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Project Summary

Chronic Toxicity of Lead and Cadmium: III. Effects of Chronic Intoxication on the Reproductive Function of the F_1 Generation and on the Central Nervous System of the F_2 Generation of Rats

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Wistar rats, descended from animals used in previous toxicity studies, were administered lead and cadmium via drinking water and examined for changes in locomotor activity, neurochemical levels in various brain regions, concentrations of heavy metals in liver and kidney, blood components, and reproductive ability and viability of offspring.

Locomotor activity was seen to vary greatly after heavy metal treatment, but with no clear pattern of response evident. Neurotransmitter levels also showed wide variations in response to heavy metal exposure, with the most notable being increases in noradrenaline in the hypothalamus and dopamine in the striatum. Serotonin and 5-hydroxyindoleacetic acid levels were less variable. Acetylcholinesterase activity in the brain was generally depressed by cadmium and long-term exposure to lead, while brain monoaminooxidase activity appeared elevated by lead and depressed by cadmium. Erythrocyte levels in the blood, as well as free erythrocyte porphyrin levels, were elevated for animals of both sexes exposed to either metal, as were hemoglobin and hematocrit levels, but the numbers of leukocytes were sometimes reduced in animals given low dosages of both metals.

Weight gains during gestation, litter sizes, survival of offspring and average size of surviving offspring were all depressed in groups of animals treated with the metals, and eye-opening was delayed in these groups.

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

Introduction

In previous studies the toxic effects of lead and cadmium in parent and offspring Wistar rats were examined (Herman et al., 1980, 1981) In those studies high concentrations of lead were seen generally to lower the noradrenaline concentrations in all areas of the brain

except the limbic system, to alter the locomotor activity of the animals, and to enhance the levels of free erythrocyte porphyrins The present studies are a continuation and extension of the above-mentioned studies and were conducted on the progeny of the animals studied previously The goals of the current work are (1) to determine which biochemical or physiological parameters studied previously yield reproducible responses when rats are exposed to lead or cadmium, (2) to determine if any long-term alteration in response results from exposure of successive generations to the heavy metals, and (3) to assess the effect of heavy metal exposure on reproductive ability

Materials and Methods

All studies were performed on Wistar rats descended from animals used in the previous toxicity studies. The parent animals had been administered lead or cadmium, or both, in low or high concentrations in the drinking water beginning on their 40th day and pregnant females continued on this regimen through gestation and lactation Their offspring (the F_1 generation) were similarly exposed beginning 30 days after birth, again through gestation and lactation, and their offspring (the F_2 generation) were subjected to the same exposure beginning on their 30th day.

Animal activity was monitored by use of photocells connected to a cage, with motor activity considered proportional to the number of times light beams were interrupted Individual rats were placed in this actionmeter for 24 h at a time, and total impulses were recorded from counters every 12 h, corresponding to daytime and nighttime activity

Rats were sacrificed by cervical dislocation and their brains were removed and stored on ice The brains were dissected into the following regions hypothalamus, pons and medulla oblongata (brain stem), hippocampus with nucleus accumbens (limbic system) and striatum Spectrofluorometric analysis was performed on each brain section to determine the concentrations of the following amines noradrenaline, serotonin, dopamine and 5-hydroxyindoleacetic acid (5-HIAA)

Blood samples were collected from anesthetized animals by heart puncture, after which the carotid and subclavian arteries were perfused with 4° C saline solution Brains were removed, frozen in dry ice, weighed, homogenized in five parts of 0 067 sodium phosphate buffer, and chilled The following enzyme activities were assayed aminolevulinic acid dehydratase (E.C 4 2.1 2 4), free erythrocyte porphyrins, acetylcholinesterase, and monoaminooxidase

Animals of the F_1 generation received lead and/or cadmium in their drinking water at the same concentrations administered to the parents Levels of peripheral blood components (erythrocytes, leukocytes, hemoglobin and hematocrit) were estimated in 90- to 100-day-old animals At the same time vaginal smears were made for females to determine the mean time of the estrous cycle Animals were mated in the proestrous phase, and when vaginal plugs were proved, the females were housed in separate cages The offspring of these females were the F_2 generation

Parameters used to quantify the generative ability of the treated rats include weight gains in pregnant and offspring rats, number of pups per litter, number of live births and survivors at the 4th and 21st day, and the implantation and resorption number for fetuses in females sectioned on the 20th day of pregnancy

Samples of liver (2g) and kidney (1g) were taken immediately after animal sacrifice. The tissues were dried and burnt in a muffle furnace at 550° C for 30 min, after which 2.5 volumes of nitric acid were added and the samples were evaporated and burned again This process was repeated several times until only a white residue remained. This residue was dissolved in 50 ml of 0 1 N nitric acid and water was added to yield a final volume of 100 ml. Cadmium and lead were complexed with ammonium pyrrolidine dicarbonium (APDC) and the complex was extracted by shaking with methylisobutyl ketone The ketone layer was decanted and centrifuged, and the lead and cadmium concentrations were estimated by comparison of the atomic absorption spectra with those of known standard solutions For lead, the wavelength 228 8 nm was the reference point

Results and Discussion

Values of locomotor activity in F_2 generation rats varied widely within the different test groups, with no consistent pattern discernible Comparison of changes from control groups values in this generation and in the F_1 generation (Herman et al., 1981) also gave inconclusive results at least as many groups showed opposite as similar effects when considered pairwise, and the values for the control groups in the two generations also showed wide variations

Table 1 summarizes the results of assays of noradrenaline levels in four brain regions from 30-, 60- and 90-day-old F_2 generation rats Each entry in the table represents an average value for eight animals The noradrenaline concentrations for each brain region remained fairly constant for the control animals over the course of the study, but greater variations were seen in the noradrenaline levels for the animals treated with the heavy metals No consistent pattern of response was readily apparent for any brain region throughout the study, except in the hypothalamus, where high dosages of cadmium plus lead resulted in enhanced noradrenaline levels Of the 72 test groups, nearly half showed statistically significant changes in noradrenaline levels, all but two being enhancement Similarly, very large (over double) increases of dopamine levels in the striatum were noted in some treated groups (Table 1), although here the assay uncertainties were rather large

The levels of serotonin and 5-hydroxyindoleacetic acid (5-HIAA) from the same four brain regions are listed in Tables 2 and 3, respectively The range of variation in the levels of these two neurotransmitters and the number of groups exhibiting significant differences from the control groups were less than for the dopamine assay

Table 4 lists the results of the biochemical assays on groups of F_2 generation rats given cadmium or lead, or both, and sacrificed after 30, 60 or 90 days With but one exception, the aminolevulinic acid dehydratase activity in the blood appeared unaffected by administration of the metals Acetylcholinesterase activity in brain tissue appeared generally depressed by cadmium and by longer exposures to lead Brain monoaminooxidase activities varied greatly for different test groups and appeared highly elevated by long-term exposure to lead, reduced by long-term exposure to high cadmium concentrations, and unpredictable after exposure to both metals The most notable and consistent change was in the levels of free erythrocyte porphyrins, where exposure to lead or lead plus cadmium uniformly resulted in large increases in porphyrin levels

Rats of the F_1 generation treated with lead and cadmium for 90 to 100 days

Table 1. *Noradrenaline (NA) And Dopamine (DA) Levels In Various Brain Regions Of F_2 Generation Rats After Exposure To Lead Or Cadmium. Each Entry Represents An Average For Eight Animals Assayed, In Units of µg/g Of Tissue*

Part of Brain	Age (days)	Metals Administered (ppm)						
		Control	Pb(5)	Pb(50)	Cd(0.1)	Cd(5)	Pb(5)+Cd(0.1)	Pb(50)+Cd(5)
	30	2.27	3.48	2.06	2.48	4.17	3.32	3.18
NA in	60	2.50	2.37	3.00	2.54	2.59	2.44	3.10
Hypothalamus	90	2.32	3.17	2.81	1.45	3.03	2.24	2.95
NA in Pons	30	0.67	1.06	0.70	0.80	1.18	0.86	0.88
+ Medulla	60	0.66	0.65	0.68	0.70	0.75	0.67	0.75
Oblongata	90	0.71	0.83	0.81	0.55	0.78	0.61	0.77
	30	0.48	0.62	0.55	0.50	0.59	0.65	0.63
NA in Limbic	60	0.51	0.45	0.43	0.50	0.54	0.49	0.55
System	90	0.51	0.73	0.74	0.42	0.67	0.50	0.66
	30	0.22	0.25	0.23	0.31	0.17	0.20	0.47
NA in Striatum	60	0.22	0.27	0.37	0.13	0.26	0.30	0.21
	90	0.19	0.32	0.19	0.12	0.32	0.11	0.35
	30	5.99	12.17	7.09	14.33	10.93	8.65	10.81
DA in	60	6.22	7.13	7.33	8.99	9.12	4.14	6.54
Straitum	90	6.17	8.93	7.51	6.50	16.23	6.58	10.06

Table 2. *Serotonin Levels (µg/g Of Tissue) From Various Brain Regions For F_2 Generation Rats Treated With Lead And Cadmium Each Entry Represents The Average For Eight Animals Assayed*

Part of Brain	Age (days)	Metals Administered (ppm)						
		Control	Pb(5)	Pb(50)	Cd(0.1)	Cd(5)	Pb(5)+Cd(0.1)	Pb(50)+Cd(5)
	30	1.53	1.64	1.27	1.30	1.63	1.70	1.60
Hypothalamus	60	1.55	1.40	1.57	1.87	1.40	1.39	1.48
	90	1.77	1.95	1.43	1.99	1.57	2.12	1.46
	30	0.95	1.38	0.93	0.96	1.41	1.31	1.24
Pons +	60	0.86	0.95	0.96	0.91	0.97	1.01	0.88
Medulla Oblongata	90	1.05	1.15	1.11	0.84	0.87	1.14	0.82
	30	0.85	1.01	1.03	0.90	0.44	0.78	0.76
Limbic System	60	0.71	1.18	1.05	1.13	1.21	1.22	0.67
	90	0.87	0.56	0.69	0.79	0.74	0.83	0.48
	30	0.93	1.10	1.01	0.96	1.23	1.17	1.04
Straitum	60	0.97	0.96	0.94	0.90	1.22	1.05	1.06
	90	0.92	0.95	0.68	0.64	0.90	0.82	1.00

Table 3. *5-Hydroxyindoleacetic Acid Levels (µg/g Of Tissue) From Various Brain Regions Of F_2 Generation Rats Treated With Lead And Cadmium Each Entry Represents The Average For Eight Animals Assayed*

Part of Brain	Age (days)	Metals Administered (ppm)						
		Control	Pb(5)	Pb(50)	Cd(0.1)	Cd(5)	Pb(5)+Cd(0.1)	Pb(50)+Cd(5)
	30	1.61	1.72	1.21	1.74	1.56	1.41	1.58
Hypothalamus	60	1.63	1.66	1.65	1.99	1.49	1.30	1.45
	90	1.57	1.35	1.11	1.81	1.67	1.40	1.16
	30	0.64	0.93	0.50	0.67	0.90	0.88	0.83
Pons + Medulla	60	0.65	0.59	0.85	0.59	0.61	0.85	0.91
Oblongata	90	0.65	0.78	0.77	0.64	0.52	0.53	0.51
	30	0.71	0.48	0.83	0.71	0.61	0.58	0.52
Limbic System	60	0.69	0.41	0.44	0.39	0.46	0.46	0.53
	90	0.70	0.76	0.79	0.76	0.71	0.52	0.47
	30	0.81	0.74	0.72	0.58	0.79	0.71	0.82
Striatum	60	0.83	0.81	0.87	0.76	0.93	0.93	0.85
	90	0.83	0.95	0.87	0.89	0.94	0.82	0.67

were sacrificed and their blood was assayed for hemoglobin levels and morphological elements. Most of the male groups exhibited slight increases in number of erythrocytes as a result of metal exposure; this increase was more pronounced in females. The hemoglobin levels and hematocrit values for the various groups roughly followed the erythrocyte levels, but the number of leukocytes were in the same cases reduced in the treated animals, most notably in the groups given low levels of both cadmium and lead.

The weight gains of the females during gestation, litter size, survival of offspring and average size of surviving offspring all were reduced in test groups administered lead or cadmium, and all of these changes were more severe in the groups receiving both metals simultaneously. The appearance of postural reflexes was relatively invariant in all groups, but the appearance of eye opening was delayed by at least a day in all groups administered metals.

F_2 generation animals given both metals weighed about 8% less on average than the control animals after their first 90 days. Table 5 presents the results from the survey of F_1 generation female rats sectioned on the 20th day of pregnancy. The average number of fetuses not implanted but resorbed increased with exposure to lead and cadmium. The mean number of corpora lutea per female remained unaffected by exposure to the metals.

For most test groups, the concentration of metals in the liver was highest when the animals were 60 days old and substantially reduced at 90 days. The metal concentration in the kidney generally stayed constant or increased during the course of exposure, except in the case of simultaneous exposure to both metals. In most cases concentrations in tissue from F_1 generation animals were equal or slightly higher than those from corresponding F_2 generation groups. Little difference in cadmium accumulation was noted between the low- and high-dose groups in each generation. Large differences, but no clear pattern, were observed between groups given single metals and those given equal concentrations plus a second metal.

References

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Table 4. *Biochemical Assays For F_2 Generation Rats Administered Lead Or Cadmium. Each Entry Represents The Average For Ten Animals Assayed.*

Assay	Age (days)	Metals Administered (ppm)						
		Control	Pb(5)	Pb(50)	Cd(0.1)	Cd(5)	Pb(5)+Cd(0.1)	Pb(50)+Cd(5)
Blood ALA Dehydratase (μmole/h/l)	30	85	147	37			47	85
	60	48	33	27			44	48
	90	53	33	21			29	22
Brain Acetylcholinesterase (μmole/min/g)	30	7.2	7.4	7.2	1.9	3.6	5.1	8.5
	60	8.9	1.6	7.0	7.1	1.4	3.6	9.2
	90	9.4	4.2	3.5	4.4	5.0	5.0	6.2
Brain Monoaminooxidase (μmole/h/g)	30	111	171	73	65	386	38	323
	60	50	108	126	589	32	103	552
	90	104	425	413	185	26	461	172
Free Erythrocyte Porphyrins (μg/l)	30	190	411	348			359	300
	60	197	317	317			343	374
	90	126	295	295			612	317

Table 5. *Survey Of F_1 Generation Female Rats Sectioned On The 20th Day Of Pregnancy. Values Are Averages For 15 Animals Per Group.*

Parameter	Metals Administered (ppm)						
	Control	Pb(5)	Pb(50)	Cd(0.1)	Cd(5)	Pb(5)+Cd(0.1)	Pb(50)+Cd(5)
Mean Number of Fetuses	10.1	7.9	9.1	8.0	9.0	7.7	7.7
Mean Number of Resorption	0.4	1.2	1.1	1.0	1.2	1.3	2.0
Mean Number of Corpora Lutea	11.3	10.3	11.5	10.5	12.0	10.4	11.3

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The complete report, entitled "Chronic Toxicity of Lead and Cadmium: III. Effects of Chronic Intoxication on the Reproductive Function of the F_1 Generation and on the Central Nervous System of the F_2 Generation of Rats," (Order No. PB 82-198 409; Cost: $6.00, subject to change) will be available only from:

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