Dynamics of diethyllead excretion in the urine of rabbits after tetraethyllead administration

Z KOZARZEWSKA,¹ J CHMIELNICKA²

From the Department of Metabolism of Toxic Compounds,¹ Institute of Occupational Medicine, and Department of Toxicological Chemistry,² Institute of Environmental Research and Bioanalysis, Medical Academy, Łódź, Poland

ABSTRACT The dynamics of diethyllead excretion in rabbit urine according to the dose and method of administration of tetraethyllead were studied. Investigations were made on male rabbits which were given two doses of tetraethyllead (12 mg/kg and 3 mg/kg) using intravenous and intragastric methods. Rabbits were also exposed for five hours to tetraethyllead at a concentration of 200 $\mu\text{g}/\text{m}^3$ in the air. The results show the relation of the diethyllead excretion in rabbit urine to the dose and method of administration.

A method for determining diethyllead ($\text{Et}_2\text{Pb}^{+2}$) in urine published recently showed that this compound is a specific indicator not only for assessing acute intoxication¹ but also for evaluating occupational exposure to tetraethyllead (Et_4Pb).²

Yamamura *et al.*,³ using the method of Yamamuchi *et al.*,⁴ also identified the presence of $\text{Et}_2\text{Pb}^{+2}$ in human urine after an acute intoxication by inhalation. The results of previous studies indicate that there is a correlation between the magnitude of exposure to Et_4Pb and the level of $\text{Et}_2\text{Pb}^{+2}$ excretion in human urine.²

Bolanowska and Garczyński examined the metabolism of Et_4Pb in rabbits and suggested that the metabolism of this compound proceeds through the formation of $\text{Et}_2\text{Pb}^{+2}$ as an intermediate metabolite.⁵ Arai *et al.* confirmed this suggestion and showed that $\text{Et}_2\text{Pb}^{+2}$ is the predominant metabolite excreted in rabbit urine.^{6,7}

The aim of the current work was to establish the dynamics of $\text{Et}_2\text{Pb}^{+2}$ excretion in rabbit urine according to the dose and method of administering Et_4Pb . For comparison, the dynamics of $\text{Et}_2\text{Pb}^{+2}$ excretion in the urine of subjects occupationally exposed to Et_4Pb were also examined.

Materials and methods

ANIMAL STUDY

Investigations were made on 17 male rabbits of Dan-

ish breed with an average body weight of 3.5 kg. The animals were given one dose each of Et_4Pb , of two different amounts, using two different methods, intravenous and intragastric. Et_4Pb was administered through the auricular vein using a microsyringe in doses of 12 mg/kg and 3 mg/kg. The same doses were administered intragastrically in gelatinous capsules (Parke-Davis, Detroit, Michigan) filled with Et_4Pb . Rabbits were also exposed through the respiratory tract for five hours in a toxicological chamber to Et_4Pb at a concentration of 200 $\mu\text{g}/\text{m}^3$ in the air. The concentration was checked every hour by taking activated carbon air samples. For the determination of Et_4Pb , a modified dithizone method (M Dobecki, unpublished data) was used.

After exposure to Et_4Pb , the rabbits were kept in metabolic cages and after one, two, three, five, seven, 14, and 30 days, 24 hour urine samples were collected. Total lead (Pb-U) and $\text{Et}_2\text{Pb}^{+2}$ were determined in the urine by atomic absorption spectrometry (AAS).¹ The control group consisted of four rabbits of the same breed.

HUMAN STUDY

A group of three workers employed by a petrol company and who added Et_4Pb to petrol was also studied. The following procedure was applied to this group. The selected workers were removed for 10 days from the work area in which they were exposed to Et_4Pb . Then for two days they returned to their work area, and hence exposure, and subsequently were again withdrawn from exposure to Et_4Pb . Urine samples were taken during the first two days of

Fig 1 Mass spectrum of pure Et_4Pb .

Fig 2 Dynamics of diethyllead and total lead excretion in rabbit urine after administration of Et_4Pb . (a) 12 mg/kg dose of Et_4Pb and (b) 3 mg/kg dose of Et_4Pb .

exposure to Et_4Pb and on the five days after it. The total lead and the $\text{Et}_2\text{Pb}^{+2}$ levels were determined in the urine by AAS,¹ the results being corrected to the average specific gravity of the urine. Atmospheric samples were taken in the breathing zone of the workers with Casella individual sampling tubes. The Et_4Pb concentration in the atmosphere of the individual work areas was determined by a modified dithionite method (M Dobecki, unpublished data). The control group was composed of individuals of the same sex as those occupationally exposed the Et_4Pb .

Pure Et_4Pb was obtained by vacuum distillation of an ethyl fluid containing about 60% of the pure compound. The purity of the Et_4Pb obtained was

Table 1 Percentage of total lead excreted in rabbit urine after 30 days of observation according to the dose and method of Et_4Pb administration

Dose of Et_4Pb (mg/kg)	Method of administration Et_4Pb	Total Pb level		$\text{Et}_2\text{Pb}^{+2}$	
		$\mu\text{g}/30$ Days	% Of dose	$\mu\text{g}/30$ Days	% Of dose
12/7*	Intragastric	1278.01	10.6 (16.6)*	1076.51	9.0 (13.9)*
	Intravenous	817.68	6.2 (10.6)*	396.18	3.3 (5.14)*
3/1.9*	Intragastric	433.04	14.4 (22.8)*	130.04	4.4 (6.53)*
	Intravenous	280.47	9.3 (14.8)*	135.48	4.1 (7.1)*

*Dose of Et_4Pb in terms of inorganic lead.

confirmed by mass spectrometry (fig 1) and analysis of the physical and chemical properties of the compound.

Results

Figure 2 shows the dynamics of $\text{Et}_2\text{Pb}^{+2}$ and Pb-U excretion after intragastric and intravenous administration of Et_4Pb according to dose.

After the intragastric administration of a 12 mg/kg dose of Et_4Pb (fig 2a), excretion of $\text{Et}_2\text{Pb}^{+2}$ in the urine was observed which varied with respect to time.

Fig 3 Dynamics of diethyllead and total lead in rabbit urine after a five hour exposure to Et_4Pb at a concentration of $200 \mu\text{g}/\text{m}^3$ in air.

During the first seven days after administration of Et_4Pb , $\text{Et}_2\text{Pb}^{+2}$ constituted from 70% to 90% of the total lead excreted in the rabbit urine. Maximum $\text{Et}_2\text{Pb}^{+2}$ excretion was recorded on the first day after administration of Et_4Pb , and after 30 days only trace quantities of this metabolite were in the urine. During the 30 days of observation, $\text{Et}_2\text{Pb}^{+2}$ constituted 70% of the total lead excreted in the urine.

After intravenous administration of the same dose of Et_4Pb , $\text{Et}_2\text{Pb}^{+2}$ excretion was lower. During the first seven days this metabolite constituted about 50% of the total Pb excreted with the urine, but the maximum $\text{Et}_2\text{Pb}^{+2}$ was again recorded on the first day after administration of Et_4Pb . After 30 days only small quantities were found in the urine. Over the whole 30 day period, only about 40% of the total lead was excreted in the form of $\text{Et}_2\text{Pb}^{+2}$ in the urine.

After the administration of a 3 mg/kg dose (fig 2b), the excretion of $\text{Et}_2\text{Pb}^{+2}$ did not vary so much between the intragastric and the intravenous methods. During the 30 days of observation, excreted $\text{Et}_2\text{Pb}^{+2}$ constituted about 40% of the total lead excreted with urine. It should be emphasised, however, that for both the intragastric and intravenous methods of administration, maximum excretion of this metabolite in the urine was observed during the first three days after administration of Et_4Pb . Table 1 shows the data concerning total lead excretion during the 30 days of observation according to the dose and method of administration. The intragastric method results in a total lead excretion in the urine proportional to the dose. Moreover, during the 30 days of observation the largest quantity of $\text{Et}_2\text{Pb}^{+2}$ in relation to the dose and level of total lead was excreted after introducing a large dose intragastrically.

Figure 3 shows the dynamics of $\text{Et}_2\text{Pb}^{+2}$ and total lead excretion in rabbit urine after a five hour exposure to Et_4Pb at a concentration of $200 \mu\text{g}/\text{m}^3$ in the air. Maximum $\text{Et}_2\text{Pb}^{+2}$ excretion was recorded on the second day after exposure, and on this day the metabolite constituted about 20% of total lead excreted in the urine. On the seventh day after the end of exposure, only trace quantities of this metabolite

Table 2 Numerical values of $\text{Et}_2\text{Pb}^{+2}$ to total lead ratio in rabbit urine after administration of Et_4Pb

Method of administration of Et_4Pb	Dose of Et_4Pb (mg/kg)	Days after administration						
		1	2	3	5	7	14	30
Intragastric	12	0.90	0.89	0.90	0.87	0.77	0.04	0.04
	3	0.13	0.39	0.29	0.41	0.31	0.24	0.01
Intravenous	12	0.65	0.47	0.46	0.61	0.53	0.10	0.13
	3	0.28	0.68	0.56	0.42	0.47	0.12	0.05
Inhalation	200 $\mu\text{g}/\text{m}^3$	0.04	0.11	0.15	0.14	*	*	*

Results in $\mu\text{g Pb/l}$ were corrected according to 24 hour excretion in urine.

Ratio of mean values ($n = 3$).

*Traces of $\text{Et}_2\text{Pb}^{+2}$ found in urine.

were found in the urine, and constituted a much smaller fraction of the total lead excreted in the urine.

Table 2 shows the ratio of $\text{Et}_2\text{Pb}^{+2}$ to total lead in rabbit urine according to the doses and methods of Et_4Pb exposure used in these experiments. The numerical value of this ratio throughout almost a week for a dose of 12 mg/kg Et_4Pb introduced intragastrically is close to one. The reduced ratio observed after the administration of the 3 mg/kg dose of Et_4Pb may indicate the further dealkylation of $\text{Et}_2\text{Pb}^{+2}$ to ionic lead.

After the intravenous introduction of a 12 mg/kg dose of Et_4Pb , the numerical value of the ratio is lower than after the intragastric administration, which may be due to the lower activity of the liver in the Et_4Pb dealkylation. The value of this ratio after intravenous administration is less affected by the size

of the dose, which could be connected with the limited ability of the liver to dealkylate a high dose of Et_4Pb .

In the experiments designed to evaluate exposure of the rabbits to Et_4Pb by inhalation the degree of Et_4Pb dealkylation to $\text{Et}_2\text{Pb}^{+2}$ is much reduced, which could be connected both with the low dose and the accelerated dealkylation process.

Analysis of variance (three factor experiment with repeated measures) served to identify the importance of the dose and intragastric administration on the efficiency of Et_4Pb metabolism into $\text{Et}_2\text{Pb}^{+2}$ ($p < 0.05$). Also, an important interdependence ($p < 0.05$) between the dose and method of administration was observed.

The application of the Newman test⁸ indicated statistically significant differences in $\text{Et}_2\text{Pb}^{+2}$ excretion in rabbit urine in the days subsequent to inhalation

Table 3 Dynamics of $\text{Et}_2\text{Pb}^{+2}$ excretion in urine of workers occupationally exposed to Et_4Pb

Observation time (days)	Concentration of Et_4Pb in the air (mg/m^3)	$\mu\text{g Pb/l}$		$\text{Et}_2\text{Pb}^{+2}$ Total lead
		Pb-U	$\text{Et}_2\text{Pb}^{+2}$	
1	0.156	52.7	4.50	0.08
2	0.147	61.49	13.44	0.21
3	—	129.76	26.24	0.21
4	—	101.53	7.60	0.08
5	—	42.1	2.57	0.06
6	—	66.1	6.85	0.06
7	—	44.8	6.86	0.15
1	0.274	61.49	8.64	0.14
2	0.350	103.0	6.50	0.06
3	—	116.8	18.46	0.15
4	—	79.3	10.12	0.12
5	—	42.9	3.50	0.08
6	—	72.0	5.13	0.07
7	—	76.64	8.90	0.12
1	0.450	49.15	7.2	0.14
2	0.810	190.51	15.36	0.05
3	—	135.7	4.28	0.03
4	—	110.1	25.0	0.22
5	—	53.3	5.70	0.10
6	—	113.46	27.20	0.23
7	—	14.9	4.00	0.26

exposure to Et_4Pb ($p < 0.05$). On the second day after administration of Et_4Pb a considerably higher excretion of this metabolite in urine, by comparison with results from the other days of observation, was noted.

Table 3 shows the results of the study of $\text{Et}_2\text{Pb}^{+2}$ excretion in the urine of subjects occupationally exposed to Et_4Pb ; total lead concentrations in urine are given after the subtraction of the concentrations of lead found in the control group ($24.8 \pm 11 \mu\text{g Pb/l}$). The lead found in the urine is thus only an effect of exposure to Et_4Pb . The percentage of $\text{Et}_2\text{Pb}^{+2}$ in proportion to total lead is variable. The increase of $\text{Et}_2\text{Pb}^{+2}$ excretion in urine is considerably influenced, however, by a high concentration of Et_4Pb in the air. It should be emphasised that in the urine of exposed workers, even five days after the end of exposure, considerable $\text{Et}_2\text{Pb}^{+2}$ excretion was observed, especially in the workers exposed to high concentrations of Et_4Pb . The numerical values of the $\text{Et}_2\text{Pb}^{+2}$ (total lead ratio) varied between 0.05 and 0.29.

Discussion

The results presented show the relations between the dose and method of Et_4Pb administration and the excretion of $\text{Et}_2\text{Pb}^{+2}$ in rabbit urine. After the intravenous administration of a 12 mg/kg dose of Et_4Pb , the results obtained for excreted total lead and $\text{Et}_2\text{Pb}^{+2}$ during the first week of observation are about the same as those of Arai *et al.*^{3,4,6,7} Total lead excreted with urine after 24 hours constituted about 3%, 70% of which was in the form $\text{Et}_2\text{Pb}^{+2}$. Prolongation of the observation time of the excretion dynamics of Et_4Pb metabolites to 30 days (fig 2) permitted the detection of $\text{Et}_2\text{Pb}^{+2}$ in rabbit urine at the level of $7 \mu\text{g}/24$ hours.

An important result was to show that $\text{Et}_2\text{Pb}^{+2}$ is also a dominant metabolite after intragastric administration of Et_4Pb . Moreover, the metabolic capacity of this compound in rabbits is higher after intragastric than after intravenous administration (table 2). The liver is the main site of Et_4Pb dealkylation to Et_3Pb^+ (triethyllead).^{5,6} This metabolite is labile and undergoes a change to $\text{Et}_2\text{Pb}^{+2}$ which is excreted into the bile. A further stage of dealkylation to ionic lead takes place in the intestines. Over 90% of the Et_4Pb dose is excreted in the rabbit faeces in the form of ionic lead, with minimal excretion of Et_3Pb^+ and $\text{Et}_2\text{Pb}^{+2}$. The Et_4Pb and Et_3Pb^+ biotransformation probably proceeds from hydroxylation of carbon with the participation of monooxygenase and further dealkylation processes occur, probably without the

presence of enzymes.⁹

It appears from our studies that the smaller the dose of Et_4Pb , the smaller the amount of $\text{Et}_2\text{Pb}^{+2}$ metabolite excreted in the urine. Even in rabbits exposed by inhalation to Et_4Pb concentrations exceeding twice the TLV, however, the method described was successful in tracing this metabolite in urine after five days. The metabolite constituted only a small percentage of total lead, which indicates that at low doses, dealkylation of $\text{Et}_2\text{Pb}^{+2}$ to ionic lead takes place quickly.

Previous studies² and those presented in table 3 indicate that the magnitude of the Et_4Pb dose absorbed by workers during working hours determines the rate of $\text{Et}_2\text{Pb}^{+2}$ excretion. This metabolite could be determined in urine even after the fifth day in the case of workers exposed to the lowest concentration of Et_4Pb .

To sum up the research results, it should be emphasised that $\text{Et}_2\text{Pb}^{+2}$ in urine is a new indicator of exposure to Et_4Pb . A strong correlation was found between the concentration of Et_4Pb in the air and excretion of $\text{Et}_2\text{Pb}^{+2}$ in the urine.²

The studies carried out with regard to various applied doses and methods of Et_4Pb administration to rabbits show that these animals are suitable experimental material for the interpretation of results on man.

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