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SHORT COMMUNICATION

METAL EXPOSURE STUDIES: ROLE OF TOXICOLOGY AND EPIDEMIOLOGY IN PUBLIC HEALTH POLICY

MARKO ŠARIĆ AND MARTINA PIASEK

Institute for Medical Research and Occupational Health, Zagreb, Croatia

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Following the idea of interrelated role of epidemiology and toxicology in risk assessment and dose response evaluation, this paper presents certain experiences from the authors' studies of health effects of environmental exposure to lead and manganese. Epidemiologic observations of adverse effects on female reproductive integrity in a lead smeltery area agree with the experimental data. Animal studies show that the adverse reproductive effects in females are time and dose-related, and reversible after exposure has ceased. Field studies show that effects of manganese on the respiratory system in a polluted region are dose, age and season-related. Cytotoxic effects of manganese, including the inhibitory effect on alveolar macrophages described in *in vitro* studies on mammalian cells confirm the epidemiologic observations. Authors conclude that in the process of risk assessment, toxicology and epidemiology have to act together. Available human data should be combined with experimental findings and data on the mechanism of toxic action.

Key words:
cytotoxic effects, lead exposure, manganese exposure, reproductive effects, respiratory effects

The definition and objectives of toxicology and epidemiology are well known. Toxicology is concerned with the nature and mechanisms of toxic lesions and quantitative evaluation of the spectrum of biological changes produced by exposure to chemicals. Epidemiology studies the real-life situation, that is, the rate and distribution of a disease in population groups and the risk factors for its occurrence and development.

As a rule, toxicological studies are conducted on experimental animals. Different extrapolative methods may be used to apply animal data on man. However, great caution should be exercised in using the existing methods, and their inherent limitations should always be kept in mind (1, 2).

Epidemiologic findings should be subject to consideration of possible errors in design and analysis. Concerns about uncontrolled confounding factors are often. An epidemiologic study usually results with an indication which is to be confirmed by repeated studies or by other approaches such as field experiment or experimental studies on animals.

When discussing the role of toxicology and epidemiology in the process of risk assessment, it has to be emphasised that they should act together. Good experimental data combined with human data (if available) and an understanding of the mechanism of toxic action are essential for satisfactory low dose extrapolation (3).

This paper presents and discusses our experience from the studies of health effects of environmental exposure to lead and manganese in the light of complementary role of toxicology and epidemiology in risk assessment and dose response evaluation.

DESCRIPTION OF THE STUDIES

1. EFFECT OF LEAD ON REPRODUCTION

EPIDEMIOLOGIC STUDY

In our study of possible adverse health effects in lead-exposed population, we focused on the evaluation of the rates of spontaneous abortions and twin births in a lead smeltery area (4, 5). The obtained data referred to considerable pollution caused by emission from the smeltery. Annual mean concentrations of lead in the air were between 20 and 22 $\mu g/m^3$.

In 1978, an efficient bag filter system was installed to control emissions. After that, concentrations of lead in the air fell radically (by about 90%) and generally remained below the values of 2 $\mu g/m^3$. Despite the normalisation of lead concentrations in the air, however, lead in the soil remained high. Lead enters the food chain and, when redistributed, contaminates other surfaces.

Subjects and methods

The spontaneous abortions study was carried out retrospectively for a 20-year period (1961–1980). The obtained data are related to the period (years) with high concentrations of lead in the air, except for the last three years following the introduction of efficient emission abatement in 1978. Since the records on spontaneous abortions were kept on the district level, the obtained rates relate to the district as a whole. As only about one third of the population in the district with the lead smeltery was actually exposed to lead, the rates of spontaneous abortions for the female population in the entire district could not represent the real situation with regards to the potential effects of lead on reproduction. Consequently, the investigation was combined with the study of the twin birth rate. The rate of twin births is inversely proportional to the rate of spontaneous abortions. As the rate of twin births is rather low, it

was possible to trace each individual birth by place of family (mother) residence. This made the analysis possible for the entire district as well as for the lead exposed area.

Population living in another district with very similar geographic and demographic profile but without a specific source of exposure to lead was chosen as control for both parts of the study (spontaneous abortions and twin births).

Results and conclusion

Figure 1 shows the results referring to spontaneous abortions. The rate of spontaneous abortions in the district with the lead smeltery was constantly higher than in the control district during the study period. Curiously enough, starting from 1978 when the bag filters were introduced to control the smeltery emissions, the difference in the rate of spontaneous abortions between the two districts disappeared.

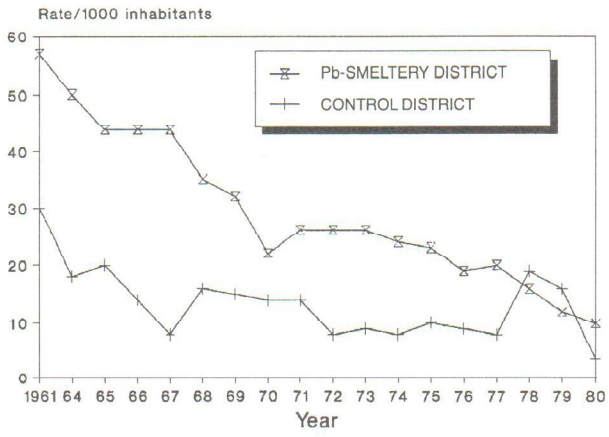


Figure 1 *The annual rate of spontaneous abortions in the district with a lead smeltery vs. control district (4)*

Cumulative rate of twin births per thousand inhabitants did not differ in the studied districts (2.4 in the lead smeltery district vs. 2.6 in the control district). However, the rate of twin births in the area close to the smeltery was smaller (1.7) than in the rest of the smeltery district (2.7) and in the control district. These findings support the assumption that lead exposure increased the rate of spontaneous abortions.

ANIMAL STUDIES

Concurrent experiments studied the effects of oral lead exposure on the reproductive integrity. The study evaluated the influence of sex, dose, exposure duration, and possible reversibility of the effects after cessation of exposure.

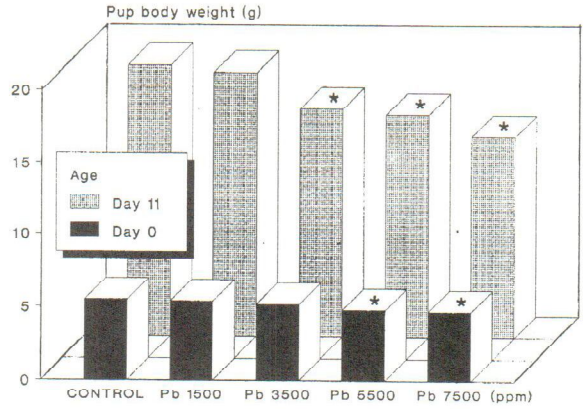
Material and methods

Experiments were conducted on albino laboratory rats of both sexes (Wistar-Zgim, from the Institute's outbred rat unit). The female rats were exposed to 0, 1,500, 3,500, 5,500, and 7,500 ppm lead (as acetate, "Kemika", Zagreb) in deionised drinking water (minimum 100 animals per group). Two principal types of protocols were applied with either shorter (subchronic) exposure, when female rats were exposed in total 9 weeks, and/or longer (chronic) exposure of up to 20 weeks (6). Each protocol involved exposure prior to mating, and followed by exposure during gestation (three weeks), parturition, and lactation (up to 11 days after birth). Similarly, male reproduction in rats was evaluated after either shorter (four) or longer (13 weeks) period of exposure to the same doses of 1,500–7,500 ppm lead in drinking water (7). Biomarkers of reproductive effects of lead were: fertility index (percentage of pregnancy) and indicators of pup viability (number and weight of pups per litter, and pup's survival from Day 0 to Day 11 after birth).

Results and conclusions

No significant changes in body weight and food consumption were observed in adult male and/or female rats during either subchronic or chronic lead exposure. Only the average daily fluid consumption in exposed females (measured out of the periods of pregnancy and lactation) decreased by up to 30% as compared to the controls. This result was not attributed to a toxic effect but to the odd flavour of the test drinking solution of lead acetate which the animals hesitate to drink. It took two weeks for the animals to grow accustomed to the drinking solution. Meanwhile, some animals died of dehydration.

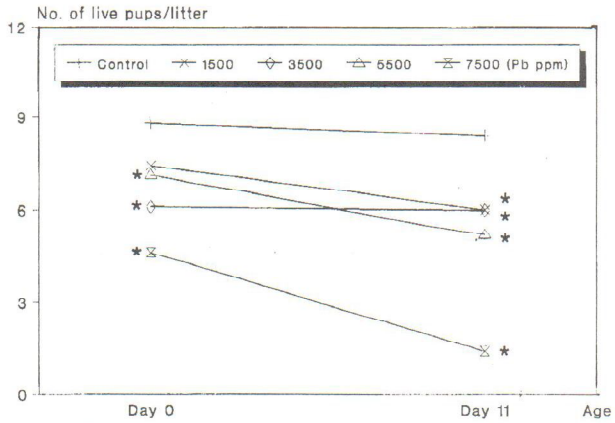
The fertility index (percentage of pregnant of mated females) did not change at any level and duration of lead exposure and was between 65% and 75% (5). Simple biomarkers of reproductive effect did not detect any effect on male reproduction (7).



*Statistically different from control (P<0.01)

Figure 2 *Average pup body weights (g) after chronic (20-week) maternal exposure to lead (1500–7500 ppm in drinking water). Number of litters: N≥25.*

Live, 0-day-old newborn and 11-day-old suckling pups born to the rats chronically exposed to lead (in total 20 weeks) manifested significantly reduced body weights (Figure 2). The effects on body weight became visible in 11-day-old pups at 3,500 ppm lead exposure, and were expressed at higher lead doses (5,500 and 7,500 ppm) in both newborn (0-) and 11-day-old sucklings. Generally, the effect was dose-dependent.



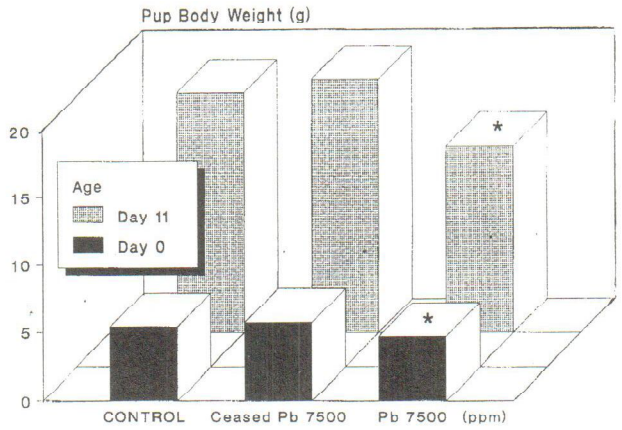
*Statistically different from control (P<0.01)

Figure 3 *Average number of live pups per litter after chronic (20-week) maternal exposure to lead (1500–7500 ppm in drinking water). Number of litters: N≥25.*

The effects of chronic lead exposure of the mother rat on the number of live 0- and 11-day-old suckling pups per litter are shown in Figure 3. Significant decrease in the number of live pups per litter appeared at 1,500 ppm dose in 11-day-old pups, while at higher lead doses (3,500–7,500 ppm), it was found in both 0- and 11-day-old sucklings.

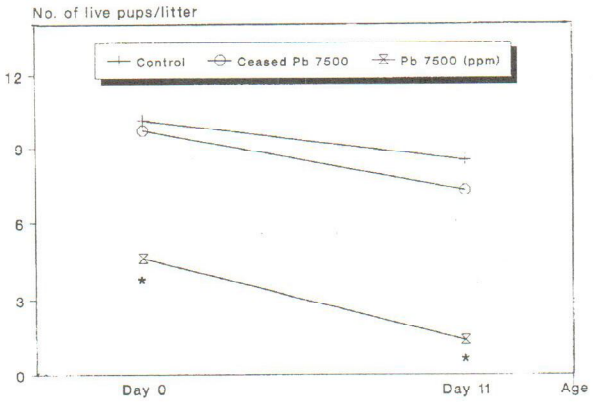
Figure 4 shows the effects on the body weight and Figure 5 shows the number of live pups per litter born to the mother rats exposed to the highest lead dose (7,500 ppm) for nine weeks after which followed an 11-week period without exposure to lead (marked as »ceased Pb 7500«). The data are compared with the results for the pups born to the mother rats continuously (20 weeks) exposed to the same level of lead (marked as »Pb 7500«). It was found that either the body weight (Figure 4) or the number of pups per litter (Figure 5) did not differ from control values if lead exposure to the highest lead dose (7,500 ppm) for nine weeks was followed by the 11-week period without lead.

Beside the dose, the exposure duration was found to be significant for the female reproductive effects of lead. It was found that in conditions of continuous oral exposure to lead, reductions in pup body weight, survival rate, and litter size were more pronounced after the longer exposure (up to 20 weeks) than after the shorter (9-week) exposure (5, 8). Fortunately, an important factor found in animal studies of



*Statistically different from control (P<0.01)

Figure 4 *Average body weights (g) after subchronic (9-week) lead exposure (in drinking water) followed by an 11-week period without lead (ceased Pb 7500), and after continuous chronic (20-week) maternal exposure to lead (Pb 7500 ppm). Number of litters: N≥21 (9).*



*Statistically different from control (P<0.01)

Figure 5 *Average number of live pups per litter after subchronic (9-week) lead exposure (in drinking water) followed by an 11-week period without lead (ceased Pb 7500), and after continuous chronic (20-week) maternal exposure to lead (Pb 7500 ppm). Number of litters: N≥25 (9).*

the reproductive lead effect was its reversibility. Reproductive lead effects in progeny born to the exposed mother rats were reversible after cessation of the maternal oral exposure (9).

The above experimental results support the evidence that the females and the young represent groups at particularly high risk to lead. Recent studies confirm more

clearly the sensitivity of perinatally exposed young rats to toxic metals (lead or cadmium) causing imbalance of the trace elements (zinc and iron), essential for their growth and development (6).

The evidence gained from these results is that sex, exposure level, duration of exposure, and the reversibility of maternally mediated effects after cessation of the exposure are relevant when assessing reproductive toxicity of lead. These aspects should be taken into consideration when setting lead exposure standards for potentially exposed women and children.

To sum up, the presented findings of epidemiologic and experimental studies on reproductive effect of lead in females are in agreement. The results from the epidemiologic study lead to a conclusion that higher exposure to lead (by about 20 $\mu g/m^3$ air) may cause an adverse effect on reproduction in women. Both types of studies indicate that the adverse reproductive action of lead is reversible after exposure has ceased.

2. MANGANESE EXPOSURE AND THE EFFECT ON RESPIRATORY SYSTEM

EPIDEMIOLOGIC STUDY

Another study dealt with exposure to manganese in an area with a ferromanganese plant. Manganese causes an irreversible disease similar to Parkinson's. Neurological effects of manganese have been registered only in association with occupational exposure by now. Manganese also affects the lungs. This effect of manganese has been described for excessive exposure. However, studies on population living in the vicinity of a plant emitting manganese have also indicated such an effect. Adverse effects on other organs are described as well, but they are of minor importance. On the basis of literature data, it seems appropriate to consider lungs as the main target organ of environmental exposure to manganese (10).

Subjects and methods

Measurements performed in the town (31,000 inhabitants), where the atmosphere was polluted with emissions from a manganese alloy plant, showed annual mean concentration of manganese range between 0.2 to 0.4 $\mu g/m^3$ of air within the plant perimeters of up to 3.5 km. The concentrations in the part of the town about 5 km SSE from the plant were almost ten times lower (between 0.04 and 0.08 $\mu g/m^3$). Owing to the sampling techniques (low volume samplers), these values related only to particles of approximately respirable size. Annual mean concentrations of sulphur dioxide in the ambient air ranged from 15 to 27 $\mu g/m^3$.

With respect to manganese concentrations, the town area was divided arbitrarily into three zones: • the zone nearest to the ferromanganese plant with the highest manganese concentration and 8,680 inhabitants (I); • the central part of the town with somewhat lower manganese concentrations and 17,105 inhabitants (II); and •

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Table 1 *Accumulated incidence of acute respiratory diseases during 1972–1975 (10)*

	Zones		Age groups 0–4	5–9	10–19	20–39	49–59	60 or more	Total
Acute bronchitis and peribronchitis	I	W[a]	139 (20.8)[b]	94 (13.5)	61 (4.0)	106 (3.4)	127 (7.0)	61 (6.9)	588
		S[a]	79 (11.8)	57 (8.2)	29 (1.9)	80 (2.6)	79 (4.3)	46 (5.2)	370
	II	W	271 (23.3)	237 (19.5)	155 (5.7)	309 (5.7)	322 (8.3)	183 (6.9)	1477
		S	130 (11.2)	104 (8.6)	90 (3.3)	213 (3.9)	213 (5.5)	130 (4.9)	880
	III	W	61 (14.8)	65 (15.2)	28 (3.0)	70 (3.7)	71 (6.4)	16 (3.2)	311
		S	28 (6.8)	45 (10.5)	24 (2.6)	32 (1.7)	42 (3.8)	20 (4.0)	191
Significance of difference		W	I–III P<0.05 II–III P<0.01	I–II P<0.01 II–III P<0.05	I–II P<0.05 II–III P<0.01	I–II P<0.01 II–III P<0.01	II–III P<0.05	I–III P<0.01 II–III P<0.01	
		S	I–III P<0.01 II–III P<0.01		I–II P<0.01	I–II P<0.01 I–III P<0.05 II–III P<0.01	I–II P<0.05 II–III P<0.05		
Pneumonia	I	W	10 (1.5)	12 (0.7)	11 (0.7)	17 (0.5)	6 (0.3)	8 (0.9)	64
		S	4 (0.6)	10 (0.5)	8 (0.5)	9 (0.3)	10 (0.5)	7 (0.8)	58
	II	W	22 (1.9)	22 (1.8)	20 (0.7)	34 (0.6)	23 (0.6)	12 (0.5)	133
		S	14 (1.2)	31 (2.6)	36 (1.3)	25 (0.5)	24 (0.6)	18 (0.7)	148
	III	W	4 (1.0)	14 (3.3)	1 (0.1)	10 (0.5)	2 (0.2)	3 (0.6)	34
		S	2 (0.5)	11 (2.6)	2 (0.2)	4 (0.2)	2 (0.2)	4 (0.8)	25
Significance of difference		W			I–III P<0.05 II–III P<0.05		II–III P<0.05		
		S		I–II P<0.01 I–III P<0.01	I–II P<0.01 II–III P<0.01	II–III P<0.05	II–III P<0.05		

[a] W = Winter; S = Summer.

[b] Numbers in parentheses denote percentage of the total number of inhabitants in particular zones and in particular age groups.

the zone 3.6 to 6 km from the plant with the lowest manganese concentrations and 5,300 inhabitants (III). On the basis of such subdivision of the town area, the incidence of acute bronchitis, peribronchitis, and pneumonia were followed for a four-year period (10).

Results and conclusions

The obtained results with respect to the zones of residence and season (summer – winter) showed the lowest incidence of acute bronchitis (peribronchitis) in the zone with the lowest manganese concentration (Table 1). With regard to age, the higher incidence of disease was registered, as expected, in the age groups 0–4 and 5–9 years. In these, as in almost all other age groups, the incidence tended to be higher in winter than in summer. The difference in the incidence of acute bronchitis (peribronchitis) in more polluted zones compared with the least polluted zone was particularly evident in the age groups of 0–4 and over 60. Pneumonia was also more frequent in children of up to 9 years of age. However, this rate did not statistically differ between the zones of residence in these and other age groups. The incidence of pneumonia did not significantly differ due to either pollution or season. As manganese concentrations were usually higher in summer than in winter, the question arose whether this fact could be associated with higher summer concentration of manganese. No significant difference was observed in incidence of the studied diseases by years of follow-up.

The results of this epidemiologic study indicated that, at the level of manganese not exceeding 1 $\mu g/m^3$ of air, effects on respiratory organ can be expected. As sulphur dioxide reacts readily with manganese dioxide to form manganese sulphate, the question is whether this respiratory effect can be exclusively attributed to manganese or perhaps to the joint action of manganese aerosol and sulphuric acid, i.e. sulphates adsorbed on the surface of manganese particles. Furthermore, some data indicate catalytic action of Mn^{2+} ions on the oxidation of SO_2 in aqueous solution. There was also a possibility that certain confounding factors (which were not under control) could influence the results. Assuming that the results were in connection with manganese exposure, the interpretation could be that manganese, even at such rather low level, disturbs some protective functions in the lungs, making the organism more susceptible to respiratory infection.

EXPERIMENTAL STUDIES

The concurrent toxicological studies supported the findings of the described epidemiologic study and corroborated the assumption as to the mechanisms of manganese action. *In vitro* studies on cells showed a cytotoxic effect of manganese, including the inhibitory effect of manganese on alveolar macrophages (11, 12). Experimental studies on animals revealed a decrease in the resistance toward respiratory infections caused by consecutive exposure to manganese dioxide and pathogenic bacteria with significantly higher rate of respiratory infections in comparison with animals separately exposed to either manganese or pathogenic bacteria (13, 14).

CONCLUDING REMARK

The presented studies on health effects of lead and manganese in humans and animals illustrate the interaction between toxicology and epidemiology in the process of risk assessment as a basis of risk management.

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Sažetak

ISTRAŽIVANJA IZLOŽENOSTI METALIMA: POVEZANOST TOKSIKOLOGIJE I EPIDEMIOLOGIJE U PROCJENJIVANJU ZDRAVSTVENIH RIZIKA

Slijedeći misao o ulozi uzajamnog djelovanja epidemioloških i toksikoloških istraživanja u procjenjivanju rizika, doza i učinaka, autori iznose svoja vlastita iskustva stečena u istraživanjima zdravstvenih učinaka izloženosti olovu odnosno manganu u okolišu. Na prvom primjeru pokazana je podudarnost rezultata epidemioloških istraživanja koji su pokazali štetne učinke olova na žensku reprodukciju u području s talionicom olova s eksperimentalno dobivenim podacima *in vivo*. U pokusima na štakoricama supkronično izloženim rastućim dozama olova u piću, nađeno je da je za nastanak reprodukcijskih učinaka značajna doza i dužina izloženosti, te da su promatrani učinci reverzibilni nakon prestanka izloženosti. Drugi primjer odnosi se na epidemiološka istraživanja u području onečišćenom manganom. Nađeno je da štetni učinci mangana na dišni sustav slijede odnos doze i reakcije, kao i da su ovisni o dobi ispitanika i o godišnjem dobu. I u ovom primjeru pokazano je kako su eksperimentalni podaci potvrdili epidemiološka opažanja. *In vitro* istraživanja na stanicama sisavaca pokazala su citotoksičnost mangana uključujući inhibitorni učinak na alveolarne makrofage. Autori zaključuju da u procesu procjenjivanja rizika toksikologija i epidemiologija moraju djelovati združeno. Dostupni podaci iz ispitivanja u ljudi moraju se povezivati s eksperimentalnim rezultatima i podacima o mehanizmima otrovnih djelovanja.

Ključne riječi:
citotoksični učinci, izloženost manganu, izloženost olovu, reprodukcijski učinci, respiracijski učinci

Requests for reprints:

Martina Piasek, M.D., Ph.D.
Institute for Medical Research
and Occupational Health
2 Ksaverska cesta, P.O. Box 291,
10001 Zagreb, Croatia
E-mail: *mpiasek@mimi.imi.hr*