Arh. hig. rada, *26* (1975) 119.

# IS THE TOTAL CONCENTRATION OF LEAD IN BLOOD A SIGNIFICANT TEST FOR LEAD POISONING?

HALKA BILINSKI

*Department of Physical Chemistry, Ruđer Bošković Institute, Zagreb*

*(Received for publication April 3, 1974)*

By the method of inverse polarography maximal concentration of soluble lead in blood plasma, at experimental conditions *in vitro*, 25°C, t = 48 h, $pCO_2 = 10^{-3.5}$ atm was found to be 101,5 μg/l. It was compared with the concentration of lead in »normal« man, at 37° C to get an approximate relationship. Because of low solubility of lead and of important time factor great care is necessary if the total lead concentration in blood is to be used as a significant test for lead poisoning.

Using a model concept of heterogeneous mixture of dissolved and solid inorganic salts, preliminary information about the behaviour of lead and calcium in blood plasma regarding solubility, ionic state and interdependence was obtained. In the examined model it was calculated that thermodynamically stable solid phases are $Pb_3(PO_4)_2$ and $Ca_5(PO_4)_3OH$. Predominance order of soluble lead and calcium complexes is $PbSO_4^0 > PbCO_3^0 > PbOH^+ > PbCl^+ > Pb(SO)_4)_2^{2-} > Pb^{2+}$, $Ca(SO)_4)_2^{2-} > Ca^{2+} > CaSO_4^0 > CaPO_4^-$.

The importance of an improved model concept as well as of such experimental technique which would enable direct determination of different ionic species of metal pollutants in blood is emphasized.

The application of lead in 1923 as an automotive fuel additive involved exposure of the whole population. As a result of studying the consequences of lead in the environment, many scientific and review articles have been published. Some of them are highly controversial. There has

---

This paper is a revised version of the one presented at the First Yugoslav Symposium on Chemistry and Environment, Zagreb, 14—16 February, 1973.
Experimental part and calculations were performed at EAWAG, ETH Dübendorf, Switzerland

been no clear evidence by now that the whole population is at risk (as believed by pessimists), but there is likewise no evidence that it is not (as claimed by optimists).

Some detailed reviews (1—7) contain most of the information presently available about lead as an environmental pollutant. About 436 $\mu$g of lead is ingested and inhaled per day by an urban air nonsmoker and of this 31 $\mu$g is absorbed (5). Most of lead dissolves in blood (1). Lead appears to exist in blood partly as peptized lead phosphate sol, which is aggregated on the surface of erythrocytes, and partly as intracellular lead of an unknown form (8). In the presence of lead blood plasma albumin content is reduced, and abnormalities in the heme synthesis occur (9). Lead is absorbed by the bone in the form of the tertiary lead phosphate. Like calcium it can be released from the bone and therefore bones act as a reservoir for lead (10). Although all organs contain some lead, about 90% is found in the skeleton while blood contains less than 1% (6). It was shown that lead concentrates in the kidney as well as in the bone marrow (11). At the cellular level, lead concentrates in the nuclei, mitochondria and microsomes. It is eliminated from the body through urine, faeces, saliva, sweat and hair (12).

For better understanding of the behaviour of trace metal ions in blood, it is very important to know not only their total concentration but which ionic species they form with blood components (chemical speciation) and which factors control their maximum solubility. The first approach to solve the problem can be analogous to the one used for natural waters (13—15).

Only a very few attempts have been made up to now to determine the chemical speciation of standard blood substances (16) and of metal ions in living organisms (17—20). In computer models of complexing equilibria present during the chelation therapy of metal contaminated blood plasma (21, 22) only organic ligands, which are a part of drugs are considered in calculation, in addition to some amino acid ligands. In the presence of strong artificial ligands, simple inorganic ligands naturally occurring in man are of minor importance for calculation and are neglected. However, to authors' knowledge there is no computer model in the literature of complexing equilibria between lead in blood plasma and inorganic and organic ligands, which are naturally occurring in man.

The model concept of heterogeneous mixture of dissolved and solid inorganic salts shown in this work is a very simplified picture of blood plasma and can serve only as a preliminary orientation. The assumption was, similar to natural waters (13, 15) that most of the organic complex forming donor groups are bound to calcium and magnesium which are present in blood in high excess over lead. Computer calculations gave thermodynamically stable solid phase of lead and calcium, their speciation and interdependence. The solubility of lead in blood plasma was determined *in vitro*, after 1 and 48 hours at 25 °C.

## MATERIALS AND METHODS

Experimental solutions were prepared from 0.1 M lead nitrate, $Pb(NO_3)_2$, Merck *p.a.*, stock solution, using micropipettes and 50 ml of human blood plasma as an electrolyte. The constant temperature of 25°C was maintained in all the experiments. Gas mixture of $N_2$ and $CO_2$ (350 ppm) was used for deaeration. Measurements were completed in fresh ($t = 1$ h) and in aged solutions ($t = 48$ h). To determine reducible lead in blood plasma, $[Pb]_{m.l.}$, anodic stripping voltametry was used (HMDE surface 0.054 $cm^2$, constant voltage sweep 0.0167 V $s^{-1}$, cathodic process with 3 min stirring and 1 min stabilizing period).

## RESULTS

a) *Experimental results*

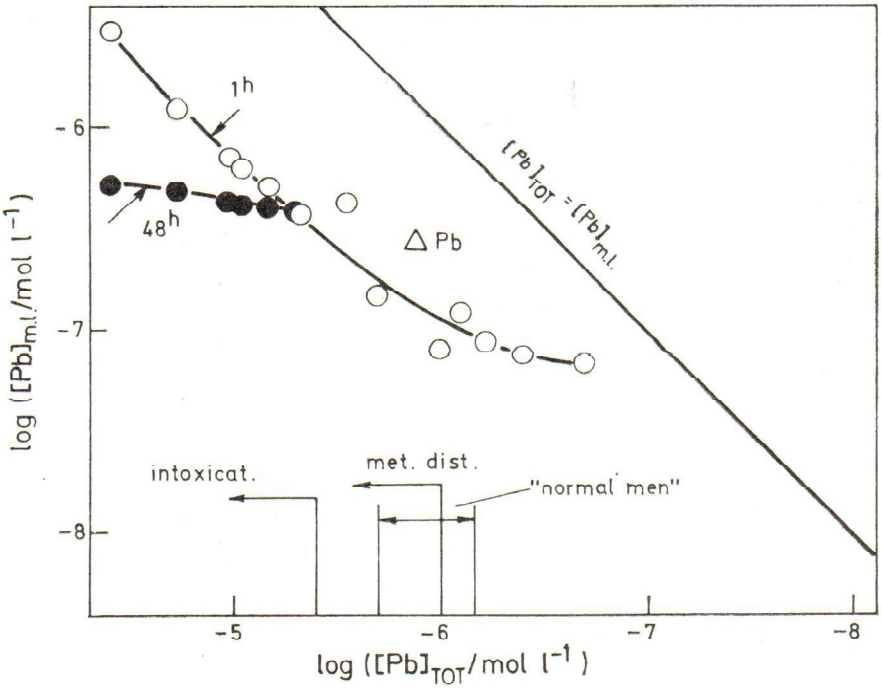


Fig. 1. *Dependence of reducible lead, $[Pb]_{m.l.}$ on added total concentration of lead, $[Pb]_{tot}$ to 50 ml of blood plasma of healthy humans. Measurements were performed in vitro, 1 hour (circles) and 48 hours (dots) after equilibration. Temperature was 25°C; gas mixture $N_2 + CO_2$ (350 ppm).*
*Concentration of $[Pb]_{tot}$ which cause intoxication and metabolic disturbances are compared with that in »normal« men. Log. units are used.*

In Fig. 1 log ($[Pb]_{m.l}$/mol $l^{-1}$) is plotted vs. log ($[Pb]_{tot}$/mol $l^{-1}$), where $[Pb]_{tot}$ corresponds to the total concentration of lead added to human blood plasma and $[Pb]_{m.l.}$ corresponds to determined reducible lead. Circles are for measurements of 1 hour and dots for 48 hours after equilibration. The difference between these two concentrations, $\Delta$Pb, concerns the non-reducible lead in solution and is due either to a solid phase formed, or to lead organic complexes or to both of them.

The amounts of the total lead in the blood, which are considered to cause pronounced effects (800 $\mu$g/l) and metabolic disturbances (200 $\mu$g/l), are compared with those existing in »normal« man (150—400 $\mu$/l). After 48 hours the value of $[Pb]_{m.l.}$ approaches approximately the constant value of $5 \times 10^{-7}$ M (101.5 $\mu$g/l). Visible colloids are formed.

b) *Model calculation:*

Table 1

*Model concept of heterogeneous mixture of dissolved and solid inorganic salts*

| $[Conc]_{tot}$ mole/l | Solid | -log $K_{so}$ | Complex | log $\beta$ |
|---|---|---|---|---|
| Na: 0.143 | 0 | 0 | $NaSO_4^-$ | 0.2 |
| | | | $NaCO_3$ | 0.7 |
| K: $4.6 \times 10^{-3}$ | 0 | 0 | $KSO_4^-$ | 0.5 |
| Ca: $2.5 \times 10^{-3}$ | $CaCO_3$ | 8.4 | $CaCO_3^0$ | 3.2 |
| | $Ca_5(PO_4)_3OH$ | 51.5 | $CaHCO_3^+$ | 10.8 |
| | $Ca(OH)_2$ | 4.9 | $CaHPO_4^0$ | 12.6 |
| | $CaHPO_4$ | 18.9 | $CaH_2PO_4^+$ | 19.9 |
| | $Ca_3(PO_4)_2$ | 26.0 | $CaPO_4^-$ | 6.46 |
| | | | $CaSO_4^0$ | 1.2 |
| | | | $Ca(SO_4)_2^{2-}$ | 3.5 |
| | | | $CaOH^+$ | 0.9 |
| Mg: $8.2 \times 10^{-4}$ | $MgCO_3$ | 4.3 | $MgCO_3^0$ | 2.05 |
| | $Mg_3(PO_4)_2$ | 23.1 | $MgHCO_3^+$ | 10.5 |
| | $Mg(OH)_2$ | 10.4 | $MgHPO_4^0$ | 12.4 |
| | | | $MgSO_4^0$ | 1.3 |
| | | | $MgOH^+$ | 2.1 |
| $CO_3$: $2.5 \times 10^{-2}$ | $CO_2$(g) | 17.0 | $HCO_3^-$ | 9.6 |
| $p\,CO_2 = 10^{-3.5}$ atm | | | $H_2CO_3^0$ | 15.4 |
| $PO_4$: $3 \times 10^{-3}$ | 0 | 0 | $HPO_4^{2-}$ | 11.8 |
| | | | $H_2PO_4^-$ | 18.5 |
| | | | $H_3PO_4^0$ | 20.4 |
| $SO_4$: $2.5 \times 10^{-2}$ | 0 | 0 | $HSO_4^-$ | 1.8 |
| Cl: $9.8 \times 10^{-2}$ | 0 | 0 | 0 | |

Table 1 shows concentrations of metal ions and inorganic ligands used in computation, possible solid phases and soluble complexes and the values of equilibrium constants. The values of the constants at 25 °C

are taken from the literature (23) and are corrected for ionic strength $I = 0.1$ mol $l^{-1}$. Similar concentrations of metals and ligands are found in blood plasma.

Table 2

*Hypothetical solids and complexes of lead which are added to heterogeneous mixture in Table 1*

| $[Pb]_{tot}$, mole/l | Solid | $-\log K_{so}$ | Complex | $\log \beta$ |
|---|---|---|---|---|
| $10^{-8}$—$10^{-5}$ | $PbCO_3$ | 12.5 | $PbCO_3^0$ | 6.4 |
| | $Pb_3(OH)_2(CO_3)_2$ | 45.0 | $Pb(CO_3)_2^{2-}$ | 9.8 |
| | $PbHPO_4$ | 22.2 | $PbSO_4^0$ | 2.52 |
| | $Pb_3(PO_4)_2$ | 42. 1 | $Pb(SO_4)_2^{2-}$ | 3.47 |
| | $PbSO_4$ | 7.6 | $PbCl^+$ | 1.18 |
| | $PbCl_2$ | 4.7 | $PbCl_2^0$ | 1.84 |
| | $Pb(OH)_2$ | 18.7 | $PbCl_3^-$ | 2.08 |
| | | | $PbCl_4^{2-}$ | 1.57 |
| | | | $PbOH^+$ | 7.0 |
| | | | $Pb(OH)_2^0$ | 11.5 |

Table 2 shows possible solids and soluble complexes of lead as well as the values of equilibrium constants (23, 24). Concentration of lead varied from $10^{-8}$ to $10^{-5}$ mol $l^{-1}$. Computer program (25) was used for equilibrium calculation. The calculation shows that the maximum solubility of lead in the model system is $1.6 \times 10^{-7}$ M. It is determined by solubility product of $Pb_3(PO_4)_2(s)$.

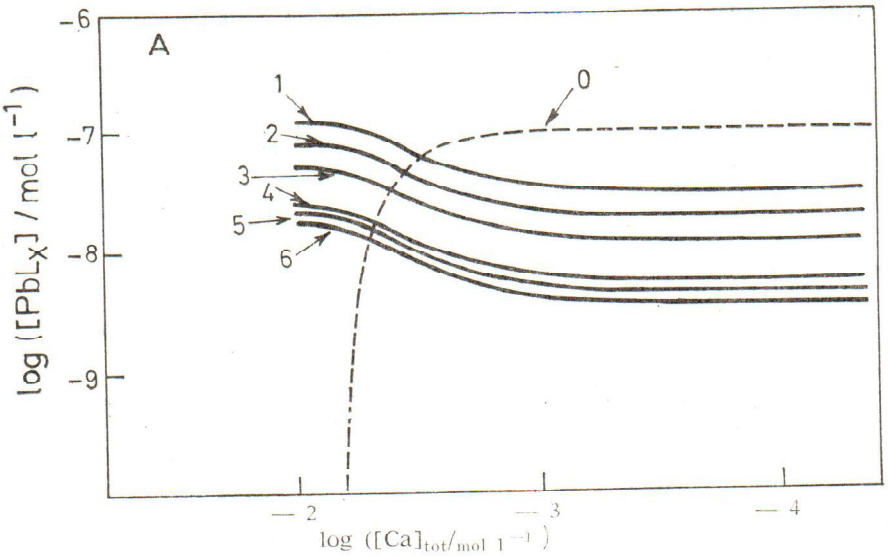


Fig. 2a

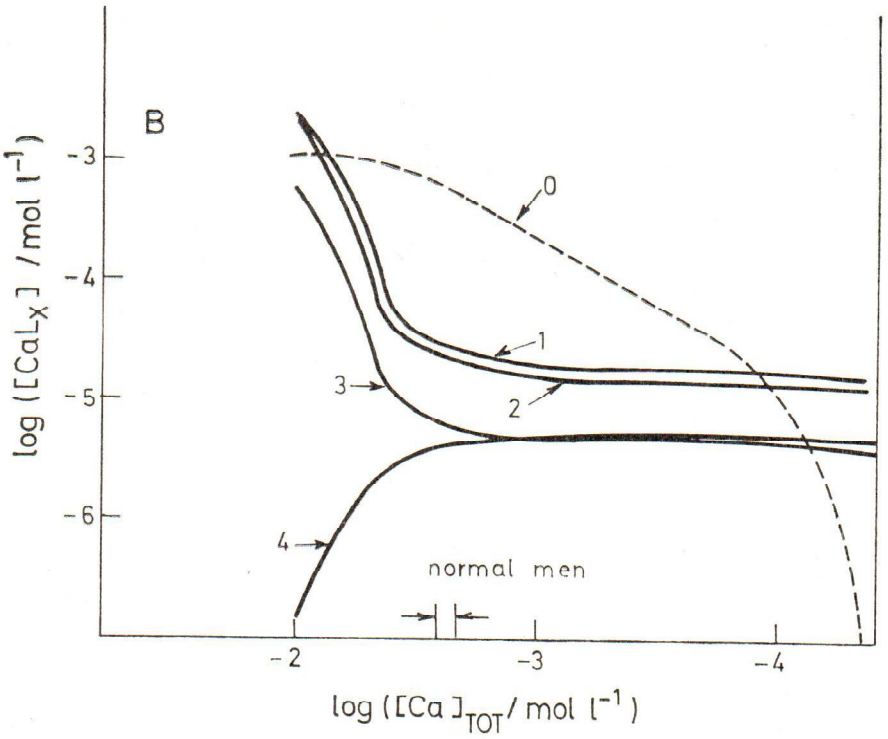


Fig. 2a

*Fig. 2. Model calculation in the system: Metal and ligand concentrations from Table 1, $[Pb]_{tot} = 3.2 \times 10^{-7}$, $[Ca]_{tot}$ var., pH = 7.4, 25 °C, I = 0.1, $pCO_2 = 10^{-3.5}$ atm. Part A. Dependence of lead speciation on concentration of calcium, plotted in log. units: 0. $Pb_3(PO_4)_2(s)$; 1. $PbSO_4^0$; 2. $PbCO_3^0$; 3. $PbOH^+$; 4. $PbCl^+$; 5. $Pb(SO_4)_2^{2-}$; 6. $Pb_2^{+}$; Part B. Dependence of calcium speciation on concentration of calcium, plotted in log. units: 0. $Ca_5(PO_4OH(s)$; 1. $Ca(SO_4)_2^{2-}$; 2. $Ca^{2+}$; 3. $CaSO_4^0$; 4. $CaPO_4^-$; Concentrations of calcium existing in »normal« men are shown for comparison*

In Fig. 2/A the influence of increased calcium concentration on dissolution of lead phosphate is shown for $[Pb]_{tot} = 3.2 \times 10^{-7}$ mol $l^{-1}$, pH = 7.4 and $I$ = 0.1 mol $l^{-1}$. Precipitated $Pb_3(PO_4)_2(s)$ dissolves and the concentrations of soluble lead complexes increase. The predominance order of soluble complexes is found to be $PbSO_4^0 > PbCO_3^0 > PbOH^+ > PbCl^+ > Pb(SO_4)_2^{2-} > Pb^{2+}$. Other complexes are present in negligible amounts.

Maximum solubility of calcium is determined by solubility product of hydroxyapatite. In Fig. 2/B the predominance order of soluble calcium complexes $Ca(SO_4)_2^{2-} > Ca^{2+} > CaSO_4^0 > CaPO_4^-$ is shown. If the con-

centration of calcium increases above that of a normal man the dissolution of lead phosphate occurs (Fig. 2/A) and calcium species in equilibrium with hydroxyapatite are changed dramatically.

## DISCUSSION AND CONCLUSION

Like any model, this one also presents a significant oversimplification of the behaviour of lead and calcium in blood plasma. It is incomplete and needs a great deal of improvement before exact answer to that complicated question is obtained. The aim of this work is to induce researchers to study *in vivo* and *in vitro* the chemical speciation of metal pollutants and their competition with essential elements in addition to their total concentrations in blood plasma. The development of new experimental techniques seems to be necessary. The knowledge of metal ion speciation is very important to predict their adsorbability and penetration into living cells (26).

Some literature data show that there is an insignificant variation in the concentration of lead in the blood of persons with different lead exposure (5, 27, 28). Cases of lead poisoning in children and adults whose blood has been found to contain relatively low concentrations of lead (29, 30) have been reported. The question is, which factors control the solubility of lead in blood plasma? Model calculations show that under the defined simplified conditions thermodynamically stable solid phases are only $Pb_3(PO_4)_2$ and $Ca_5(PO_4)_3OH$. The two solids are found in bones and teeth (8—10) respectively (31, 32). Thus in blood plasma naximum solubility of lead might be controlled with the solubility product of $Pb_3(PO_4)_2$, which is the most insoluble thermodynamically stable solid phase. Further addition of lead causes its precipitation and the concentration of soluble lead remains constant.

Disagreement in the literature regarding the most useful diagnostic test for lead poisoning seems to be significant. Namely, there are many papers based only on analyses of the total lead in blood, used as the most useful diagnostic test for lead poisoning (33), showing significant variation of lead blood concentration. One has to be aware that inadequate analytical methods have made many published data open to suspicion. The dithizone method is subject to interference by other trace substances (9). Experimental results in Fig. 1 show that the time factor must not be forgotten. The interval between the onset of illness and the time of the blood analysis may well be and often is sufficient to enable the blood level to decrease (34). Blood and hair lead levels correlate quite well considering that blood lead primarily reflects an active transport mechanism and recent exposure whereas hair lead most probably represents a longer term exposure integral (35) and should be therefore recommended as a more useful test for lead exposure.

*Literatura*


1. *Schroeder, H. A., Balassa, J. J.:* Chronic Dis., *14* (1961) 408.
2. *Hammond, P. B.:* Chapter 4. Lead Poisoning. An Old Problem with a New Dimension, in Blood, R. F. (ed.): Essays in Toxicology, Vol. I, Academic Press, New York and London, 1969.
3. *Goyer, R. A.:* Chapter 3, in *Lee, D. H. K.* (ed.): Lead in Metallic Contaminants and Human Health, Academic Press, New York, 1972.
4. *Scanlon, J.:* Clin. Pediat., *11* (1972) 135.
5. *Hankin, L.:* J. Milk Food Technol., *35* (1972) 86.
6. Chapter 12. Selected Environmental Pollutants, in Health Hazards of the Human Environment, World Health Organization, Geneva, 1972, 174.
7. *Truffert, L.:* Ann. Fals. Expert. Chim., *65* (1972) 51.
8. *Clarkson, T. W., Kench, J. E.:* Biochem. J., *69* (1958) 432.
9. *Snyder, R. B., Wuebbles, D. J., Pearson, J. E., Ewing, B. B.:* U. S. Nat. Tech. Inform. Serv. P. B. Rep., 1971, No. 205239.
10. *Berman, E.:* Clin. Pediat., *5* (1966) 287.
11. *Goyer, R. A., Leonard, D. L., More, J. F.* et al.: Arch. Environ. Health, *20* (1970) 705.
12. *Lagerwerff, J. V.:* Micronutr. in Agricult., (1972) 594.
13. *Stumm, W., Bilinski, H.:* Trace Metal in Natural Waters. Advances in Water Pollution Research, 6th International Conference, Jerusalem (1972); Pergamon Press, Oxford and New York, 1973.
14. *Stumm, W., Brauner, Ph.:* Chemical Speciation, in *Riley, J. P., Skirow, G.* (eds.): Chemical Oceanography, 2nd edition, Academic Press, 1973.
15. *Bilinski, H., Schindler, P., Stumm, W., Zobrist, J.:* Vom Wasser, *43* (1974) 107.
16. *De Land, E. C.:* The Classical Structure of Blood Biochemistry, Proc. Alfred Benson Symp., 4th (1971), 74.
17. *Tsuchiya, K.:* Some Industrial Problems in Absorption and Excretion of Metal Ions (Lead, Cadmium, Mercury), in *Skoryna, S. C., Waldron-Edward, D.* (eds.): Intestinal Absorption of Metal Ions Trace Elements and Radionuclides, Pergamon Press, Oxford and New York, 1970, 399.
18. *Pfordte, K.:* Acta Biol. Med. Germ., *27* (1971) 527.
19. *Hendel, R. C., Sunderman, F. W.:* Research Commun. Chem. Pathol. Pharm., *4* (1972) 141.
20. *Williams, D. R.:* Chem. Reviews, *72* (1972) 203.
21. *Williams, D. R.:* South African Journal of Science, *70* (1974) 252.
22. *Corrie, A. M., Touche, M. L. D., Walker, M. D., Williams, D. R.:* Computer Models of the Complexing Equilibria Present During the Chelation Therapy of Cadmium-, and of Lead-, Contaminated Blood Plasma, in Proceedings of the XVI ICCC, Dublin, Ireland (1974).
23. *Sillén, L. G., Martell, A. E. (compilers):* Stability Constants of Metal-ion Complexes. The Chemical Society Burlington House, London; Special Publication No. 17, 1964; Supplement No. 6- Special Publication N. 25, 1971.
24. *Bilinski, H., Stumm, W.:* Eawag News, Jan. (1973).
25. *Morel, F., Morgan, J. J.:* Env. Sci. Technol., *6* (1972) 58.
26. *Dwyer, F. P., Meller, D. P.:* Chelating Agents and Metal Chelates, Acad. Press, London, 1964, 383.
27. *Thomas, H. V., Milmore, B. K., Heidbreder, G. A., Kogan, B. A.:* Arch. Environ. Health, *15* (1967) 695.
28. *Goldwater, L. J., Hoover, A. W.:* Arch. Environ. Health, *15* (1967) 60.
29. *Moncrieff, A. A., Koumides, O. P., Clayton, B. E., Patrick, A. D., Renwich, A. G., Roberts, G. E.:* Arch. Dis. Child., *39* (1964) 1.
30. *Beritić, T.:* Arch. Environ. Health, *23* (1971) 289.
31. *Neuman, W. F., Neuman, M. W.:* Chemical Dynamics of Bone Mineral. Chicago University Press, 1953.

32. *Boyd, W.:* A Text Book of Pathology, Lea and Febiger, Philadelphia, 1962, 428.
33. The Surgeon General's Policy Statement on Medical Aspects of Childhood Lead Poisoning, Dept. of Health Education and Welfare Public Service, Nov. 1970; quoted in Michrochem. J., 18 (1973) 77.
34. *Kehoe, R. A.:* J. Occup. Med. *14* (1972) 390.
35. *Hammer, D. I., Finklea, J. F., Hendricks, R. H., Hinners, T. A., Riggan, W. B., Shy, C. M.:* Trace Substance in Environmental Health. V. 1972. A Symposium D. D. Hemphill, Ed., University of Missouri, Columbia.

*Sažetak*

## JE LI UKUPNA KONCENTRACIJA OLOVA U KRVI VALJAN KRITERIJ ZA TROVANJE OLOVOM?

Određena je maksimalna koncentracija 101,5 $\mu$g/l topivog olova u krvnoj plazmi *in vitro*, 25 °C, t = 48 h, $pCO_2$ = 10—3.5 atm, metodom inverzne polarografije i uspoređena je s koncentracijom olova u »normalnom« čovjeku, pri 37°C. Zbog niske topivosti olova i važnosti vremenskog faktora, potrebna je velika opreznost pri upotrebi ukupne koncentracije olova u krvi kao kriterija za trovanje olovom.

Pomoću modelne koncepcije heterogene smjese otopljenih i krutih anorganskih soli, dobivena je preliminarna orijentacija za ponašanje olova i kalcija u krvnoj plazmi, u pogledu topljivosti i ionskog stanja. U promatranom modelu izračunane su termodinamički stabilne krute faze $Pb_3(PO_4)_2$ i $Ca_5(PO_4)_3OH$ i topljivi kompleksi olova i kalcija, koji prevladavaju ovim redoslijedom:

$$PbSO_4^0 > PbCO_3^0 > PbOH^+ > PbCl^+ > Pb(SO_4)_2^{2-} > Pb^{2+};$$

$$Ca(SO_4)_2^{2-} > Ca^{2+} > CaSO_4^0 > CaPO_4.$$

Naglašena je važnost usavršavanja modelne koncepcije, kao i pronalaženja eksperimentalne tehnike, koja bi omogućila direktno određivanje različitih ionskih vrsta metala-zagađivača u krvi.

*Odjel za fizikalnu kemiju,*
*Institut »Ruđer Bošković«, Zagreb*

*Primljeno 3. IV 1974.*