced developmental toxicity in mice. Physiol Behav 58:979–984

DeSesso JM, Jacobson CF, Scialli AR, Farr CH, Holson JF (1998) An assessment of the developmental toxicity of inorganic arsenic. Reprod Toxicol 12:385–433

Domingo JL (1994) Metal-induced developmental toxicity in mammals: a review. J Toxicol Environ Health 42:123–141

Domingo JL (1995) Prevention by chelating agents of metal-induced developmental toxicity. Reprod Toxicol 9:105–113

Domingo JL, Gomez M, Sanchez DJ, Llobet JM, Jones MM, Singh PK (1995) Effects of monoisoamyl *meso*-2,3-dimercaptosuccinate on arsenite-induced maternal and developmental toxicity in mice. Res Commun Mol Pathol Pharmacol 89:389–400

Gale TF (1984) The amelioration of mercury-induced embryotoxic effects by simultaneous treatment with zinc. Environ Res 35:405–412

Garcia M, Lee M (1981) Interaction of cadmium and zinc during prenatal development in the rat. Biol Trace Elem Res 3:149–156

Golub MS (1994) Maternal toxicity and the identification of inorganic arsenic as a developmental toxicant. Reprod Toxicol 8:283–295

Golub MS (1998) Developmental and reproductive toxicity of inorganic arsenic: animal studies and human concerns. J Toxicol Environ Health 1:199–241

Gomez M, Sanchez DJ, Colomina MT, Domingo JL, Corbella J (1994) Evaluation of the protective activity of BAL (2,3-dimercaptopropanol) and DMPS (sodium 2,3-dimercaptopropane-1-sulfonate) on methylmercury-induced developmental toxicity in mice. Arch Environ Contam Toxicol 26:64–68

Hoffman DJ, Sanderson CJ, LeCaptain LJ, Cromartie E, Pendleton GW (1992) Interactive effects of arsenate, selenium, and dietary protein on survival, growth, and physiology in mallard ducklings. Arch Environ Contam Toxicol 22:55–62

Holson JF, Stump DG, Ulrich CE, Farr CH (1999) Absence of prenatal developmental toxicity from inhaled arsenic trioxide in rats. Toxicol Sci 51:87–97

Holson JF, Stump DG, Clevidence KJ, Knapp JF, Farr CH (2000a) Evaluation of the prenatal developmental toxicity of orally administered arsenic trioxide in rats. Food Chem Toxicol 38:459–466

Holson JF, Desesso JM, Jacobson CF, Farr CH (2000b) Appropiate use of animal models in the assessment of risk during prenatal development: an illustration using inorganic arsenic. Teratology 62:51–71

Ince NH, Dirilgen N, Apikyan IG, Tezcanli G, Üstün B (1999) Assessment of toxic interaction of heavy metals in binary mixtures: a stastical approach. Arch Environ Contam Toxicol 36:365–372

Jacobson CF, Stump DG, Nemec MD, Holson JF, Desesso JM (1999) Appropiate exposure routes and doses in studies designed to assess developmental toxicity: a case study of inorganic arsenic. Int J Toxicol 18:361–368

Krishnan K, Brodeur J (1994) Toxic interactions among environmental pollutants: corroborating laboratory observations with human experience. Environ Health Perspect 102(suppl 9):11–17

Layton WM Jr, Ferm VH (1980) Protection against cadmium-induced limb malformations by pretreatment with cadmium or mercury. Teratology 21:357–360

Llobet JM, Granero S, Schuhmacher M, Corbella J, Domingo JL (1998) Biological monitoring of environmental pollution and human exposure to metals in Tarragona, Spain. II. Levels in autopsy tissues. Trace Elem Electr 15:44–49

Madden EF, Fowler BA (2000) Mechanisms of nephrotoxicity from metal combinations: a review. Drug Chem Toxicol 23:1–12

Mason RW, Edwards IR, Fisher LC (1989) Teratogenicity of combinations of sodium dichromate, sodium arsenate and copper sulphate in the rat. Comp Biochem Physiol 93C:407–411

Naruse I, Hayashi Y (1989) Amelioration of the teratogenicity of cadmium by the metallothionein induced by bismuth nitrate. Teratology 40:459–465

Nelson BK (1994) Interactions in developmental toxicology: a literature review and terminology proposal. Teratology 49:33–71

Pagano G, His E, Beiras R, De Biase A, Korkina LG, Iaccarino M, Oral R, Quiniou F, Warnau M, Trieff NM (1996) Cytogenetic, developmental, and biochemical effects of aluminum, iron, and their mixture in sea urchins and mussels. Arch Environ Contam Toxicol 31:466–474

Preston S, Coad N, Townend J, Killham K, Paton Gl (2000) Biosensing the acute toxicity of metal interactions: are they additive, synergistic, or antagonistic? Environ Toxicol Chem 19:775–780

Simmons JE (1995) Chemical mixtures: challenge for toxicology and risk assessment. Toxicology 105:111–119

Stanley Jr TR, Spann JW, Smith GJ, Rosscoe R (1994) Main and interactive effects of arsenic and selenium on mallard reproduction and duckling growth and survival. Arch Environ Contam Toxicol 26:444–451

Staples RE, Schnell VL (1968) Refinements in rapid clearing technique in the KOH- alizarin red S method for fetal bone. Stain Technol 43:61–63

Stump DG, Holson JF, Fleeman TL, Nemec MD, Farr CH (1999) Comparative effects of single intraperitoneal or oral doses of sodium arsenate or asenic trioxide during in utero development. Teratology 60:283–291

Trieff NM, Romaña LA, Esposito A, Oral R, Quiniou F, laccarino M, Alcock N, Ramanujam VMS, Pagano G (1995) Effluent from bauxite factory induces developmental and reproductive damage in sea urchins. Arch Environ Contam Toxicol 28:173–177

Wilson JG (1965) Embryological considerations in teratology. In: Wilson JG, Warkany J (eds) Teratology: principles and techniques. University of Chicago Press, Chicago, pp 251–277

Yang RS (1994) Introduction to the toxicology of chemical mixtures. In: Yang RS (ed) Toxicology of chemical mixtures. Academic Press, San Diego, CA, pp 1–10

Yang RS (1998) Some critical issues and concerns related to research advances on toxicology of chemical mixtures. Environ Health Perspect 106(suppl 4):1059–1063