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LEAD TOXICITY IN MICE

by

E.A. EWEIS and N.M. IBRAHIM.

Economic Entomology and pesticides Department. Faculty of Agriculture. Cairo University. Giza.

SUMMARY

Effect of long term (180 days) treatment with different concentrations of lead nitrate (0.25, 0.5, 0.75 and 1.0 mg/kg/day) dissolved in drinking water was studied on male adult white mice *Mus musculus*. All the concentrations used caused decrease in the body weight of the animals. This decrease was more noticeable in the higher concentrations. Also both the daily body weight gain and the daily food intake was affected.

Introducing of lead in the drinking water resulted in different percentages of mortality at the end of the exposure period. Mortality percentages were 20, 20 and 27% for the concentrations 0.5, 0.75 and 1.0 mg/kg/day, respectively. The organ weight/total body weight ratio of the treated mice was affected. the ratio either decreased or increased in contrast to control.

The maximum ratio observed in the case of liver and kidney weights were 103 and 109% in mice exposed to 1.0mg/kg/day lead, respectively.

A gradual inhibition which correlated with lead concentration was observed in the cholinesterase (ChE) activity of all the tested organs (liver, brain, kidney and plasma). Lead has been shown signs of toxicity included ataxia, diarrhea and hypertension.

The data indicated that the enzyme activity of acid phosphatase (Acp), alkaline phosphatase (Ap) and lactic acid dehydrogenase (LDH) of the different organs of the lead treated animals was affected as compared with control.

INTRODUCTION

Metals are ubiquitous in the modern industrialized environment. Some metals (lead, cadmium, mercury, etc.) have no beneficial in humans and there is no known homeostasis mechanism for them. In contrast, other metals such as chromium, copper, zinc, manganese and iron are essential for man. However, these essential trace elements can also be dangerous at high levels (Schumacher et al., 1994). Lead and its compounds are known to negatively affect human and animal life (Nriagu 1988). the advent of modern industrialization and in particular, the motor vehicle, has witnessed dramatic increases in lead usaged both as component of lead-acid storage battery and from 1923 as organic lead alkyl "anti-knock" additiv in petroleum (Ratcliffe 1981, and Onyari et al 1991). Rodrigues and Castellon (1982) reporte

the average content of lead in gasoline to be 0.19 g/l and estimated that 70-80% of the gasoline lead content is emitted to the atmosphere in automobile exhaust fumes. Several workers have established a correlation between increasing lead concentration in roadside soils and vehicular traffic density (Yassoglou et al., 1987; Ho and Tai 1988). Vegetables absorb metals from the soil (Bosque et al., 1990; Schumacher et al., 1993). When such metals are transferred from soil to plants to animals and/or humans, there is selectivity for some metals or elements and barriers to others in their movement from lower to higher trophic levels within the human food chain (Welch and House 1984).

It is obvious that the effects of toxic levels of lead are diverse and represent several functional systemic alterations (Mokhtar et al., 1994). The toxicity of lead at high levels of exposure is well known, but a major concern of today is the possibility that continual exposure to relatively low level may entail adverse health effects. Since lead is used as a petrol additive in Egypt. It is necessary to document the extent and magnitude of its toxicity. The adverse effects of lead and other heavy metals on insects were indicated in a previous investigation (Ibrahim et al., 1994). Thus, this study was intended as a next attempt to provide a much needed model for future investigations concerning the toxic effects which lead may cause in all animal species. Lead nitrate pb $(No)_3$ was dissolved in male mice drinking water during the early mature period and till 6 months. Growth, body weight gain, food consumption, development of various major body organs, enzyme activity in addition to other toxicological observations were the criteria studied.

MATERIAL AND METHODS

Male adult white mice *Mus musculus* weighing 20± 2 gram were housed in an air conditioned room of the Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University.

Groups of 15 mice each received daily for 180 days lead nitrate (0.25, 0.50, 0.75, and 1.0 mg/kg/day) in drinking water. A control (untreated) was run in parallel. Mice were allowed free excess of water and were fed on adequate stable diet. Each mice was weighed every day its daily food intakde was assessed. Treated adult white mice were observed daily. The animals were observed daily. The animals were killed by decapitation at the end of the experimental period (180 days). Blood, liver, brain, kidney and intestine were collected and tissues were dissected out and weighed. Blood and tissues were used to determine activity of cholinesterase (ChE), Alkaline, acid phosphatase (Ap), (Acp) and lactic acid dehydrogenase (LDH).

Tissue samples were homogenized in an ice bath with polter-Elvehjem homogenizer in 10 ml phosphate buffer solution pH 7.3. The homogenate was collected an then centrifuged at 3.000 rpm at OC. The colorimetric determination of cholinesterase activity (ChE) was assayed by the method of Ellman et al. (1961), while alkaline, acid phosphatase was assayed by the method of Kind and King (1954). Lactic acid dehydrogenase was determined by the method of Wrobblewski and Lndae (1955). Determination of proteins was carried out by the method of Captain and Weichselpaum (1946). All the results were subjected to statistical analysis. The significance

of difference between means was calculated by using "t" test. The differences between means were considered of low significance when the calculated p values were <0.05, significant when p values <0.01, and highly significant when p values were <0.001.

RESULTS and DISCUSSION

The toxic observations obtained from mice received different concentrations of lead in drinking water are presented in the Tables from 1 to 6.

The results in Table (1) show the effect of lead on the mice body weight It could be noticed that all the concentrations tested caused decrease in the body weight and the daily food intake.

Additionally, introducing of lead in the drinking water caused different percentages of mortality at the end of the exposure period. Mortality percentages were 20, 20 and 27% for the concentrations 0.5, 0.75 and 1.0 mg/kg/day, respectively.

From the results presented in Table (2) it could be seen that the mice groups received lead treated water exhibited different degrees of response which discrepance from increasing or decreasing of the animal organ weight total body weight ratio as compared to control. The maximum ratio observed in the case of the liver and kidney weights were 103 and 109% in mice exposed to 1.0 mg/kg/day lead, respectively. the highest ratio of brain weight was in mice exposed to 0.25 mg/kg/day lead.

Toxic effects of lead on animals has been reported by many investigators Maehara et al. (1986) studied the effect of various doses of lead acetate solution when injected in male albino rats. The mean body weight in the 10mg exposed group decreased slightly up to the 14th day and then increased gradually. The mean body weights of the 20 mg exposed group showed the minimum levels between 14th - 21th day and then increased slightly up to the 52th day.

Table (3) represents the cholinesterase activity percntage (ChE) of adult male mice exposed to different concentrations of lead nitrate. Agradual inhibition could be observed in the activity of (ChE) in all the tested organs (liver, brain and plasma). the inhibition increased as lead concentration increased in the drinking water.

In this respect, Kobayashi et al. (1980 a) reported that MMC inhibited potently the choline acetyl transferase (ChA) activity, and also the high affinity uptake of choline into the brain synaptosomes. Athough it had little effect on the cholinesterase (ChE) activity and Ach release from the brain slices.

Similarlly, a long-term with MMC (5mg Hg/kg/day) induced nervous signs and decreased Ach in striatum and cerebral cortex, and conversion rations of Ach in cerebellum, striatum, and cerebral cortex of mice (Kobayashi et al., 1980 b).

Schmidt and Ibrahim (1994) found that of the (ChE) in lead treated adult of *Aiolopus thalassinus* exhibited an increase in activity to about 14% in all treatments in contrast to control.

Table (1) : Effect of lead nitrate given in drinking water to mice on body weight gain and daily food intake (N = 15)

Dose mg/kg/day	Initial body weight (g)	Final body weight (g)	Body weight gain (g)	Daily food intake g	%	Daily body weight gain g	%	%Mortality
control	22.0 ± 0.88	35.3 ± 1.40	13.3±0.37	1.18 ± 0.12	100	0.07 ± 0.007	100	----
0.25	21.4 ± 0.85	29.5 ± 1.18	**8.1 ± 0.32	0.95 ± 0.08	80.5	0.05 ± 0.005	71.4	-----
0.50	20.5 ± 0.82	28.2 ± 1.12	**7.7 ± 0.31	0.88 ± 0.08	74.6	0.04 ± 0.004	57.1	20
0.75	21.0 ± 0.84	26.1 ± 1.04	***5.1 ± 0.20	0.82 ± 0.08	69.4	0.02 ± 0.002	28.6	20
1.00	20.0 ± 0.80	26.3 ± 1.05	***6.3 ± 0.25	0.80 ± 0.07	67.7	0.03 ± 0.003	42.9	27

** P < 0.01 Significant

*** P < 0.001 Highly significant .

Table (2) : Organ weight /body weight ratio of male mice administered different lead concentrations

Dose mg/kg/day	Liver weight (g)	%	Liver/body w. ratio Ratio	%	Brain weight (g)	%	Brain W/ body w.ratio Ratio	%	kidney weight (g)	%	kidney w/ body w. ratio Ratio	%
Control	2.70±0.26	100	0.08±0.006	100	0.43±0.04	100	0.01±0.0008	100	0.54±0.05	100	0.015±0.001	100
0.25	2.66±0.25	98.5	0.09±0.007	113	0.44±0.04	102	0.01±0.0008	100	0.53±0.05	98	0.017±0.001	113
0.50	2.73±0.26	101	0.10±0.008	125	0.43±0.04	100	*0.02±0.0010	200	0.56±0.05	103	0.019±0.002	126
0.75	2.79±0.27	103	0.11±0.008	138	0.40±0.03	93	*0.02±0.0010	200	0.59±0.06	109	0.022±0.002	146
1.00	2.79±0.27	103	0.11±0.008	138	0.42±0.04	98	*0.02±0.0010	200	0.59±0.06	109	0.022±0.002	146

P < 0.05 Slightly significant

Table (3): Effect of lead nitrate added in drinking water to male mice on Liver brain , kidney . and plasma cholinesterase (ChE) activities (N = 15)

Dose mg/kg/day	cholinesterase activity %			
	Liver	Brain	Kidney	Plasma
Control	100	100	100	100
0.25	97.0	98.5	97.2	98.0
0.50	94.4	95.6	96.0	95.1
0.75	92.6	94.8	95.6	94.7
1.00	90.0	94.0	95.0	92.5

Table (4) : Effect of lead nitrate added in drinking water to male mice on acid and alkaline phosphatase activities (unit / g) (N = 15)

Dose mg/kg/day	* Liver		Brain		kidney		plasma	
	Acp	Ap	Acp	Ap	Acp	Ap	Acp	Ap
Control	8.5± 0.4	9.4± 0.1	5.3± 0.4	5.9± 0.3	7.6± 0.2	8.7± 0.6	9.8± 0.5	10.3± 0.1
0.25	8.0± 0.37	8.5± 0.09	4.7± 0.35	5.5± 028.	7.1± 0.19	8.1± 0.55	9.1± 0.46	9.5± 0.09
0.50	7.1± 0.33	8.0± 0.08	4.3± 0.32	4.9± 0.20	6.3± 0.17	7.4 ± 0.51	8.2± 0.41	8.7± 0.08
0.75	*6.4± 0.30	*7.2± 0.08	*3.9± 0.29	*4.2± 0.21	*5.6± 0.15	*6.8 ± 0.47	7.4± 0.37	*8.4± 0.08
1.00	**5.3± 0.25	**6.7± 0.07	**3.4± 0.26	**3.8± 0.19	*5.1± 0.13	*6.1± 0.42	*6.8± 0.34	**7.3± 0.07

The activities are expressed as kind and king activity of untreated animals
Mean values ± SE * unit / ml .

*P<0.05 Slightly significant

** P<0.01 Significant

Table (5) : Effect of lead nitrate added in drinking water to male mice on lactic acid dehydrogenase (LDH) activities (N = 15)

Dose mg/kg/day	LDH Activity									
	*Liver	%	* brain	%	* kidney	%	** plasma	%	*Intestine	%
Control	740±11.9	100	635±12.0	100	760±23.0	100	890±28.4	100	720±3.2	100
0.25	680±10.9	91.7	600±11.3	94.5	685±20.7	90.1	810±25.5	91	660±2.9	91.7
0.50	*590±9.5	79.7	530±10.0	83.5	*605±18.3	79.6	765±24.4	85.9	*605±2.7	84
0.75	**430±6.9	58.1	*470±8.9	74	*540±16.3	71	*690±22.0	77	*550±2.4	76.4
1.00	**310±5.0	41.9	**340±6.4	53.5	**490±14.8	64.5	**620±19.8	69.7	**480±2.1	66.7

* per gram of tissue
** per gram of protein
Mean values± SE

* P < 0.05 Slightly sinificant

** P < 0.01 Significant

*** P < 0.001 Highly significant

Additian of lead through drinking water to the male mice has affected on the acid phosphatase (Acp) and alkaline phosphatase (Ap) activity in the different organs (Table 4). The activity of both the two enzymes was depressed as compared with control. There is a relationship between the reduction in activity of the (Acp) and (Ap) in liver, brain, kidney and plasma and increasing lead concentration in the water.

Schenk (1989) found that a 10-or 30 day cd_{+2} exposition decreased the activity of alkaline phosphatase (Ap), which depends on zn+2, becaused cd+2 has a higher affinity to proteins and enzymes than zn+2, cadmium displaces zn+2 from its bindings, where by an inactive cd2+ - Ap complex result (Applybury et al.,1970).

Table (5) show the activity of liver, brain, kidney and plasma lactic dehydrogenase (LDH) in lead treated male mice. there was a Lactic decrease in the of activities the different organs. It is noteceable that the maximum decrease in activity could be observed in the highest concentration (1mg/kg/day) of lead in liver, brain, kidney, intestine and plasma respectively.

Iannaccone et al. (1976) found unmodified LDH activity in Kidney homogenate of rats fed for 6 months with 50% lead acetate. The effect of following subacute intoxication of rats with pb, cu and zn-salts (separately or in mixture) for 5 weeks, the chelating agent D-penicillamine was administered for 3 weeks. In the course of the 3-month experiment, lactate dehydrogenase (LDH) was estimated in serum and in cytoplasmic fraction of the kidney pb2+ treatment resulted in an increase of LDH activity, cu2+ in a slight decrease, whereas zn2+ had no effect, respectively. Mixture of these metals caused a significant rise in the enzymatic activity. In the experiments in vitro cu2+ inhibited significantly the kidney LDH activity, pb2+ and zn2+ being 100 and 400 times less efficient, respectively (Dobryszcka and Owczdrek 1981).

It could be concluded that, drinking of lead in water (0.25 mg/kg/day) for 180 day produced no discernible toxic effects in experimental animals, and considered as the least toxic dose, whereas other concentrations (0.5, 0.75 and 1.0 mg/kg/day) produced moderate to serve toxicity, such as development of signs of intoxication and mortality, organ body weight ratio and enzymatic changes.

REFERENCES

Applybury, M.L., B.P. Johnson, and J.F. Conleman (1970): Phosphatebinding to alkaine phosphatase. Metal ion dependence J. Biol. Chem. 245: 4968-4970.

Bosque, M. A., M. Schumacher, J.L. Domingo and J.M. Llobet (1990): Cocentrations of lead and cadmium in edibl vegetables from Tarragona province, Spain. Sci. Tatal Envirom. 95: 61-67.

Captain, T. and S. C. Weichselbaum (1946): An accurate and rapid method for the determination of proteins in small amount of blood seum and plasma. Am. J. Clin. Path. 16: 40-49.

Dobryszycka, W. and H. Owczarek (1981): Effects of lead, copper, and Zinc on the rats lactate dehydrogenase in vivo and in vitro. Arch Toxicol. 48: 21-27.

Ellman, G.L., K.D.C.V., Andres and R.M. Feathersonte (1961): A new and rapid colourimetric determination of acetyl cholinesterase activity. Arch. Biophys. 82: 88-92.

HO,4B, and KM tai (1988): Elevated levels of lead and other metals in roadside soil and grass and their use to monitor aevial metal depositions in Hong kong. Envirom. Pollut-49: 34-51.

Iannaccone A.P. Boscolo, and G. Bombardieri (1976): Comparative effects of experimental lead poisoning on enzymatic activites of kidney and liver in rat. Life Sci. 19: 427-432.

Ibrahim, N.M.E.S. Risha, and M. Rabie (1994): Toxic effect of the hbeavy metals (cadmium, zinc and lead) on embryonic development of *Locusta migratoria* (Danica L.). Bull. ent. Soc. Egypt, Econ. Ser. 21: 95-101.

Kind, P.R.N. and E.G. King (1954): Estimation of plasma phosphatase by determination of hydrolysed phenol with aminoantipyrine. J. Clin. Path 7: 322-326.

Kobayashi, H.A. Yuyama, N. Matsusaka, K. Takeno, and I. Yanagiya (1980 a): Effect of methylmercury on brain acetylcholine concentration and turnover in mice. Toxicology and Applied Pharmacology 54: 1-8.

Kobayashi, H., A. Yugama, N. Matsusaka, K. takeno, and I. Yanagiya (1980 b): Effects of methylmercury chloride on various cholinergic parameters in vitro. J. toxicol. Sci. 4: 351-362.

Maehara, N.E. Uchino, K. terayama, H. Ohnoand K. Yamamura(1986): Motor nerve conduction velocity (MCV) and lead content in sciatic nerve of lead. exposed rats. Bull. Environ. Contam. Toxicol. 37: 47-52.

Mokhtar, M.V., A. Bt. Awaluddin, C.W. Fong, and WB. Woojdg (1994): Lead in blood and hair of population near an operational and a proposed area for copper Mining, Malaysia. Bull. Environ. Contam Toxicol, 52: 149-154.

Nriagu Jo (1988): A silent epidemic of environmental metal poisoning Environ. Pollut, 50: 139-161.

Onyari, JM, S.O. Wandiga, G.K. Njenga and J.O. Nyatebe (1991) Lead contamination in street soils of Nairobi city and Mombasa island, Kenya. Bull. Environ. Contam, Toxicol, 46: 782-789.

Ratcliffe, JM. (1981): Lead in man and environment. El Horwood Ltd England.

Rodrigues MF, and Castellon (1982): Lead and cadmiu levels in soil and plants near highways and the correlation with traffic density. Environ. Pollut. (B) 281-290.

Schenke, J. (1989): Tier experimentelle Untersuchung zum EinfluB von Cadmium auf die Aktivit zinkabhangiger Enzyme in verschiedenen Organe Med. Dissertaion, Unirersitat Heidelberg.

Schmidt, G.H. and N.M. Ibrahim (1994) : Heavy met content (Hg2+, cd2+, pb2+) in various body parts: i impact on cholinesterase activity and bindin glycoproteins in the grasshopper *Aiolopus thalassinu* adults. Ecotoxicology and Environmental Safety 2 148-164.

Schumacher M.J.L. Domingo, J.M.L. lobet and J. Corbell (1993): Chromium, copper, and zinc concentrations i edibl Vegetables grown in Tarragona Province, Spair Bull. environ. Contam. Toxicol. 50: 514-521.

Schumacher, M , J.L. domingo, J.M.L. lobet and J. Corbell (1994): Cadmium, chromium, copper, and zinc in ric and rice field soil from southerm Catalonia, Spain. Bull Environ. Contam. Toxicol 53: 54-60.

Tamashiro, H., M. Arakaki, H. Akagi, K. Hiragamd an M.H. Smolensky (1986): Methylmercury toxicity i spontaneously hypertensive rats (SHR). Bull. Enviror Contam. Toxicol. 36: 668-673.

Welch, R.M. and W.A. House (1984) : Factors affecting th bioavailability of mineral nutrients in plant foods. In Welch R.M. Gabelman wH (eds) Crops as sources o nutrients for humans American Society of Agronomy Madison, WI, p 37.

Wroblewski, F. and J.S. Ladue (1955): Lacti dehydrogenase acitivity in blood, Proc. Soc. exp. Biol Med. 90.: 210-217.

Yassoglou N. C. Kosmas, Asimakopoulos, and C. Kollinor (1987): Heavy metal contamination of roadside soils i the Greater Athens area. Environ. Pollut, 47: 293-304.