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Project Summary

Chronic Toxicity of Lead and Cadmium: II. Changes in the Central Nervous System of the F_1 Generation of Rats After Chronic Intoxication With Lead and Cadmium

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Lead (Pb) and cadmium (Cd) are hazardous trace elements found in the present environment of man. Taking into account possible individual and synergistic effects of chronic exposure to Pb and/or Cd, we have previously studied the effect on the central nervous system in rats chronically exposed to drinking water containing Pb and/or Cd in trace amounts. Previously, we have shown that a brief Pb and/or Cd exposure affects the subtle functions of the nervous system. In this paper we present the changes in behavior and biogenic amine concentrations in discrete brain areas of rats from the F_1 generation.

Forty-day old male and female rats received one of the following treatments in their drinking water: Group I (control), 0.005 M acetate buffered drinking water; Group II, 5 ppm Pb; Group III, 50 ppm Pb; Group IV, 0.1 ppm Cd; Group V, 5 ppm Pb and 0.1 ppm Cd; Group VI, 50 ppm Pb and 5 ppm Cd. Pb and Cd acetate salts were dissolved in drinking water buffered to pH 7 with an 0.005 M acetate buffer. Animals were exposed for 40 days and pregnant females continued on these regimens throughout gestation and lactation. Rats born from these animals (F_1 generation) were similarly exposed for 40 days beginning 20 days after birth. Each control and experimental group of animals of the F_1 generation consisted of 10 rats. All evaluations described were made at 30, 60 and 90 days of age.

This Project Summary was developed by Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project which is fully documented in a separate report of the same title (see Project Report ordering information at back).

Behavior

Locomotor activity from all treatment groups during Pb/Cd exposure is reported as a percentage of control values (Table 1). Increased diurnal and nocturnal locomotor activity was evident in 30-day old rats treated with 5 ppm Pb, while at 60 and 90 days a decrease in these activities was observed. Fifty ppm Pb treatment increased diurnal locomotor

Table 1. *Locomotor Activity Changes (Percent of Control)[a] Following Exposure to Lead and/or Cadmium*

Measurement Time	Age (Days)	Control (Counts/ 12 hr ± S.D.)	5 ppm Pb	50 ppm Pb	0.1 ppm Cd	5 ppm Cd	5 ppm Pb + 0.1 ppm Cd	50 ppm Pb + 5 ppm Cd
Day	30	929±118	181[b]	142[b]	163[b]	84	132[c]	87
	60	1096±105	87	161[b]	68[c]	107	82	61[b]
	90	950± 89	80	120	73[c]	124[c]	63[c]	99
Night	30	1690±182	280[b]	90	186[b]	183[b]	161[b]	132[c]
	60	1708±175	44[b]	138[b]	116	85	88	48[b]
	90	1670±152	65[c]	62[c]	95	112	54[b]	91

[a] - $n = 10$ for all groups
[b] - $p < 0.01$
[c] - $p < 0.05$

activity in 30-, 60-, and 90-day old rats. In 90-day old rats there was an evident decrease in nocturnal activity. Cd (0.1 ppm) also increased locomotor activity of 30-day old rats, while the diurnal activity in 60- and 90-day old animals decreased. Changes in locomotor activity after Cd exposure (5 ppm) were seen as an increase of nocturnal activity in 30-day old rats. After simultaneous treatment with lower doses of Pb and Cd, an increase in activity of 30-day old animals was observed and a decrease in diurnal and nocturnal activity of 90-day old rats was observed. Higher doses of Pb and Cd in 60-day old rats produced a depression in locomotor activity.

Biogenic Amines In Discrete Areas Of Brain

Regional brain NA and DA concentrations are presented in Tables 2 and 3. Overall, the intoxication by Pb caused a decrease in NA content in the examined brain areas while Cd caused an increase. Pb and Cd applied concomitantly in lower doses caused an increase in NA in 30- and 90-day old animals. Higher doses usually caused a decrease of NA in 30- and 60-day old rats and an increase in 90-day old animals.

After 5 ppm Pb, DA level in the striatum increased in 30- and 60-day animals. Exposure to 50 ppm Pb decreased striatal DA in 30- and 60-day old rats and increased DA in 90-day old rats. Cd (0.1 ppm) increased striatal DA concentrations in 30-day old rats and decreased it in 60- and 90-day old animals. Cd (5 ppm) increased DA in 60-day old rats. Combined Pb and Cd exposure to lower concentrations significantly increased DA in 30-day old rats, while exposure to 50 ppm Pb and 5 ppm Cd increased striatal DA in 30- and 60-day, but not in 90-day old rats.

In general, treatment with 5 ppm Pb decreased 5-HT and increased 5-HIAA in specific areas of the brain (Tables 4 and 5). Thirty-day old rats exposed to 50 ppm Pb showed an increase in 5-HT and 5-HIAA in the brain stem while a decrease in 5-HT was observed in the limbic system. In 60- and 90-day old rats, exposed to 50 ppm Pb, 5-HT and 5-HIAA levels were increased in specific areas of the brain.

Increased striatal 5-HIAA was noted in animals receiving 0.1 ppm Cd. In 60-day old rats, a decrease in 5-HT in the hypothalamus, limbic system, and brain stem were seen. Decreased 5-HIAA in the hypothalamus and striatum was also observed in these 60-day old animals. In 90-day old rats, an increase of striatal 5-HT and a decrease of 5-HIAA in the hypothalamus, limbic system, and striatum were seen.

An increase in brain stem 5-HT and 5-HIAA was observed in 30-day old rats treated with 5 ppm Cd. In 60-day old rats, the level of 5-HT decreased in the

Table 2. *Noreadrenalin (NA) Changes (Percent of Control)[a] Following Chronic Exposure to Lead and/or Cadmium*

Brain Area	Age (Days)	Control NA Concentration (μg/g ± S.E.)	5 ppm Pb	50 ppm Pb	0.1 ppm Cd	5 ppm Cd	5 ppm Pb + 0.1 ppm Cd	50 ppm Pb + 5 ppm Cd
Hypothalamus	30	2.28±0.08	96	54[b]	105	77[c]	120[c]	90
	60	2.40±0.07	141[c]	56[b]	129[b]	115[c]	92	89
	90	2.30±0.06	95	114	103	103	98	98
Pons plus	30	0.69±0.02	101	96	89	90	89	74[b]
Medulla	60	0.69±0.02	132[b]	99	109	112	95	95
	90	0.67±0.01	120[b]	46[b]	109	88	120[c]	103[b]
Limbic	30	0.61±0.03	60[b]	50[b]	80[c]	79[c]	71[b]	103
System	60	0.59±0.01	135[b]	54[b]	126[c]	113	94	64[c]
	90	0.55±0.01	136[b]	110	103	96	122[c]	125[c]
Striatum	30	0.32±0.04	135[b]	105	85[c]	84[c]	162[b]	84[c]
	60	0.32±0.04	137[b]	113	71[b]	93	98	100
	90	0.31±0.01	135[b]	80[c]	107	104	131[b]	140[c]

[a] - $n = 8$ for all groups
[b] - $p < 0.01$
[c] - $p < 0.05$

Table 3. *Dopamine (DA) Changes (Percent of Control)[a] Following Chronic Exposure to Lead and/or Cadmium*

Brain Area	Age (Days)	Control NA Concentration (μg/g ± S.E.)	5 ppm Pb	50 ppm Pb	0.1 ppm Cd	5 ppm Cd	5 ppm Pb + 0.1 ppm Cd	50 ppm Pb + 5 ppm Cd
Striatum	30	5.58±0.33	298[b]	70[b]	402[b]	93	265[b]	133[b]
	60	5.66±0.20	120[c]	62[b]	70[b]	122[b]	90	121[c]
	90	5.14±0.20	118	223	72[b]	85[c]	87	89

[a] - $n = 8$ for all groups
[b] - $p < 0.01$
[c] - $p < 0.05$

Table 4. *5-Hydroxytryptamine (5-HT) Changes (Percent of Control)[a] Following Chronic Exposure to Lead and/or Cadmium*

Brain Area	Age (Days)	Control 5-HT Concentration (μg/g ± S E)	5 ppm Pb	50 ppm Pb	0 1 ppm Cd	5 ppm Cd	5 ppm Pb + 0 1 ppm Cd	50 ppm Pb + 5 ppm Cd
Hypothalamus	30	2.1 ± 0.1	93	90	95	104	113	114
	60	2.0 ± 0.1	97	103	82[c]	102	92	104
	90	2.0 ± 0.1	92	138[b]	95	101	89	107
Pons plus	30	0.98 ± 0.04	125[c]	155[c]	108	155[b]	112	119[c]
Medulla	60	0.96 ± 0.1	59[c]	47[c]	85	81[c]	185[b]	76[c]
	90	0.91 ± 0.03	86	40[c]	109	97	120[c]	76[c]
Limbic	30	0.95 ± 0.04	86	70[c]	104	96	167[b]	100
System	60	0.93 ± 0.06	70[b]	75[c]	84[c]	88[c]	128[b]	81[c]
	90	0.95 ± 0.05	142[b]	131[b]	102	114	134[b]	69[b]
Striatum	30	0.92 ± 0.02	98	101	110	97	88[c]	130[b]
	60	0.98 ± 0.08	51[b]	73[c]	87	106	115[c]	119[c]
	90	0.93 ± 0.10	107	37[b]	133[b]	100	140[b]	67[b]

[a] - n = 8 for each group
[b] - $p < 0.01$
[c] - $p < 0.05$

Table 5. *5-Hydroxyindoleacetic Acid (5-HIAA) Changes (Percent of Control)[a] Following Chronic Exposure to Lead and/or Cadmium*

Brain Area	Age (Days)	Control 5-HIAA Concentration (μg/g ± S E)	5 ppm Pb	50 ppm Pb	0 1 ppm Cd	5 ppm Cd	5 ppm Pb + 0 1 ppm Cd	50 ppm Pb + 5 ppm Cd
Hypothalamus	30	2.5 ± 0.2	97	96	92	54[b]	102	76[c]
	60	2.5 ± 0.1	72[b]	97	76[c]	119[c]	88	96
	90	2.3 ± 0.1	70[b]	86	43[b]	89	77[c]	74[b]
Pons plus	30	0.63 ± 0.03	141[b]	170[b]	115[c]	159[b]	73[b]	138[b]
Medulla	60	0.61 ± 0.05	165[b]	214[b]	105	145[b]	143[b]	183[b]
	90	0.65 ± 0.02	106	110	129[b]	93	173[b]	97
Limbic	30	0.62 ± 0.04	91	97	80[c]	151[b]	50[b]	161[b]
System	60	0.63 ± 0.04	136[b]	127[b]	107	130[b]	115[c]	127[b]
	90	0.59 ± 0.03	101	88	57[b]	117[c]	119[c]	93
Striatum	30	0.95 ± 0.03	149[b]	174[b]	237[b]	98	95	105
	60	0.09 ± 0.05	125[b]	98	69[b]	97	103	83[c]
	90	1.0 ± 0.1	104	152[b]	65[b]	126[b]	101	77[c]

[a] - n = 8 for each group
[b] - $p < 0.01$
[c] - $p < 0.05$

Table 6. *Specific Biochemical Changes (Percent of Control)[a] Following Exposure to Lead and/or Cadmium*

	Age (Days)	Control Mean ± S E	5 ppm Pb	50 ppm Pb	0 1 ppm Cd	5 ppm Cd	5 ppm Pb + 0 1 ppm Cd	50 ppm Pb + 5 ppm Cd
ALA-	30	121 ± 25	32[b]	15[b]	—	—	21[b]	90
dehydratase	60	48 ± 18	52[b]	73[b]	—	—	25[b]	46[b]
blood	90	49 ± 17	42[b]	71[b]	—	—	50[b]	63[b]
Acetyl-	30	5.8 ± 1.3	146[b]	70[b]	—	—	81[c]	55[b]
cholinesterase	60	6.7 ± 0.5	100	100	152[b]	148[b]	42[b]	80[c]
brain	90	6.0 ± 1.1	62[b]	100	151	180[b]	100	72[b]
Monoamine	30	64 ± 15	330[b]	25[b]	180[b]	100	61[b]	100[b]
oxidase	60	69 ± 19	50[b]	64[b]	160[b]	158[b]	58[b]	170[b]
brain	90	60 ± 15	50[b]	72[b]	158[b]	316[b]	63[b]	176[b]
Free	30	183 ± 58	327[b]	391[b]	—	—	247[b]	290[b]
Erythrocyte	60	103 ± 23	448[b]	412[b]	—	—	473[b]	110
Porphyrins	90	120 ± 15	339[b]	224[b]	—	—	360[b]	318[b]

[a] - n = 8 for each group
[b] - p < 0.01
[c] - $p < 0.05$

brain stem and limbic system while the 5-HIAA level increased in the brain stem and limbic system. Increased 5-HIAA levels were found in the limbic system and striatum of 90-day old animals.

Effects of the combined Pb-Cd exposures were primarily synergistic or protective and were not related to age or dose.

Biochemical Changes

ALA-dehydratase activity in the blood was significantly decreased and free erythrocyte porphyrins were significantly increased in Pb-treated rats (Table 6). AChE and MAO activity in whole brain were variously affected in rats intoxicated by Cd and/or PB (Table 6). LDH activity and AlPh activity were not significantly changed in any examined group of animals.

Conclusion

Our behavioral studies have shown that both Pb and Cd alter locomotor activity. In control animals, age had no effect on either diurnal or nocturnal activity. In animals treated with Pb or Cd singly there was no pattern to the alterations in locomotor activity with dose or age at testing. When treated with the Pb-Cd combination, 30-day activity was generally elevated; whereas, 60- and 90-day activity was depressed. These CNS mediated activity changes seen in laboratory animals are similar to the clinical syndrome of Pb intoxication in children.

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John Laskey is the EPA Project Officer (see below).
The complete report, entitled "Chronic Toxicity of Lead and Cadmium: II. Changes in the Central Nervous System of the F_1 Generation of Rats After Chronic Intoxication With Lead and Cadmium," (Order No. PB 81-150 989; Cost: $5.00, subject to change) will be available only from:
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