ACTA DE REUNION DE COMITÉ OPERATIVO Y COMITÉ AMPLIADO

FECHA REUNION : 21 de junio de 1999
LUGAR : CONAMA -Santiago
HORARIO :.9:30 – 13:30 hrs.

ASISTENCIA :

Bartolomé Alfaro	CODELCO
Anibal Mege	SOFOFA
Fernando Cacho	Intendencia R.M.
Richard Vargas	Servicio de Salud de Concepción
Francisco Bernasconi	PETROX S.A
Ramón Gutierrez	SEC
Jaime Retamal	MTT (Subsec. Tranporte)
Alejandro Barra H	CONAMA II Región
Manuel Cortés	S. Salud Antofagasta
Walter Folch	MINSAL
M. de la Luz Vásquez	Min. Minería
Rodrigo Cerda Candia	O.P.S
Andrea Varas	CNE
Andrei N. Tchernitchin	Colegio Médico de Chile
Andrea Muñoz	CONAMA
Rodrigo Lucero	CONAMA
Maritza Jadrijevic	CONAMA
Andrea Urrutia	Memorista Universidad de Chile

Tabla :

1. Información general del proceso de elaboración de la Norma, Andrea Muñoz, Depto. Descontaminación, Planes y Normas

2. Presentación sobre efectos del plomo en la salud, Dr. Andrei Tchernitchin, Colegio Médico.

3. Presentación de avances de los grupos de trabajo:

    I - " Plomo en Población", Walter Folch, MINSAL
    II- " Plomo en Aire y Metodología de Medición", Andrea Urrutia y Maritza Jadrijevic
    III.- " Normas Internacionales de plomo en aire, Andrea Muñoz

Comentarios.

1.- Andrea Muñoz inicia la reunión dando a conocer sus objetivos, presenta los avances del proceso, e informa sobre las reuniones realizadas de los distintos grupos de trabajo. Detalla los objetivos del grupo de trabajo de fiscalización y recuerda el cronograma de trabajo de cada grupo y del proceso en general.

2.- El Dr. Tchernitchin presenta los contaminantes atmosféricos que son cancerígenos, explica lo que es el imprinting, y presenta en detalle los efectos del plomo en la salud humana.

3.- El Sr. Folch se refiere a 4 estudios nacionales que muestran valores de plomo en aire y en sangre. El Sr. M. Cortéz, del Servicio de Salud de Antofagasta acota que los estudios realizados indican que los acopios producen problemas de plomo en el material particulado sedimentable.

4.- La Srta. Andrea Urrutia expone los métodos de medición, tanto de recolección de material particulado como los métodos de análisis para medir concentraciones de plomo en el material particulado. Se pregunta respecto a la sensibilidad de los métodos.

La Sra Maritza Jadrijevic muestra los resultados de mediciones de plomo obtenidos en el Proyecto " Estudio de la calidad del aire en regiones urbano-industriales de Chile" (COSUDE). Se destaca que ninguna de las 5 ciudades presentan valores altos de plomo, en relación a la recomendación de la OMS. Se indica que los resultados de las mediciones realizadas por la Consultora Gredis serán entregadas una vez que se tenga el informe final.

5.- La Sra. Muñoz se refiere a las normas extranjeras y a la recomendación de la OMS. Se pregunta a que se deben las diferencias de normas entre los diferentes países y si estos valores de plomo permitidos son en PTS o PM10.

Andrea Muñoz
Depto. Descontaminación, Planes y Normas
CONAMA

Antiestrogenic Activity of Lead

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ABSTRACT: Lead is a widely spread environmental pollutant known to affect both male and female reproductive systems in humans and experimental animals. The present study investigated the effect of an acute exposure to lead (75 mg lead per gram of body weight) 1 or 24 h before hormone treatment on different parameters of estrogen stimulation in the rat uterus. Lead pretreatment enhanced some parameters of estrogen stimulation and inhibited other estrogenic responses, and the remaining parameters were unaltered. The interaction with responses to estrogen was different depending on whether lead pretreatment was 1 or 24 h before hormone stimulation. The estrogenic responses mostly affected were uterine eosinophilia, endometrial edema, uterine luminal epithelial hypertrophy, and mitosis in various—but not all—uterine cell types. In some cell types, estrogen-induced mitotic response developed earlier under the effect of lead exposure. Results revealed an interaction with the different mechanisms of estrogen action in the uterus at various levels, that some cell types are more sensitive to lead than others, and that the effect of exposure changed with time after lead pretreatment. The relevance of the results are discussed in relation to lead-induced infertility, mutagenicity, and carcinogenicity; possible mechanisms of action are proposed. © 1998 by John Wiley & Sons, Inc. Environ Toxicol Water Qual **13**: 43–53, 1998

Keywords: lead; pollutant; antiestrogen; estrogen; infertility; mutagenicity; carcinogenicity

INTRODUCTION

Lead is a widely spread environmental pollutant known to affect both male and female reproductive systems and other organs in humans and experimental animals. In humans, these effects are induced by low lead environmental concentrations, but the time when the individual was exposed (prenatally, during the first years of development, prepubertal age, or adulthood) has implications in the characteristics of the sequellae (International Programme on Chemical Safety, 1977; Rothemberg et al., 1989; Needleman et al., 1990, 1996; Royce,

1990; Andrews et al., 1994). Excessive lead exposure still persists in the community, not only in the workplace, but also as exposure to lead-based paint, to dust from combustion of leaded gasoline, soil, and lead-contaminated glazed pottery or water. In women, reported effects due to lead include infertility, miscarriage, pre-eclampsia, pregnancy hypertension, and premature delivery (Rom, 1976; Laudanski et al., 1991; Winder, 1993). In experimental animals, chronic exposure to lead may cause an inhibition of menstruation, ovulation, and follicular growth in monkeys (Vermande-Van Eck and Meigs, 1960), a delay in vaginal opening in pubertal rats (Kimmel et al., 1980), and a decrease in frequency of implanted ova and of pregnancies in mice (Odenbro and Kihlström, 1977). Exper-

imental studies suggest that lead affects female reproductive organs through different mechanisms. The heavy metal may interact at the enzyme level (Wiebe et al., 1988; Kempinas et al., 1994). It may interfere with the action of reproductive hormones at the target organ by modifying the activity of estrogen receptors in the pregnant uterus (Wide and Wide, 1980) and inhibiting the implantation process that is regulated by estrogens (Wide 1980). Lead may induce imprinting mechanisms (Csaba et al., 1986; Tchernitchin and Tchernitchin, 1992), causing persistent changes in uterine estrogen receptors (Wiebe and Barr, 1988) and ovary luteinizing hormone (LH) receptors (Wiebe et al., 1988) following perinatal exposure. Finally, lead may interfere at the level of the hypothalamus–pituitary, decreasing pituitary response to growth-hormone-releasing factor (Camoratto et al., 1993), affecting levels of gonadotropin-releasing hormone, somatostatin (Sierra and Tiffany-Castiglioni, 1992), follicle-stimulating hormone (FSH), and LH (McGivern et al., 1991), and increasing blood levels of glucocorticoids (Vyskocil et al., 1991). Additionally, exposure to lead is known to increase the level of stress, which, in turn, is characterized by increased levels of glucocorticoids, catecholamines, growth hormone, and prolactin. Taking into consideration that increased levels of glucocorticoid hormones selectively block some parameters of estrogen stimulation in the uterus (Tchernitchin et al., 1975, 1985b), that high prolactin levels interfere with some responses to estrogen but not others (Unda et al., 1989), and that lead interacts with estrogen receptors in the organ (Wide and Wide, 1980), it becomes clear that the effects of lead exposure on uterine responses to estrogens should be investigated to understand the mechanisms involved in lead-induced fertility impairment.

To investigate the effects of exposure to lead on responses to estrogen in the uterus, it is necessary to consider that estrogens induce separate groups of responses through independent mechanisms of hormone action in which different kinds of estrogen receptors are involved. Accordingly, increased uterine RNA and protein synthesis are genomic responses to hormone stimulation induced through hormone interaction with cytosol–nuclear receptors in the various uterine cell types (Jensen and DeSombre, 1972). Estrogen-induced uterine edema, increased vascular permeability, and release of histamine are nongenomic responses (Tchernitchin and Galand, 1982; Tchernitchin et al., 1985b) induced through hormone interaction with eosinophil leukocyte estrogen receptors (Tchernitchin et al., 1985b, 1989), which mediate the migration of these cells from the blood to the uterus (Tchernitchin et al., 1974, 1985b), their degranulation (Tchernitchin et al., 1985a, 1989), and the release of enzymes and agents involved in the development of the eosinophil-

mediated responses (Tchernitchin et al., 1985b, 1989). Additional mechanisms of estrogen action, involving lower affinity type II cytosolic and nuclear estrogen receptors (Clark and Peck, 1979), uterine membrane estrogen receptors (Pietras and Szego, 1977; Müller et al., 1979; Nenci et al., 1981), cyclic AMP (Hechter et al., 1965; Kvinnsland, 1976), and prostaglandins (Resnik et al., 1975; Penney et al., 1981; Soto-Feine et al., 1981) have been proposed as well. The existence of multiple and independent mechanisms of estrogen action for the different responses to hormone stimulation results in a dissociation of these responses under a number of conditions (Tchernitchin et al., 1975, 1985b; Tchernitchin and Galand, 1982, 1983; Galand et al., 1985). In addition, estrogenic compounds may selectively interact with some receptor systems, perhaps in some cell types but not in others, affecting some parameters of hormone stimulation only (Tchernitchin et al., 1985b; Grunert et al., 1986, 1987; Unda et al., 1989). Therefore, the study of any agent displaying estrogen action must consider the different mechanisms and the wide spectrum of responses to hormone stimulation in the target organ under study. The present study investigated the effect of acute exposure to lead on various responses to estrogen, separately in the different uterine cell types, taking into consideration the two best known mechanisms of estrogen action.

MATERIALS AND METHODS

Female rats from a Sprague–Dawley-derived colony bred at the vivarium of the Faculty of Medicine, University of Chile, were used in the present study. Eight groups of immature animals were subjected to the following procedure: at the age of 20 days (four experimental groups) or 21 days (four experimental groups) they were pretreated with lead acetate (Merck, Darmstadt, Germany) at a dosage of 75 μg Pb /g body wt, i.v. under ether anesthesia, or saline physiological solution. This dose of lead is commonly used for the study of acute effects of lead in rats and other experimental animals, since it causes slight to moderate toxicity effects (see International Programme on Chemical Safety, 1977, for a review). At the age of 21 days, 24 h after the pretreatment of rats at the age of 20 days, or 1 h after the pretreatment of rats at the age of 21 days, all animals were treated with either estradiol-17ß (Sigma Chemical Co., St. Louis, MO) at a dosage of 300 ng/g body wt, i.v. under ether anesthesia, or with estradiol's vehicle (absolute ethanol in saline physiological solution 1:9). This age is the most appropriate for the study of the effects of sex steroids on target organs, since estrogen and progesterone levels are extremely low and receptor levels and hormone responsiveness are already fully developed (Tchernitchin et al., 1985b).

The dose of estradiol-17ß was chosen because it enabled maximal responses of the parameters studied and also allowed comparison with previously published results (Tchernitchin et al., 1974, 1975, 1985b, Tchernitchin and Galand, 1982; Soto-Feine et al., 1981; Unda et al., 1989). At the time of treatment, the body weight of the animals was between 40 and 49 g. Six or twenty-four hours after treatment, the uteri were excised under ether anesthesia, fixed in 4% neutral formalin, and subjected to further histological procedure for eosinophil quantification and morphometry (Tchernitchin and Galand, 1983).

The following parameters of estrogen stimulation were investigated in the uterus: myometrial hypertrophy was measured as increase in the reciprocal value of cell density (RVCD) in circular myometrium, edema in deep and superficial endometrial stroma was evaluated as increases in RVCD in these histological locations (Grunert et al., 1984, 1986), and uterine eosinophilia

was measured as total number of eosinophils located in both uterine horns (Tchernitchin et al., 1974). Luminal epithelial and glandular epithelial cell hypertrophy were evaluated morphometrically as estrogen-induced increase in cell volume (Tchernitchin et al., 1995), and estrogen-induced mitotic response was evaluated as increase in the number of mitotic figures in every cell type investigated (Grunert et al., 1986, 1987).

Statistics

Since multiple comparisons were performed between the four experimental conditions within the same age of pretreatment, data were subjected to the least significant difference (LSD) test. The common variance used in this test was obtained from the one way analysis of variance (ANOVA), and no significant differences were declared unless the ANOVA was significant (Snedecor and Cochran, 1967).
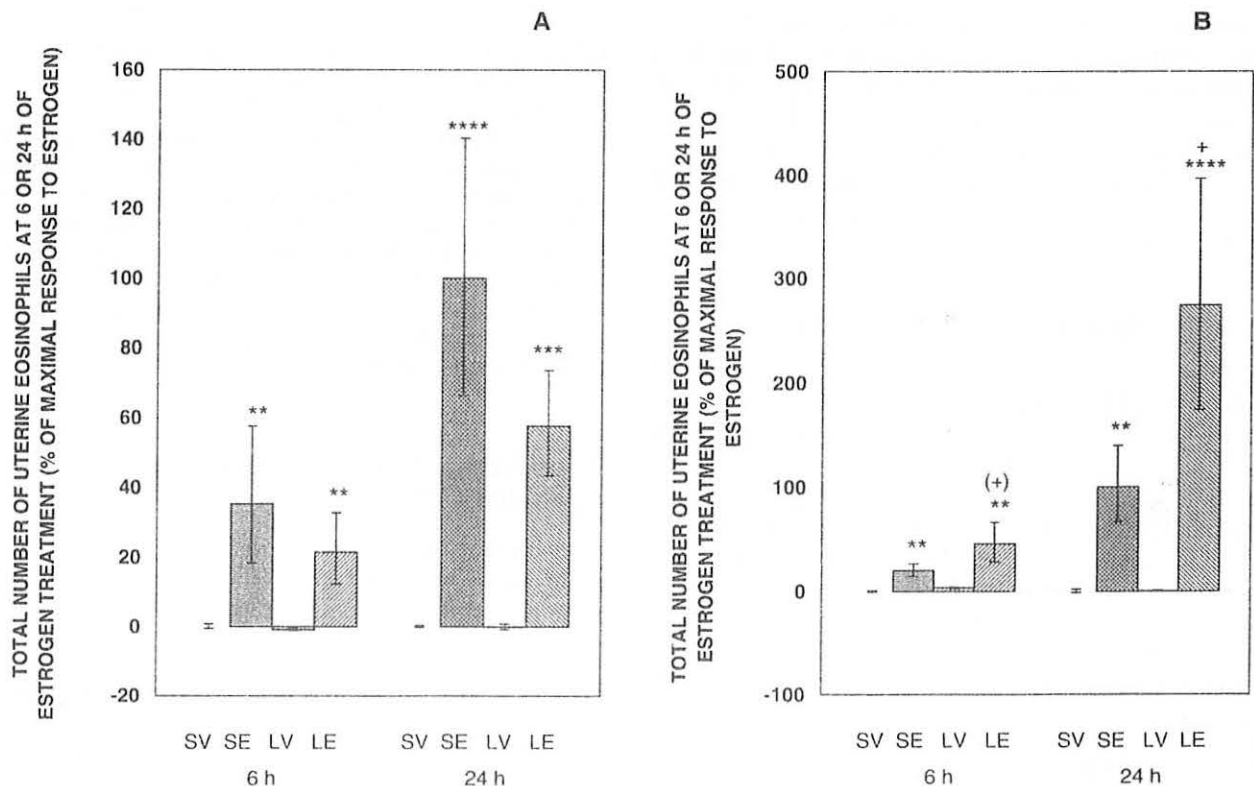

Fig. 1. Effect of lead on estrogen-induced uterine eosinophilia. Rats were pretreated with lead (L) or saline physiological solution (S) and 1 h (A) or 24 h (B) later were treated with estradiol-17ß (E) or vehicle (V). The uteri were obtained 6 or 24 h after hormone or vehicle administration. Bars indicate means (expressed as percent of maximal response to estrogen) plus or minus standard error of the mean. Statistics: LSD test. +, $P < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$; (+), $0.05 < P < 0.1$; *, comparisons to the homologous condition without estrogen; +, comparisons to the homologous condition without lead.

RESULTS

Uterine Eosinophilia

Lead exposure 1 h before estrogen treatment does not significantly modify estrogen-induced uterine eosinophilia (Fig. 1A). Lead exposure for 24 h before estrogen treatment (Fig. 1B) significantly increases uterine eosinophilia induced by a 24 h estrogen treatment, and displays a tendency for an increase at 6 h of estrogen treatment.

Edema in Superficial Endometrium

Lead exposure 1 h before estrogen treatment potentiates estrogen-induced edema in superficial endometrial stroma 6, but not 24 h, after hormone treatment (Fig. 2). This effect is not observed in animals pretreated with lead 24 h before estrogen or vehicle treatment (not shown in figure).

Edema in Deep Endometrium

Lead pretreatment 1 h before hormone treatment increases estrogen-induced uterine edema at 24 h of steroid administration (Fig. 3). At 6 h after estrogen treatment, a tendency for an increase in estrogen-induced deep endometrial edema is also observed. Lead exposure 24 h before estrogen treatment does not cause any change in estrogen-induced deep endometrial edema (not shown in figure).

Hypertrophy in Circular Myometrium

It can be observed that the reciprocal value of cell density (RVCD) in this location decreases in animals treated with the vehicle, in the group pretreated with lead 1 h before hormone or vehicle treatment (Fig. 4); estradiol causes a significant increase from this value, although RVCD values are significantly smaller than RVCD increase (hypertrophy) induced by estrogen. No interference with estrogen-induced myometrial hypertrophy is detected in animals exposed to lead 24 h before treatment (not shown in figure).

Hypertrophy of Uterine Luminal Epithelial Cells

No significant differences are detected by ANOVA in the group pretreated with lead 1 h before hormone treatment (results not shown). In the group pretreated with lead 24 h before treatment with the steroid, lead causes an increase in estrogen-induced hypertrophy of luminal epithelial cells (Fig. 5).
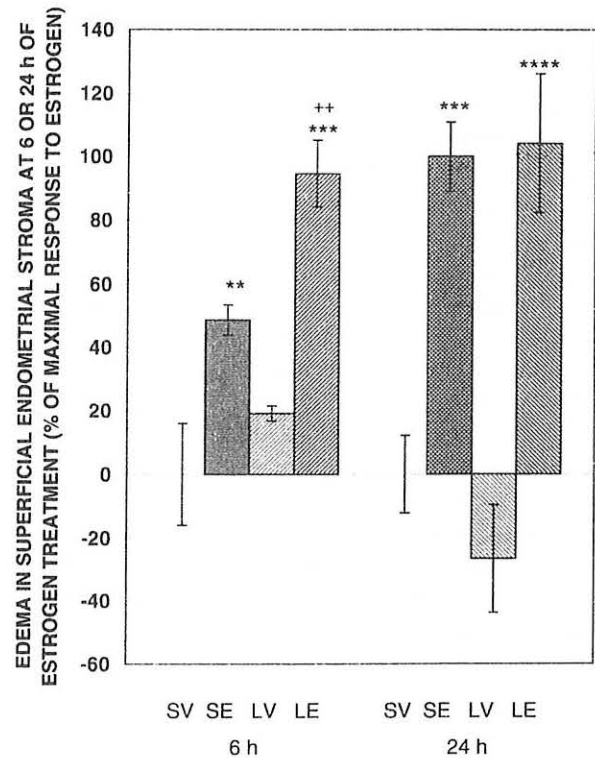

Fig. 2. Effect of lead on estrogen-induced edema in superficial endometrial stroma, measured as increases in the reciprocal value of cell density. Rats were pretreated with lead (L) or saline physiological solution (S) and 1 h later were treated with estradiol-17β (E) or vehicle (V). The uteri were obtained 6 or 24 h after hormone or vehicle administration. Bars indicate means (expressed as percent of maximal response to estrogen) plus or minus standard error of the mean. Statistics: LSD test. ∗∗ or ++, $p < 0.01$; ∗∗∗, $p < 0.001$; ∗∗∗∗, $p < 0.0001$; ∗, comparisons to the homologous condition without estrogen; +, comparisons to the homologous condition without lead.

Hypertrophy of Uterine Glandular Epithelial Cells

Lead pretreatment (1 or 24 h before estrogen treatment) does not significantly modify estrogen-induced glandular epithelial hypertrophy (not shown in figure).

Mitoses in the Different Uterine Cell Types

Figures 6 to 11 show the number of mitotic figures in the different uterine cell types under the effect of lead pretreatment and/or estrogen treatment. In animals that were not exposed to lead, estrogen does not increase the number of mitoses in any uterine cell type at 6 h after treatment. A pretreatment with lead 1 h before estrogen causes a significant increase in mitoses under the effect of hormone treatment at 6 h after
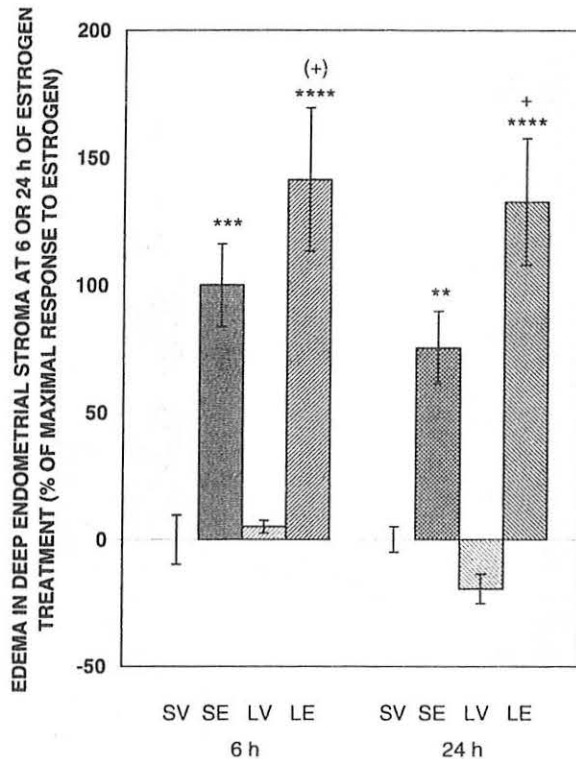
Fig. 3. Effect of lead on estrogen-induced edema in deep endometrial stroma, measured as increases in the reciprocal value of cell density. Rats were pretreated with lead (L) or saline physiological solution (S) and 1 h later were treated with estradiol-17β (E) or vehicle (V). The uteri were obtained 6 or 24 h after hormone or vehicle administration. Bars indicate means (expressed as percent of maximal response to estrogen) plus or minus standard error of the mean. Statistics: LSD test. +, $P < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$; (+), $0.05 < P < 0.1$; *, comparisons to the homologous condition without estrogen; +, comparisons to the homologous condition without lead.

Fig. 4. Effect of lead on estrogen-induced hypertrophy in circular myometrium, measured as increases in the reciprocal value of cell density. Rats were pretreated with lead (L) or saline physiological solution (S) and 1 h later were treated with estradiol-17β (E) or vehicle (V). The uteri were obtained 24 h after hormone or vehicle administration. Bars indicate means (expressed as percent of maximal response to estrogen) plus or minus standard error of the mean. Statistics: LSD test. *, $P < 0.05$; ++, $p < 0.01$; *, comparisons to the homologous condition without estrogen; +, comparisons to the homologous condition without lead.

steroid administration in superficial and deep stromal cells, circular and longitudinal myometrium, and mesometrium. A pretreatment with lead 24 h before estrogen causes a tendency for this effect at 6 h of estrogen treatment in deep endometrial stroma ($0.05 < p < 0.10$; not shown in figure). A pretreatment with lead 1 h before estrogen causes a decrease in mitoses induced 24 h after estrogen treatment in uterine luminal epithelial cells and circular myometrium, and a tendency for this effect is observed in deep endometrial stroma and in mesometrium. In superficial and deep stroma, as well as in circular myometrium, lead causes a decrease in the number of mitoses observed in animals without estrogen treatment. In the animals pretreated with lead 24 h before hormone treatment, no significant effect of lead is observed on estrogen-induced increase in the number of mitotic figures in any uterine cell type (not shown in figure).
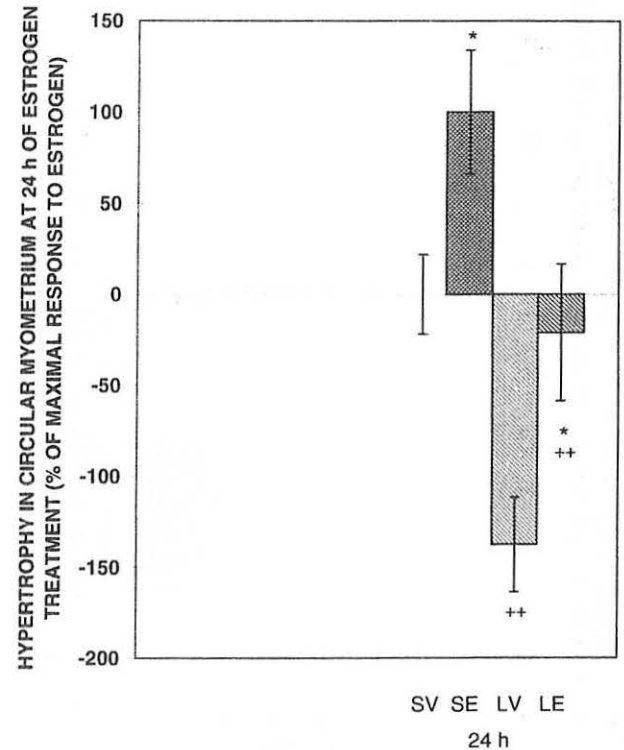
DISCUSSION

The present study provides the first evidence that an acute exposure to lead potentiates some estrogenic responses and inhibits others, while the remaining responses are unaffected. The finding that pretreatment with lead 1 h before hormone treatment enhances estrogen-induced edema in deep and superficial endometrium but inhibits mitosis in luminal epithelium suggests a different kind of interaction with the separate mechanisms of estrogen action reported to exist in the uterus (Tchernitchin et al., 1985b, 1989). The potentiation of endometrial edema may be explained by an increase in the degranulation in the eosinophils that
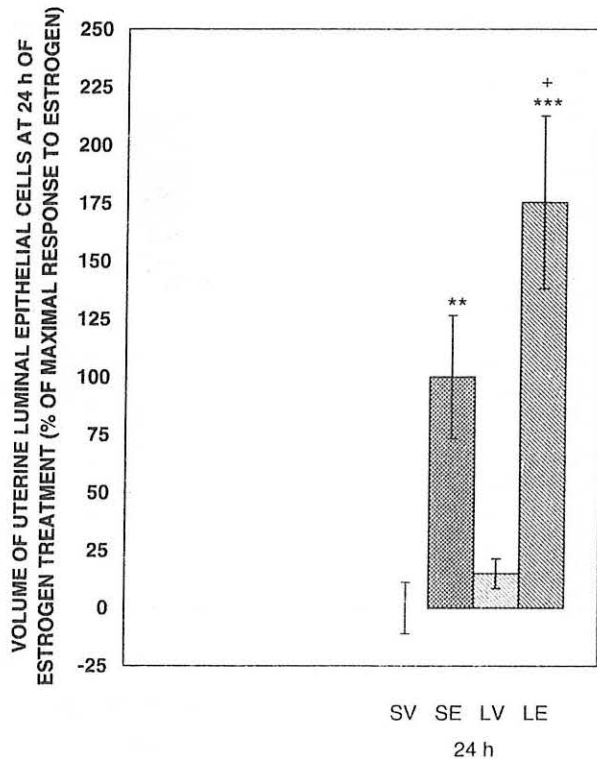
Fig. 5. Effect of lead on estrogen-induced hypertrophy of uterine luminal epithelial cells, measured as increases in cell volume. Rats were pretreated with lead (L) or saline physiological solution (S) and 24 h later were treated with estradiol-17ß (E) or vehicle (V). The uteri were obtained 24 h after hormone or vehicle administration. Bars indicate means (expressed as percent of maximal response to estrogen) plus or minus standard error of the mean. Statistics: LSD test. +, $P < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; *, comparisons to the homologous condition without estrogen; +, comparisons to the homologous condition without lead.
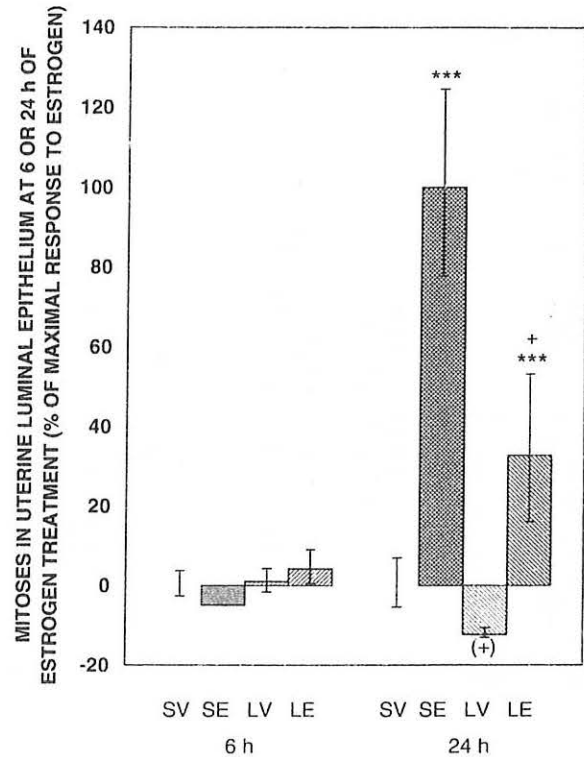

Fig. 6. Effect of lead on estrogen-induced mitoses in uterine luminal epithelium. Rats were pretreated with lead (L) or saline physiological solution (S) and 1 h later were treated with estradiol-17ß (E) or vehicle (V). The uteri were obtained 6 or 24 h after hormone or vehicle administration. Bars indicate means (expressed as percent of maximal response to estrogen) plus or minus standard error of the mean. Statistics: LSD test. +, $P < 0.05$; ***, $p < 0.001$; (+), $0.05 < P < 0.1$; *, comparisons to the homologous condition without estrogen; +, comparisons to the homologous condition without lead.

has been found to occur in the blood following chronic exposure to lead (Villagra et al., 1997), which suggests that eosinophils also increase their degranulation in the uterus, with an increased enzyme release from these cells and a potentiation of eosinophil-mediated responses (Tchernitchin et al., 1985a). The dramatic decrease in estrogen-induced mitoses in luminal epithelium only, but not in the other cell types, may be explained by the increased concentration of lead by uterine epithelium (Nilsson et al., 1991) as a first step before its secretion to uterine lumen (Jin and Nilsson, 1993), a process that may affect the blastocysts. Alternatively, the varying susceptibility of the different uterine cell types to mitotic response inhibition by lead may be explained by differences in their estrogen receptors (Tchernitchin et al., 1985b).

The finding that, in most cell types of animals exposed to lead 1 h before hormone treatment, estrogen induces an important increase in the number of mitotic figures 6 h after treatment (which does not occur in the absence of lead) and that the number of mitoses tend to decrease in some of these cell types 24 h after estrogen treatment clearly indicates that the time before estrogen induces the mitotic response is shorter in lead-exposed animals. This mitogenic activity of lead that was previously reported in the liver (Ledda-Columbano et al., 1994; Calabrese et al. 1995) may be explained by calcium substitution in the second messenger metabolism by lead and lead activation of molecules such as calmodulin-dependent phosphodiesterase, calmodulin inhibitor-sensitive potassium channels, calmodulin-independent protein kinase C (Goldstein, 1993; Long et al., 1994; Watts et al., 1995);
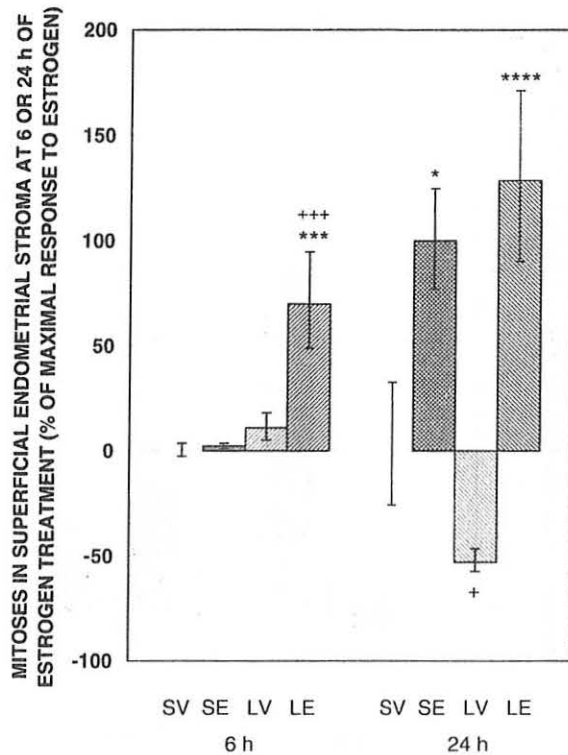
Fig. 7. Effect of lead on estrogen-induced mitoses in superficial endometrial stroma. Rats were pretreated with lead (L) or saline physiological solution (S) and 1 h later were treated with estradiol-17β (E) or vehicle (V). The uteri were obtained 6 or 24 h after hormone or vehicle administration. Bars indicate means (expressed as percent of maximal response to estrogen) plus or minus standard error of the mean. Statistics: LSD test. * or +, P < 0.05; *** or +++, p < 0.001; ****, p < 0.0001; *, comparisons to the homologous condition without estrogen; +, comparisons to the homologous condition without lead.

Fig. 8. Effect of lead on estrogen-induced mitoses in deep endometrial stroma. Rats were pretreated with lead (L) or saline physiological solution (S) and 1 h later were treated with estradiol-17β (E) or vehicle (V). The uteri were obtained 6 or 24 h after hormone or vehicle administration. Bars indicate means (expressed as percent of maximal response to estrogen) plus or minus standard error of the mean. Statistics: LSD test. ** or ++, p < 0.01; *** or +++, p < 0.001; ****, p < 0.0001; (+), 0.05 < P < 0.1; *, comparisons to the homologous condition without estrogen; +, comparisons to the homologous condition without lead.

these kind of proteins may play a role in cell-cycle regulation by hormones. The lead-induced acceleration of the mitotic process may involve a shortening of the G_2 period, where DNA-repair mechanisms take place; this may increase the possibility that unrepaired replication errors may result in mutations (Pincheira et al., 1995), which explains lead mutagenicity and lead carcinogenicity (see Needelman and Landrigan, 1981, for a review).

The present finding that changes induced by lead administration 1 h before estrogen treatment are different and sometimes opposite (uterine eosinophilia) from those caused by lead administration 24 h before hormone treatment are in agreement with the report by Kempinas et al. (1994) on time-dependent differences between the different effects of lead on reproductive changes. The slight tendency for a decrease in
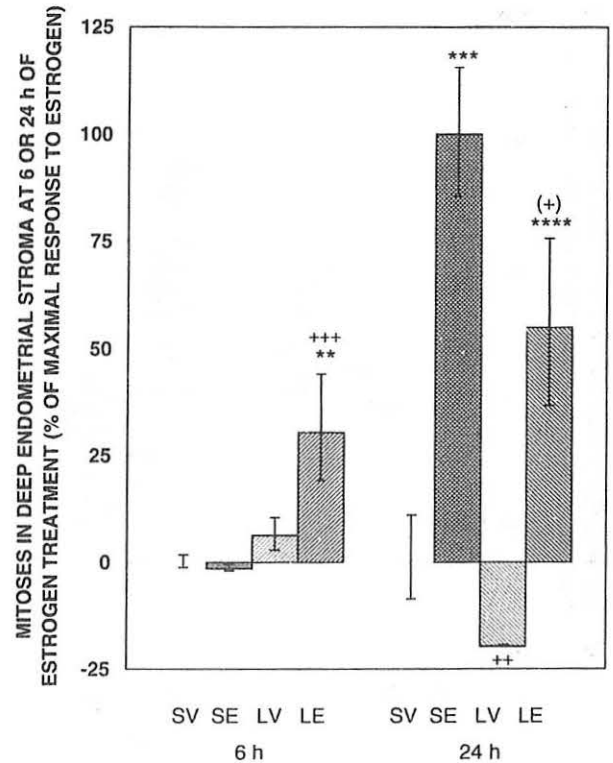
eosinophil numbers (statistically nonsignificant) during the first 24 h of lead exposure, followed by a great potentiation of uterine eosinophilia after that time, may be explained by a stress reaction known to occur after lead exposure (Nation et al., 1987), which causes increased levels of hormones such as glucocorticoids that may affect negatively eosinophil levels in the blood and the uterus during the first hours and may cause blood and uterine eosinophilia in the following days. The disappearance of effects of lead in most responses to estrogen in the group exposed to lead 24 h before estrogen treatment may be explained in part by the declining lead levels with time. The reported synthesis of various kinds of stress proteins under the effect of lead (Shelton et al., 1986), which may protect some but not all cell types, may alternatively explain the lack of
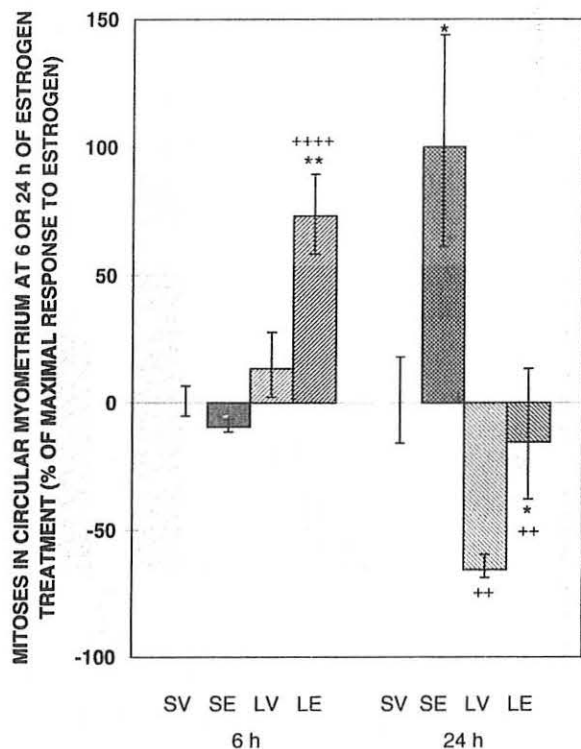
Fig. 9. Effect of lead on estrogen-induced mitoses in circular myometrium. Rats were pretreated with lead (L) or saline physiological solution (S) and 1 h later were treated with estradiol-17ß (E) or vehicle (V). The uteri were obtained 6 or 24 h after hormone or vehicle administration. Bars indicate means (expressed as percent of maximal response to estrogen) plus or minus standard error of the mean. Statistics: LSD test. *, $P < 0.05$; ** or ++, $p < 0.01$; ++++, $p < 0.0001$; *, comparisons to the homologous condition without estrogen; +, comparisons to the homologous condition without lead.

Fig. 10. Effect of lead on estrogen-induced mitoses in longitudinal myometrium. Rats were pretreated with lead (L) or saline physiological solution (S) and 1 h later were treated with estradiol-17ß (E) or vehicle (V). The uteri were obtained 6 or 24 h after hormone or vehicle administration. Bars indicate means (expressed as percent of maximal response to estrogen) plus or minus standard error of the mean. Statistics: LSD test. *, $P < 0.05$; ++, $p < 0.01$; *, comparisons to the homologous condition without estrogen; +, comparisons to the homologous condition without lead.

lead effects on most cell types after 24 h of exposure. Estrogen-induced luminal hypertrophy remains, however, potentiated in animals exposed to lead 24 h before estrogen treatment. This effect of lead exposure may be explained by a difference in sensitivity to exposure between the various cell types and the increased concentration of lead in some cell types only (uterine epithelium; Nilsson et al., 1991). Studies are in progress to investigate the effects of subchronic and chronic exposure to lead, and to evaluate the time-dependent changes in lead toxicity on the various parameters of estrogen action in the uterus, where delayed effects, such as changes in hormone receptor synthesis or replenishment or changes in the differentiation of target cells, may be detected, in addition to the acute effects of lead that may occur on steroid hormone receptors, calcium levels, or other regulatory mediators.
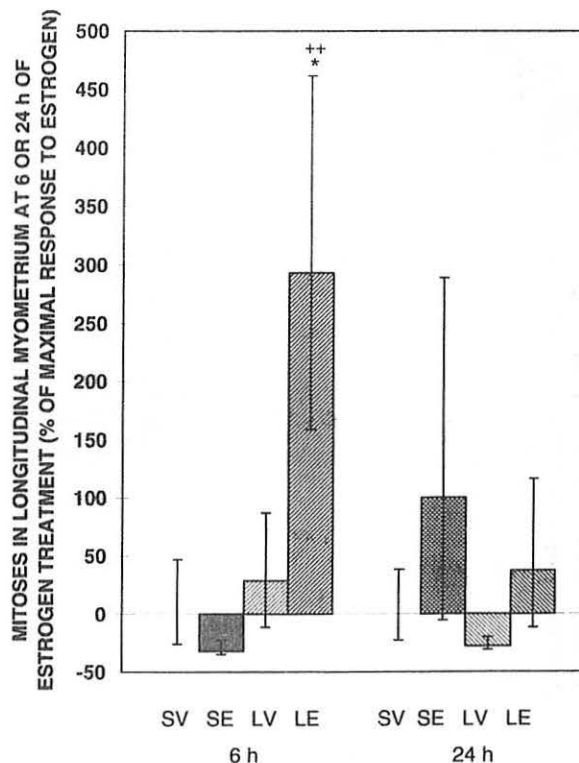
Finally, the reported changes in the action of estrogens in the uterus may explain in part the decrease in fertility in females under the effect of exposure to lead. Current studies are intended to evaluate the effects of chronic exposure, to compare these effects to those of acute exposure, to investigate whether similar effects can be detected in curettage biopsies from lead exposed women, and to establish a correlation between blood lead levels and lead exposure adverse effects. A further understanding of the mechanisms of interaction of lead and reproductive hormones at target tissues and the determination the blood lead level limit at which lead-induced adverse effects in reproductive physiology are no longer detected is specially important, taking into consideration that lead pollution affects an important part of human population and that perhaps most urban inhabitants present blood lead
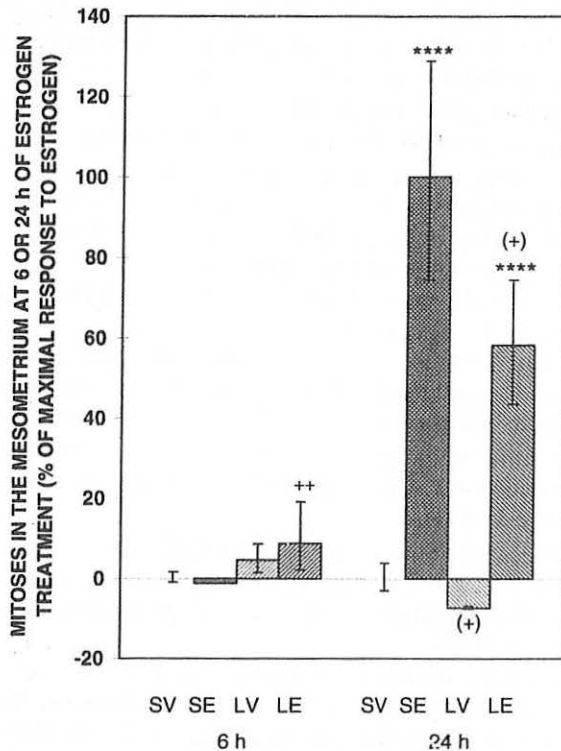
Fig. 11. Effect of lead on estrogen-induced mitoses in uterine mesometrium. Rats were pretreated with lead (L) or saline physiological solution (S) and 1 h later were treated with estradiol-17ß (E) or vehicle (V). The uteri were obtained 6 or 24 h after hormone or vehicle administration. Bars indicate means (expressed as percent of maximal response to estrogen) plus or minus standard error of the mean. Statistics: LSD test. ++, $p < 0.01$; ****, $p < 0.0001$; (+), $0.05 < P < 0.1$; *, comparisons to the homologous condition without estrogen; +, comparisons to the homologous condition without lead.

levels beyond this limit level and display the adverse effects of lead exposure.

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Forum

Imprinting of paths of heterodifferentiation by prenatal or neonatal exposure to hormones, pharmaceuticals, pollutants and other agents and conditions

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Introduction

Since the first reports linking the development of clear cell cervicovaginal adenocarcinomas in young women with diethylstilbestrol treatment of their mothers during pregnancy [1–3], later confirmed in studies with experimental animals [4, 5], it has been clear that prenatal exposure to this synthetic estrogen induces permanent changes in some cell types. These alterations become evident as late as the onset of puberty as an enhanced risk for malignant cell transformation, probably under the effect of increased post-pubertal estrogen levels in the blood.

Based on these findings, György Csaba and his co-workers started an experimental study that allowed the demonstration that exposure of fetuses to certain hormonally active agents during critical periods of their development induces permanent changes in the action of related hormones [6, 7]. These alterations can be detected later in adulthood as a modification in the activity of receptors and in the intensity of responses mediated by them [8, 9].

Csaba [7, 9] has given the name "imprinting" to this effect of hormones during fetal or neonatal life in permanently modifying the ability of cells to react to hormone stimulation during adulthood.

Causes of "imprinting"

Recent findings demonstrate, however, that imprinting may also be induced by various pharmaceuticals, polluting agents, food components ingested by the mother, maternal stress, and several other agents or conditions that interact with the different cell-types at precise stages of fetal or neonatal development. Furthermore, this process involves not only changes in the quantity and quality of hormone receptors in affected cells once they reach maturity, but also several biochemical, morphological and functional changes. Therefore, taking into consideration that the process discovered by Csaba really involves a modification of the routes of normal differentiation of these cells, we propose to rename it as 'imprinting of paths of heterodifferentiation'. The changes in cell differentiation induced by this mechanism may lead, later in adulthood, to the development of diseases such as neoplasias and endocrine abnormalities, infertility, immune diseases, psychological alterations, and/or changes in personality and behaviour. The enormous importance of this process in the determination of health conditions later in life rests in the fact that it is generated not only by agents that are easy to avoid. It is also induced by early exposure to a myriad of agents and conditions that are difficult to detect. These include stress, very low concentrations of pollutants and natural substances contained in food.

Long-term effects on organs and systems

Many of the xenobiotics that will be described below induce, at relatively high doses, permanent changes in 100% of prenatally exposed experimental animals. At much lower concentrations, the agents may affect a small percentage of exposed human population; therefore the aetiology of the diseases induced by them may remain undetected for a long time. These xenobiotics can be absorbed from drinking water (as with arsenic, for example) the air (as with lead, carbon monoxide and nicotine) or food (e.g. cadmium lead, mycotoxins, pesticides and synthetic hormones used as animal growth promoters). Such substances could possibly affect an important part of the exposed population-determining, as in the case of lead from gasoline, behavioural alterations leading to sociological changes in future years.

Research in this new field should be concerned not only with gross effects of exposure to such agents on the morphology and functions of different organs and systems. It should also focus on all of their components. This is especially important if we take into consideration the newly developed concept that several hormones interact with target organs through multiple and independent mechanisms, with different kinds of receptors for the same hormone (see [10–16] for a review).

This concept arose from the finding in our laboratory of different types of estrogen receptors [14, 17, 18], involved in separate groups of responses through independent mechanisms [10, 11, 14, 15, 19], and the dissociation of the different estrogenic responses under various experimental conditions [14, 15, 20–24]. Further studies suggested that glucocorticoids [16, 25, 26], and perhaps many other hormones [12, 15], also act through multiple mechanisms. According to this new concept, harmful agents may modify selected parameters of hormone action, but not others. The interaction may not be detected in studies evaluating only one mechanism or parameter of hormone action [14, 15, 27–30].

Examples provided below point to possible health implications in offspring caused by the exposure of pregnant mothers to various agents to which people are frequently massively exposed. Only a full understanding of the process of imprinting of paths of heterodifferentiation, and of agents determining these changes, may improve the public health conditions in the future and perhaps prevent many diseases of still unknown aetiology.

Effect of perinatal exposure to diethylstilbestrol and other estrogens on the female genital tract

The first information on the effect of prenatal exposure to diethylstilbestrol in humans was based on the observation of a new type of gynaecological malignancy developing after puberty or during adulthood in the daughters of mothers treated with this synthetic estrogen during pregnancy [1–3]. The finding was confirmed in experimental animals [4, 5].

In addition to increased tumourigenicity, other alterations have been described in the genital tract of female offspring after maternal exposure to diethylstilbestrol and other estrogens. In experimental animals, perinatal exposure to diethylstilbestrol, allylestrenol, estradiol-17β or estradiol benzoate has been shown to induce permanent changes in steroid hormone activity [9, 31–34] and histological alterations in the female genital tract [34, 35], including gross abnormalities [35], the development of paraovarian cysts [36] and infertility [37]. In the human species, women prenatally exposed to diethylstilbestrol present histological alterations in the genital tract [2], including gross abnormalities [38, 39], the development of paraovarian cysts [36], endometriosis [40] and an increased frequency of abortion [3] and infertility [38–40].

The changes in steroid receptors, explaining the modifications in response to hormone stimulation and most of the above effects, probably reflect the imprinting of routes of heterodifferentiation of genital tissues following perinatal exposure to diethylstilbestrol or other estrogens. In the mouse, the precocious appearance of estrogen receptors in the uterovaginal epithelium [34] may explain the postpubertal increase in adenocarcinomas derived from this tissue. In humans, the abnormal localisation of uterine epithelium in the cervix and vagina was considered as one of the factors increasing the risk of tumourigenicity [41].

Further, the decrease in estrogen receptors in the rodent uterus following neonatal treatment with diethylstilbestrol, allylestrenol, estradiol-17β or estradiol benzoate [9, 31–33, 42, 43] may account for the persistent underdevelopment of rat uterine glands [42]. It suggests an explanation for uterine hypoplasia in humans [42], in addition to a decrease in the ability of the uterus to respond to estrogen stimulation [31–33, 43].

Effect of perinatal exposure to androgens on the female genital tract

The perinatal exposure of experimental animals to high levels of androgens causes changes in the normal development of the fetal genitalia [44, 45], failure in ovulation and corpus luteum formation [44, 46, 47], polycystic ovary development [44, 47, 48], the presence of a constantly cornified vaginal epithelium [47, 49], changes in uterine physiology (including abnormal hormone-induced uterine growth [31, 44–46, 49, 50]), a permanent alteration in the hypothalamic cyclic centre [51], and sterility [44, 52, 53].

The literature contains conflicting reports on some of these changes. While some investigators have failed to detect alterations in estrogen receptor levels [31, 32, 43] or in estrogen action [31] in the uterus of neonatally androgenised rats, others have reported a decrease in receptor levels [50] and an impairment in hormone action [32, 43, 45, 46, 50]. Biochemical techniques not discriminating between alterations in the different uterine cell types were used in these studies.

Considering the possible dissociation of responses to estrogen under different experimental conditions [14, 15, 20–24], we have performed studies on estrogen action in the uterus of prenatally androgenised rats, using morphometrical techniques that discriminate between responses in the different uterine cell types. We found that prenatal androgenisation inhibits estrogen-induced luminal and glandular epithelium hypertrophy, and potentiates endometrial oedema, eosinophil migration to the uterus [29] and the mitotic response in the prepubertal rat uterus [30]. But it does not modify myometrial hypertrophy [29]. This dissociation of responses to estrogen can be explained by the independence between the different mechanisms of estrogen in the uterus [10–16, 19] and the independent regulation of hormone action in every cell type [14, 54].

What mechanisms bear upon alterations in the physiology of the uterus?

The mechanisms involved in the changes in uterine physiology are not well understood. Perinatal androgenisation may play a role in the alteration of the normal development of estrogen receptors [8, 9] in some cell types only [29, 30]. Other stages in the mechanisms of hormone action might also be affected [43, 46]. Perinatal androgens may modify estrogen action directly [8, 9], or indirectly, through changes in blood levels of other hormones involved in the regulation of estrogen action. This last possibility is based on alterations in blood levels of prolactin [55, 56], estrogens [57] and progesterone [58], and on a potentiation of stress-induced events, including the hyperprolactinaemic response to stress [59], in neonatally androgenised animals. Indeed, progesterone [54, 60], prolactin [28, 61], and the stress-induced hormones epinephrine [62] and glucocorticoids [63] selectively modify some but not all responses to estrogen.

The selective inhibition of estrogen action in luminal and glandular epithelium cells in androgenised rats is the most conspicuous histological change observed in the uterus. It may contribute to the decrease in fertility observed in these animals [51]. If this effect is confirmed in humans, it could explain shifts in fertility in the daughters of patients treated with androgens or other steroids during pregnancy, and alert us to the possible risk of the ingestion of meat from animals given synthetic androgens.

Neurobehavioural effects of perinatal exposure to synthetic androgens, estrogens or progestins

The development of adult sex behaviour and other sex-dependent personality characteristics is dependent on the presence of sex hormones during precise stages of intrauterine development in some regions of the brain. These hormones determine paths of neuronal differentiation that are normal for each gender.

In humans, prenatal exposure to low levels of sex steroids permanently affects personality [64]. Exposure to synthetic estrogens determine an outer-directed personality in the adult, one that in more group-oriented and group-dependent, less individualistic and more consciously identified with its group or social environment. Exposure to synthetic progestins causes an inner or self-directed personality in the adult, one that is more independent, self-assured and self-sufficient, more individualistic and less concerned with social environment [64].

Perinatal exposure to higher levels of sex steroids or non-steroidal synthetic agonists such as diethylstilbestrol determines life-long alterations in sex-dimorphic behaviour (gender role), temperamental sex differences and sexual orientations in humans [65–69]. Other changes include

alterations in personality dimensions and self-esteem, attitudes towards work and family, mental abilities [67], sex-dimorphic play behaviour in children [68] and a decrease in orientation towards parenting in adult women [69].

In countries where meat contamination with hormones is evident [30, 70–73], increased frequencies of premature ovary enlargement, ovarian cysts [71], high estrogen levels in the blood [71, 74], premature telarche and other signs of estrogen activity [71, 74], have been found in a significant percentage of girls under 3 years of age. A similar increase in sex hormone production may occur in males prenatally or postnatally exposed to synthetic sex hormones, causing behavioural abnormalities in adulthood. The presence of abnormally high androgen levels in the blood has been reported in criminals with a history of extreme violence or rape [66]. Perinatal exposure to sex hormones may contribute in the increase in sex hormones levels in some of them, causing changes in personality.

Other effects of prenatal or early postnatal exposure to synthetic estrogens or androgens

Neonatal androgenisation also abolishes clock-timed gonadotrophin release in prepubertal and adult female rodents [75]; induces changes in tuberoinfundibular dopamine nerve activity [76]; and causes several biochemical alterations in the forebrain [77], hypothalamus [78–80] and cerebellum [81], including changes in opioid control of noradrenaline release in specific brain areas [82] and alterations in the secretion of gonadotropin [51], oxytocin [83] and prolactin [55, 56]. Prenatal exposure to estrogens affects subsequent transport of α-aminobutyric acid into the rat brain [84]. This reflects important changes in the differentiation and development of the central nervous system following perinatal exposure to synthetic androgens and estrogens, and suggests an explanation for the behavioural changes in exposed experimental animals or humans.

Androgenisation induce permanent alterations in testosterone metabolism in the hypothalamus-pituitary-gonadal axis in male rats [85]. Neonatal exposure to estrogens determines developmental, structural and functional alterations in the testis, prostate and seminal vesicles [86–89].

The immune system is also affected by exposure to sex hormones. Estrogens cause changes in the development of the rat thymus gland, including premature involution of its cortex [90]. Diethylstilbestrol persistently alters natural killer (NK) cell activity in the mouse [91] and humans [92]. Changes in immune responsiveness [93] and an increased occurrence of autoimmune disease [94], in addition to the increased frequency of diseases suggesting impaired immune function, such as respiratory tract infections, asthma, arthritis, and lupus [95], have been reported in women exposed *in utero* to diethylstilbestrol.

Imprinting of paths of heterodifferentiation by polypeptide or aminergic hormones

In the rat, neonatal treatment with thyroxine (which decreases thyroid stimulating hormone (TSH) levels) [96] or high doses of TSH [6] depress subsequent responsiveness to TSH. Neonatal treatment with vasopressin [97] or met-enkephalin [98], permanently increases sensitivity to vasopressin or opioids. Neonatal exposure to insulin determine, in adult rats, altered binding of insulin and altered response to the hormone in the liver [99]. Imprinting of paths of heterodifferentiation has been reported in the chicken ovary

for follicle stimulating hormone (FSH) [100]. Neonatal treatment with catecholamines (epinephrine, isoproterenol, dopamine) alters the adrenergic vascular response in the adult rat. Isoproterenol modifies the response to norepinephrine and also to vasopressin [101].

Imprinting of paths of heterodifferentiation can also be induced by hormones that have similar molecular structure but different actions. For instance, perinatal exposure to oxytocin determine a persistent hypersensitivity to vasopressin [97]. The use of oxytocin to induce delivery will have to be re-evaluated in view of these findings.

Imprinting of paths of heterodifferentiation by polluting agents displaying hormonal action

Several estrogens, androgens and progestins are still widely used as growth promoters for farm animals or birds in several countries, mainly in the Third World, without any effective regulation [70, 72, 73]. Diethylstilbestrol is one of the hormones reported to be used, at least until recently [70, 72]. Very high estrogen levels were detected in chicken and beef meat in Puerto Rico [71]. Premature telarche, gynecomastia, other signs of precocious sexual development, ovarian or uterine enlargement, ovarian cysts and increased estrogen levels in the blood have been reported among children in several countries [71, 74, 102] as a result of estrogen contamination of the food ingested by the children or their mothers [71].

The high incidence of premature telarche reflects the action of high levels of hormones, which may imprint paths of heterodifferentiation in prenatally or postnatally exposed population, determining increased incidences of conditions such as gynaecological malignancies, infertility, immune deficiencies and autoimmune diseases. Other effects may possibly include character and behavioural alterations, including changes in sex-dimorphic behaviour and even the development of violent behaviour. The risk of exposure to high hormone doses from food is particularly high when hormones are implanted in eatable parts of the animal, and/or when cattle are killed shortly after implantation.

Imprinting of paths of heterodifferentiation by pharmaceuticals, polluting agents and other non-hormonal substances

The imprinting of paths of differentiation is not an exclusive attribute of hormones. Non-hormonal molecules interacting with hormone receptors or other equivalent structures may also induce this phenomenon, as shown by the sugar molecules glucosamine and mannose. These alter the reactivity of pancreatic beta cells in rats and the production of insulin in adults [103]. Various pharmaceuticals, polluting agents, ethanol, abused substances, food additives, and even some substances normally present in certain foods, also appear on the list of agents capable of imprinting paths of heterodifferentiation. Every day more compounds are described as sharing this characteristic; perhaps, in the years to come, several compounds currently considered to be innocuous will be added to the list. Below are a few examples.

Diazepam and related pharmaceuticals

Besides its medical applications, diazepam is frequently used in many countries by self-prescription and is a substance of abuse. Prenatal exposure to diazepam in experimental animals permanently decreases β-adrenergic receptors in

brain cortex, striatum and hypothalamus, but not in the cerebellum [104]; alters dopaminergic function in some brain areas [105]; and changes the low affinity form of gamma amino butyric acid and receptors [106].

Biochemical changes in the differentiation of cell-types affected by diazepam may explain alterations in the ability to cope with stress [105], including motivational responsiveness to environmental challenges [107]; in the copulatory activity of male rats [107]; and in the severe depression of the cellular immune response [108].

Phenobarbital

Although phenobarbital is widely used in the treatment of epilepsy, prenatal exposure induces a significant decrease in dendritic development of rat hippocampus neurons during the first postnatal month [109]. It also causes feminine sex behaviour in adult male hamsters [110] and reproductive dysfunctions in the male rat, including a delay in testis descent, a decrease in seminal vesicle weight and fertility reduction [111]. Phenobarbital elicits puberty delay, aberrant cycles, infertility and increases in estrogen levels in blood and estrogen receptors in the uterus in female rats. In humans, it exerts a negative effect on cognitive development [112].

Ethanol

Prenatal exposure resulting from maternal alcohol intake during pregnancy mainly affects the central nervous system. In the rat, this causes a decrease in the thickness of brain cortex and changes in glucose metabolism in certain brain areas, mainly affecting neurons from the thalamus and corpus callosum connections [113]. Alcohol causes permanent changes in brain receptors for benzodiazepines [114] as well as serotonergic 5-HT1 [115] receptors, while also changing enkephalin level [116] and destabilising norepinephrine secretion [117]. It induces changes in astrocyte enzymes, which may cause neuronal alterations [118], a decrease in the number of neurons [119], morphological changes in neurons [110, 120], and impairment of the development of hippocampus pyramidal cell dendrites [121].

These changes may be the biochemical and morphological substratum of the behavioural changes observed after prenatal exposure to ethanol [114], among them an increase in aggression [122]. Prenatal exposure also determines permanent immunological depression [123], alterations in sex dimorphic behaviour [124] and reproductive changes such as a decrease in hypothalamic sensitivity to testosterone feedback [125], an increase in fetal testosterone levels and disruption of the oestrus cycle [126].

Nicotine

In the rat, prenatal exposure to nicotine results in an increase in spontaneous locomotor activity [127], which can be explained by changes in striatum dopamine binding sites [128]. It causes persistent alterations in the functional state of catecholaminergic neurons, evidenced by a persistent decrease in MOPEG and, in male rats only, an increase in noradrenaline content [129]. Nicotine also elicits up-regulation of adenylate cyclase activity in membrane preparations of kidney and heart, not accompanied by β-adrenergic receptor up-regulation, that can be explained by changes in enzymes involved in membrane receptor signal transduction, leading to altered responsiveness independently of changes at the receptor level [130].

Prenatal exposure to nicotine also alters subsequent sexual behaviour in males, increasing the latency before the physiological changes that occur during intercourse and decreasing the efficiency of copulation [131]. These alterations may be caused by the decrease in blood testosterone levels observed in adult prenatally exposed animals, due to a decrease in hormone synthesis in the testis [131].

Pesticides

Prenatal exposure to polychlorobiphenyl pesticides causes persistent behavioural changes in rats [132]. Exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) elicits atrophy of the thymus gland and suppression of the immune response [133], through an alteration in the path of differentiation of lymphocyte stem cells [133].

Lead

In the rat, prenatal exposure to lead causes a permanent increase in the affinity of δ- but not μ-opioid receptors in the brain [134], which parallels the impairment of opioid but not non-opioid stress-induced antinociception in developing rats [135]. If these changes also occur in humans, they may explain behaviour changes in exposed populations [136–138], and perhaps may account for the increased frequency of addiction to opioid or other abuse drugs in environments highly contaminated with lead. The finding that dopamine and 5-hydroxyindoleacetic acid responses to amphetamine are enhanced in lead-exposed animals [139] suggests that the response to other stimulant abuse substances may be enhanced as well.

In experimental animals, exposure to lead impairs learning [140]. In humans, it causes deficits in central nervous system functioning that persist into adulthood. These include learning impairment, deficits in psychometric intelligence scores, lower IQ scores, poorer school performance, increased school failure, reading disabilities and poorer eye-hand coordination [136–138]. Imprinting of paths of heterodifferentiation in central nervous system cells may explain these alterations at least in part.

Exposure to lead also affects the reproductive system. In perinatally exposed adult rats, the numbers and characteristics of uterine estrogen receptors differ from those of non-exposed animals [141]. Permanent alterations in ovary gonadotropin receptors and in steroidogenesis have been detected as a result of exposure [142]. These alterations explain, at least in part, the known depression in fertility in lead-exposed experimental animals [143] and humans [144]. They also support the hypothesis that lead intoxication caused the fall of the Roman Empire due to the increasing infertility of the ruling class [145].

Natural food components and additives

In experimental animals, sugar molecules such as glucosamine and mannose may alter insulin production by pancreatic beta cells during adulthood [103]. High perinatal feeding of fat alters hepatic drug metabolism during adult life [146] and induces a cholesterol homeostatic memory [147]. Thirdly, a perinatal diet high in sodium chloride determines increases in the salt intake and sodium excretion in the adult [148]. These findings suggest that dietary factors in early life modify the extent of adaptative responses in later years [147].

Some foods or beverages contain active agents. For instance, coffee, besides caffeine, also includes estrogenic

agents that may imprint paths of heterodifferentiation [149]. Prenatal caffeine determines increased activity and decreased emotionality in the adult rat; higher doses of caffeine may have opposite effects [150]. Exposure of pregnant rats to caffeine inhibits the differentiation of testis interstitial tissue and Leydig cells and reduces testosterone synthesis by fetal testis [151], which may in turn imprint paths of heterodifferentiation in other tissues.

Many processed foods and beverages contain exogenous compounds that may imprint paths of heterodifferentiation. For instance, caffeine is added to soft drinks in several countries and pregnant women are usually not warned about this. Nitrates or nitrites, added to foods, may cause discrimination learning deficits and impaired retention behaviour following prenatal exposure [152]. In some countries, tetracyclines are added to frozen food [70]; prenatal exposure to these antibiotics can cause persistent immune deficits [153, 154]. Many food colour additives and other substances used to improve the organoleptic properties of food have not been yet evaluated for health risks following perinatal exposure.

Prenatal stress

Exposure to maternal stress during fetal life alters morphine- and stress-induced analgesia in male and female rats [155]. This may be explained by persistent decrease in μ-opioid receptors in the striatum but not in other brain regions in prenatally exposed adult rats [156]. Prenatal stress also affects adult sex behaviour in both experimental animals and humans. In rats, it feminises and demasculinises male behaviour [157]. In humans, it can lead to homosexuality in males [158].

Stress may also have effects through the hypersecretion of various maternal hormones. This in turn causes sex behaviour changes in imprinting paths of cell heterodifferentiation.

Possible wider effects of imprinting of paths of heterodifferentiation

We proposed above that prenatal or early postnatal exposure to lead imprints paths of cell heterodifferentiation in various organs, causing changes in their receptors and alterations in their responsiveness to hormones, neurotransmitters or exogeneous substances. In the uterus, ovary and perhaps hypothalamus, these changes may lead to shifts in the reproductive capacity of exposed populations. Alterations in estrogen and perhaps opiate receptors in the central nervous system can also trigger changes in sex behaviour and sexual orientations. In the brain, other biochemical shifts may cause neurobehavioural and psychopathic alterations. The increased affinity of brain opiate receptors and greater sensitivity for endogenous or exogenous opioids may favour a tendency towards addiction to morphine-like narcotics or, indirectly, to cocaine or amphetamine stimulants.

If, therefore, a significant part of a population is neonatally exposed to lead, increases in the incidence of infertility, sex behaviour alterations, addiction to drugs of abuse, and changes in criminality and other kinds of antisocial behaviour may be expected [159]. This could contribute to the decadence of an exposed society, its cultural decline, disorganisation and finally disappearance.

In this context, the fall of Rome was related to the wide use of lead in paints, water distribution, and the storage of wine and fruit juices. Gilfillan [145] suggested that the declining birth rate and apparently increased incidence of psychosis in Rome's ruling class, which may have been at the root of the Empire's dissolution, were a result of exposure to lead in food and wine. Recent reports on estrogen, gonadotropin and opiate receptors may explain the increase in the incidence of infertility and homosexuality, and the apparent addiction to narcotic plants, in the Roman population, as well as its incapacity to defend its world against foreign invaders.

The second part of the present century has seen an important epidemic of addiction to drugs of abuse, firstly in large cities in the USA, then in the cities of Western Europe, and currently in most large cities in South America. This addiction to narcotic or stimulant drugs, which has not affected rural or small town populations, parallels a simultaneous increase in criminality and an apparent rise in the incidence of alterations in sexual orientation.

According to our hypothesis, these changes can be explained, at least in part, by an increase in lead pollution in large cities, but not in small towns, that appeared first in North American, then in Western European and subsequently in South American cities, caused by an increase in the use of leaded gasoline. Perinatal exposure to lead may have triggered changes in brain opiate, estrogen and other receptors and subsequent neurobehavioural alterations.

The populations of several countries may also be exposed to other pollutants, such as hormonally active compounds from the meat of farm animals or poultry treated for anabolic purposes; colour and other additives in foods and beverages; pesticides, nicotine, ethanol and substances of abuse. In addition, the population may be exposed to natural products in certain food products preferentially ingested in these countries.

The effects of some of these agents are currently well known, but the possibility of imprinting of routes of heterodifferentiation by the remaining substances have not yet been investigated. It is possible that many ancient and perhaps more recent civilisations declined due to exposure to some of these agents, especially those that impair intelligence or cause psychological changes affecting the society.

Perspectives and conclusions

Many health conditions in a community, such as the incidence of various diseases and its behavioural and psychological characteristics, are in part determined by agents to which people are exposed during prenatal or early postnatal life. It has already been suggested that the focal prevalence of human diseases has social and ecological causes – *i.e.* is determined by environmental and cultural factors in a society [160]. In agreement with this proposal, Bradley [161] has highlighted the fetal and infant origins of adult diseases.

Therefore, most individuals from any human community can be exposed, during prenatal or early postnatal age, to agents or conditions that alter the paths of differentiation of various cell types, determining their health conditions, behavioural characteristics and mental abilities. Although these effects may sometimes be convenient, they are adverse if they favour an increased incidence of conditions such as immune depression, cancer, infertility, psychological changes and neurobehavioural alterations. Perhaps the behavioural characteristics of any ethnic group or society are determined, at least in part, by their food preferences and by local pollutants.

This new field may develop great importance in the medical science of the future. When the diseases that result from prenatal or neonatal exposures are identified, and when most of the potentially harmful agents imprinting paths of heterodifferentiation are recognised, we should be able to avoid these agents or neutralise their effects. Much research, and

determination to avoid exposure to toxic agents, seem necessary if we are to achieve a substantial improvement in the health condition of humankind.

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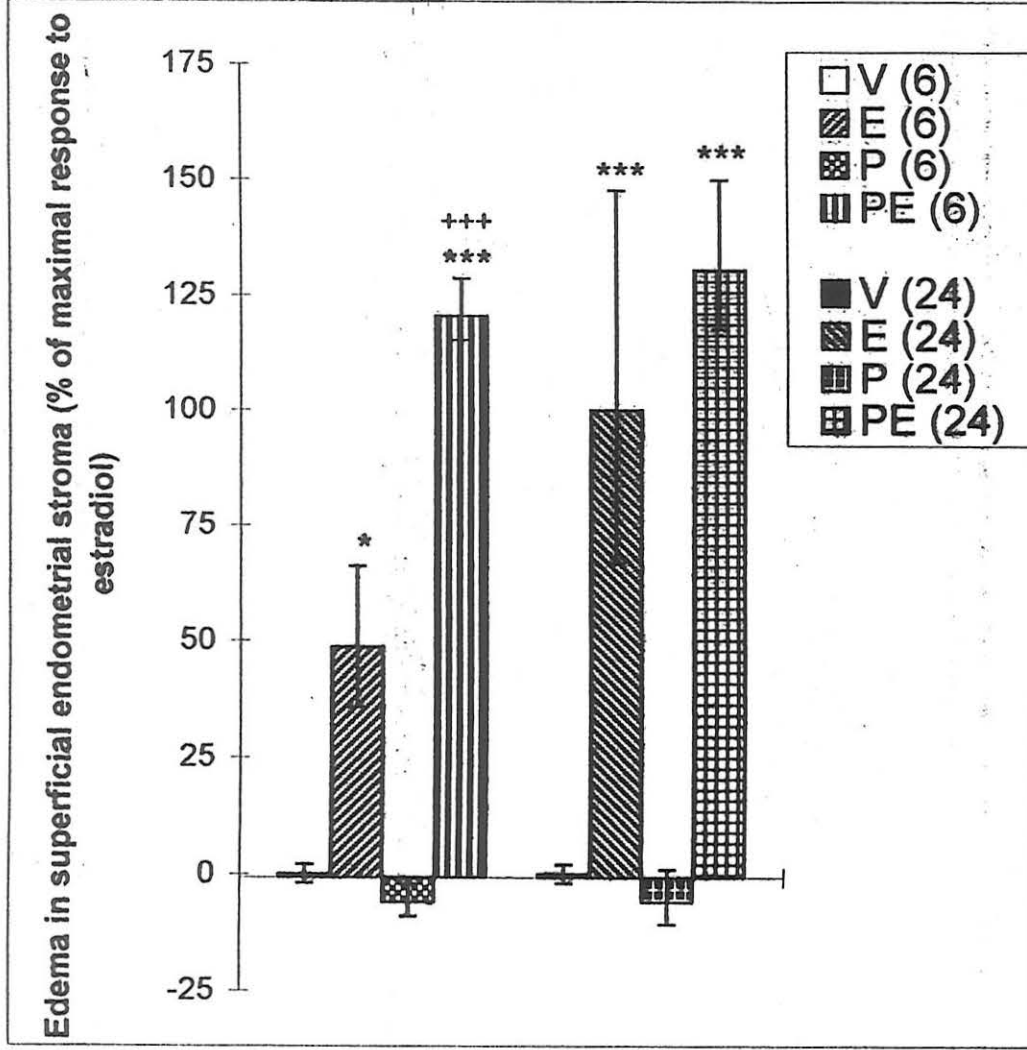
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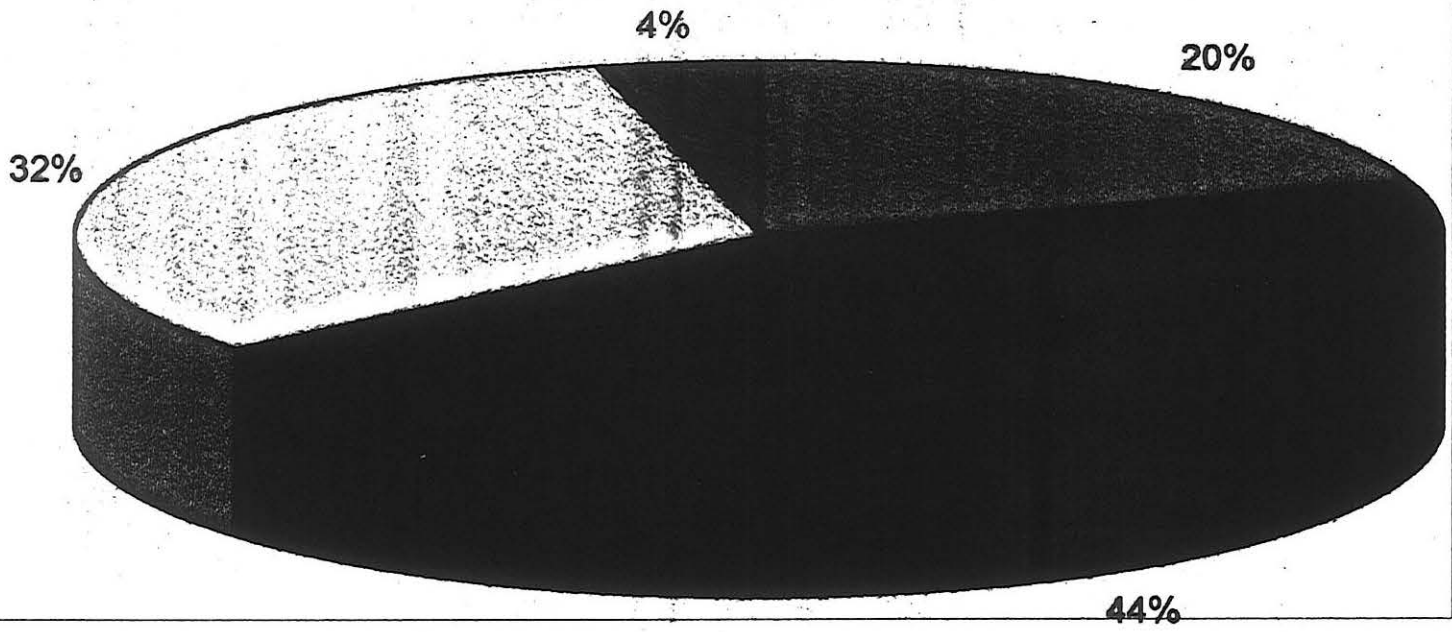
Correspondence should be addressed to:
Prof. Dr. Andrei N. Tchernitchin, Head, Laboratory of Experimental Endocrinology and Environmental Pathology LEEPA, Environment and Biomedicine Research Center CIMAB and Institute of Biomedical Sciences ICBM, University of Chile Medical School; P.O. Box: Casilla 21104, Correo 21, Santiago, Chile. Phone/FAX (56-2) 678 62 22. E-mail: atcherni@machi.med.uchile.cl
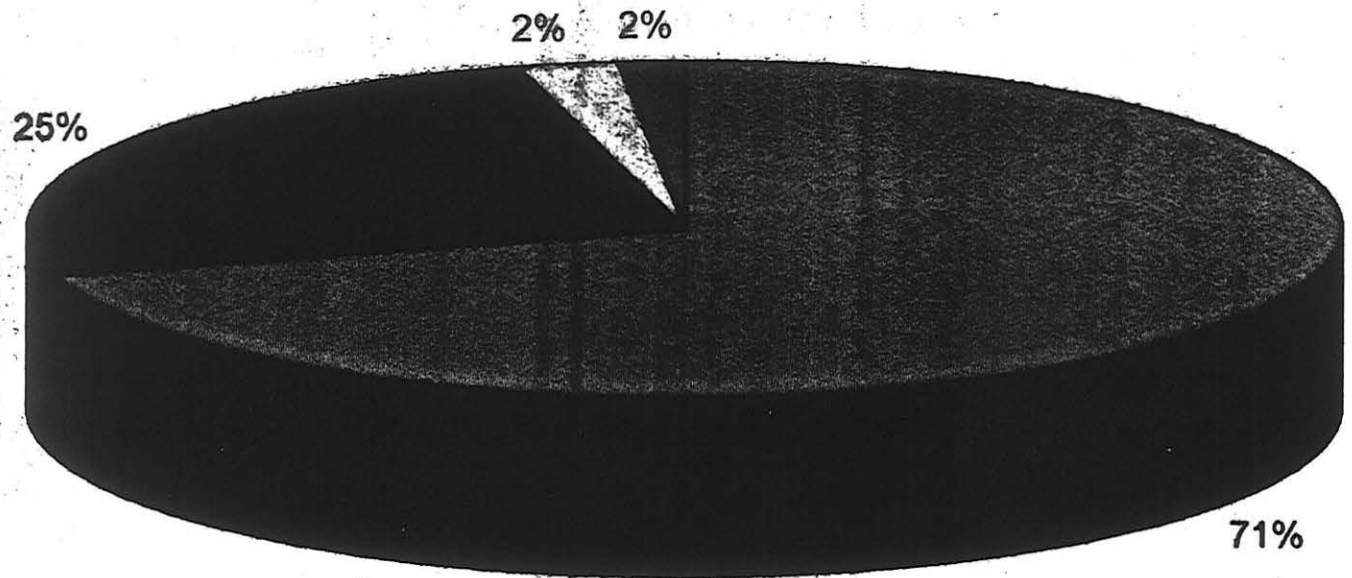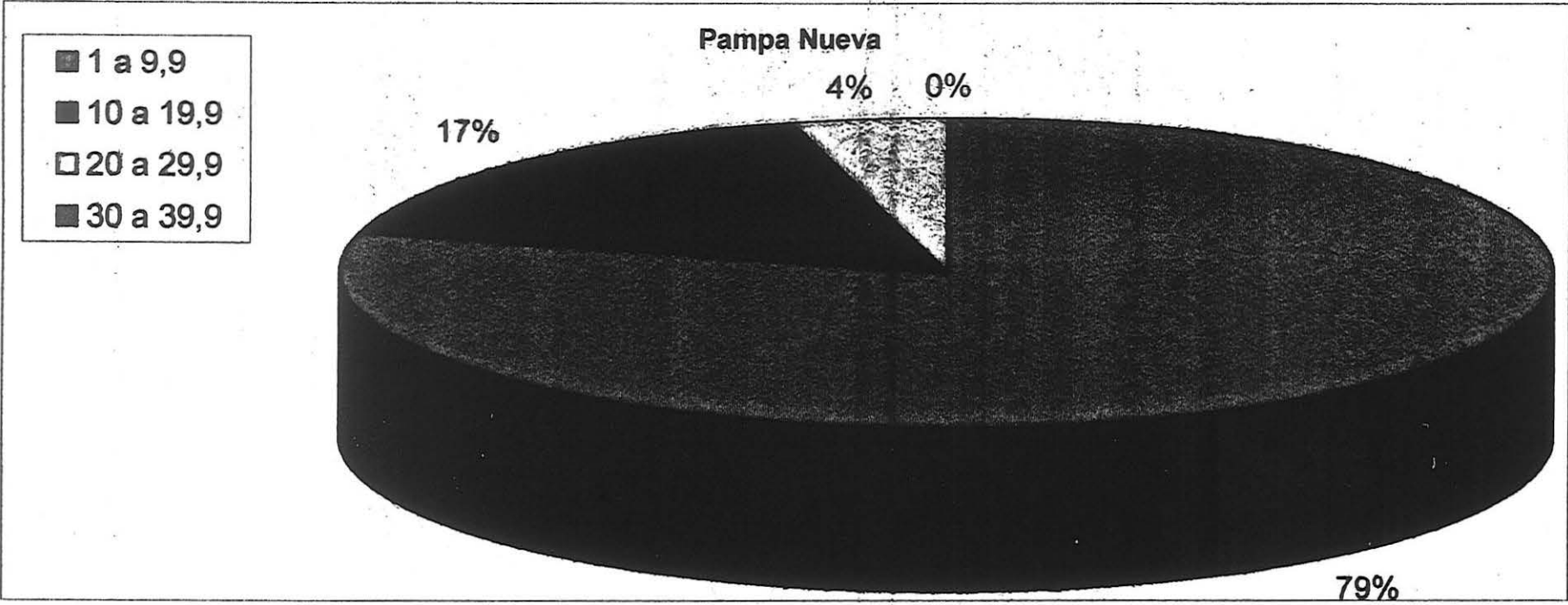
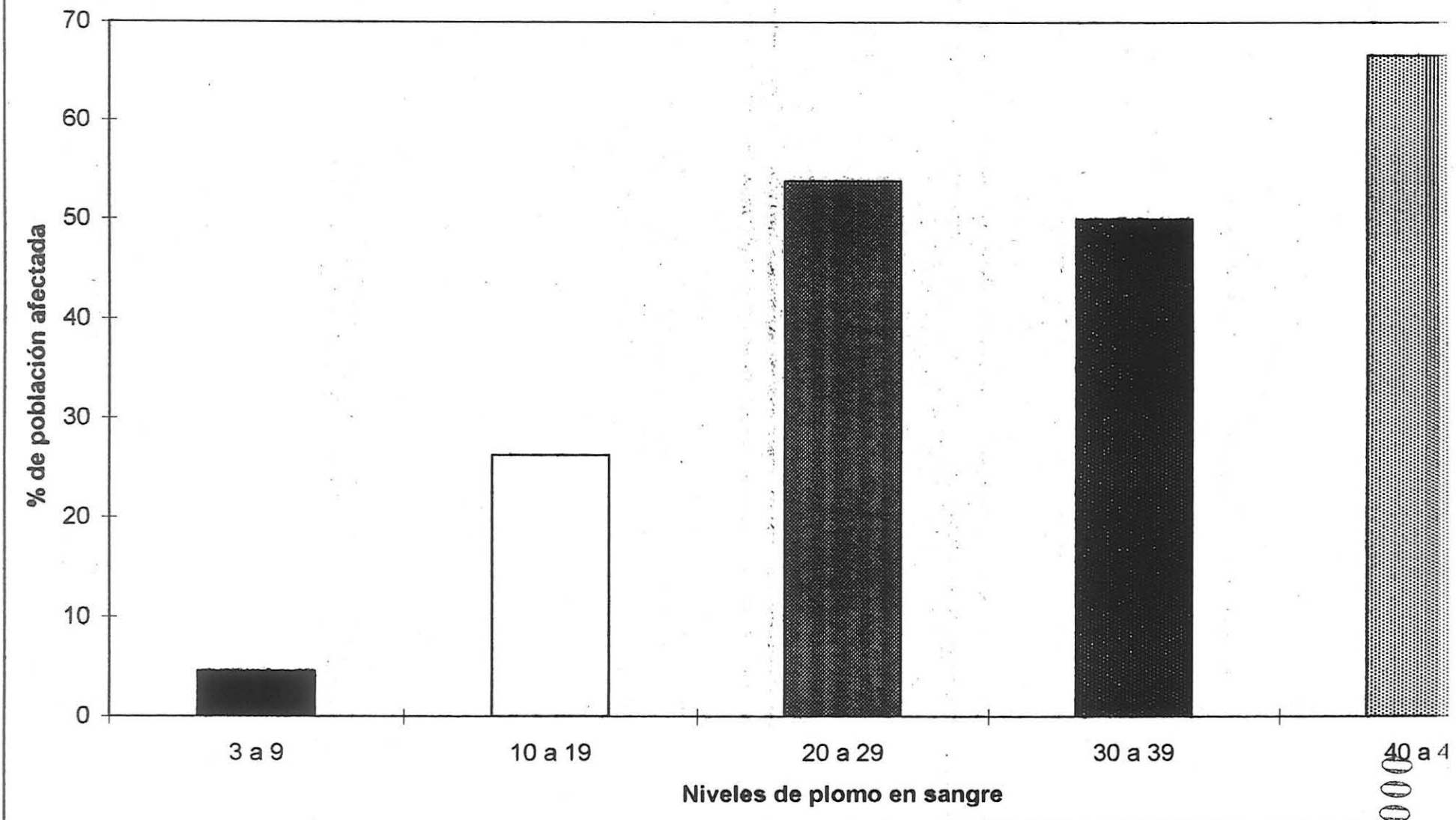Villa Sta. María

Legend:
- 1 a 9,9
- 10 a 19,9
- 20 a 29,9
- 30 a 39,9

4%
20%
32%
44%

Los Industriales

Legend:
- 1 a 9,9
- 10 a 19,9
- 20 a 29,9
- 30 a 39,9

2%   2%

25%

71%

Pampa Nueva

Legend:
- 1 a 9,9
- 10 a 19,9
- 20 a 29,9
- 30 a 39,9

4%   0%

17%

79%

% población con manifestaciones neurológicas moderadas o graves

Niveles de plomo en sangre

MANIFESTACIONES NEUROLOGICAS Y NEUROCONDUCTUALES SEGUN NIVELES DE PLOMO

Eje Y: Intensidad de efectos (0 a 3,5)

Eje X: Niveles de plomo (3 a 9, 10 a 19, 20 a 29, 30 a 39, 40 a 49)

Leyenda:
- 3 a 9
- 10 a 19
- 20 a 29
- 30 a 39
- 40 a 49
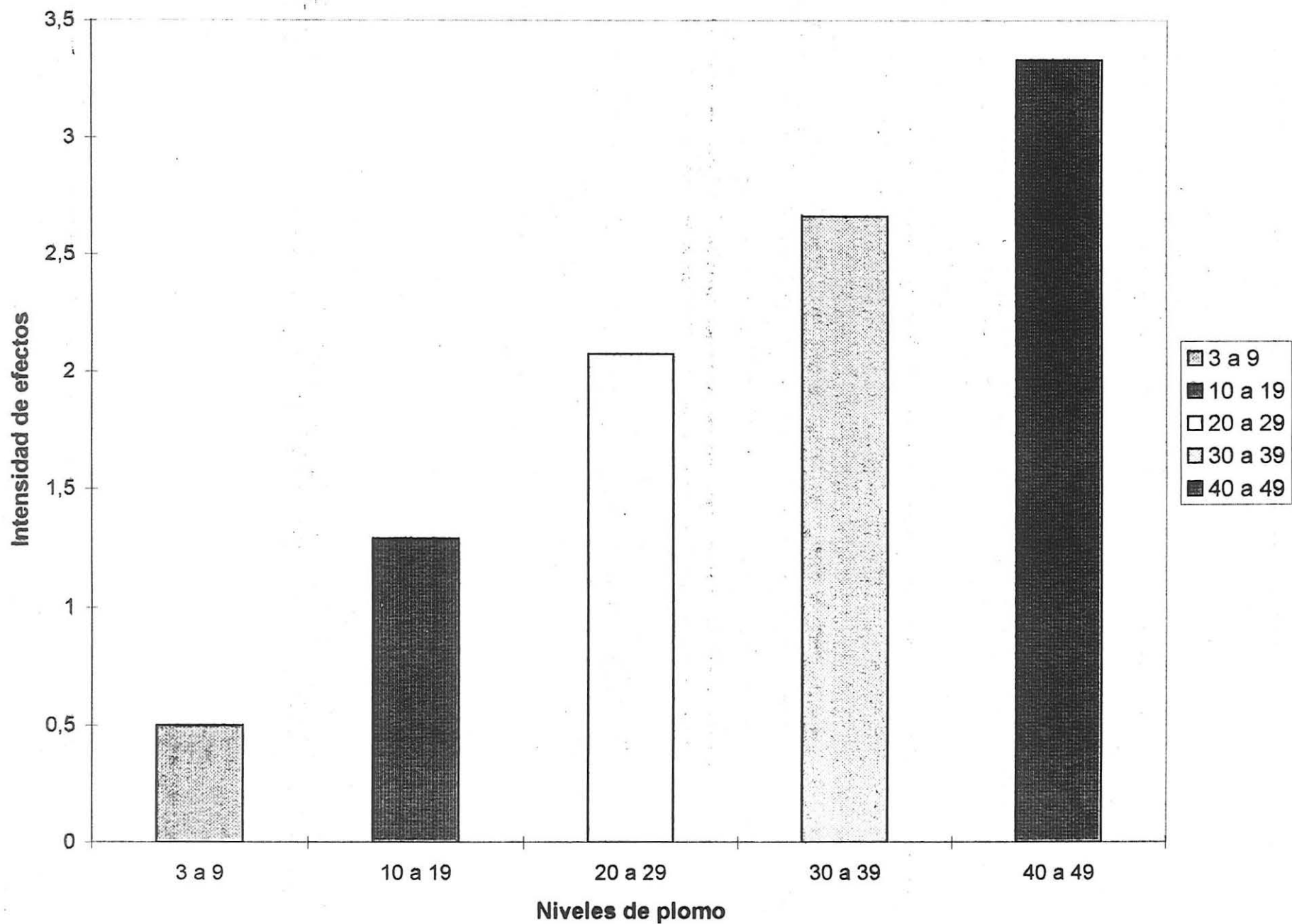
PORCENTAJE NIÑOS SEGUN NIVELES DE PLOMO EN SANGRE

Menor que 10
10 a 19
20 a 29
30 a 39
40 a 49

40 a 49
7%

Menor que 10
10%

30 a 39
14%

Menor que 10
10 a 19
35%

20 a 29
34%
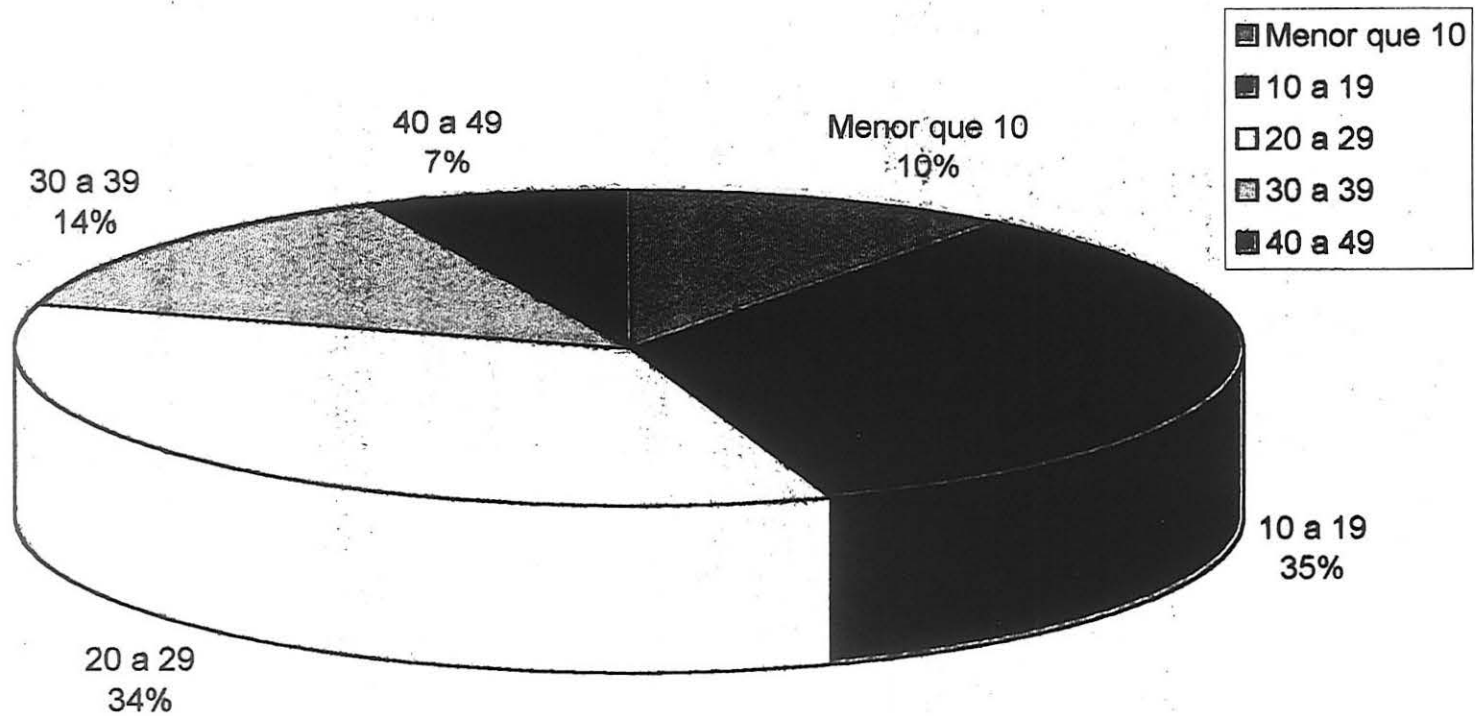
PORCENTAJE NIÑOS CON NIVELES DE PLOMO DE 10 O MÁS

Menor que 10
10%

10 a 49
90%
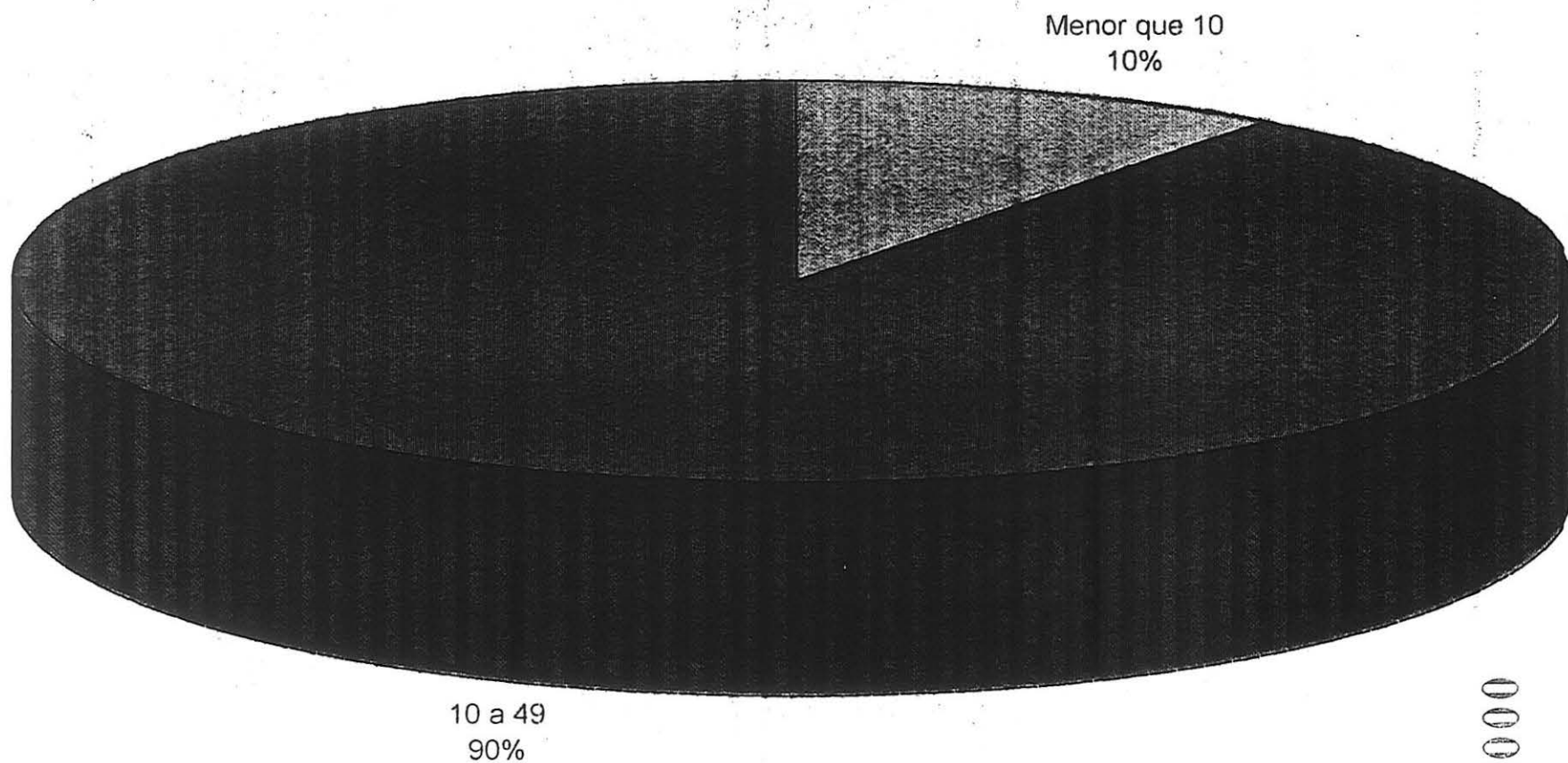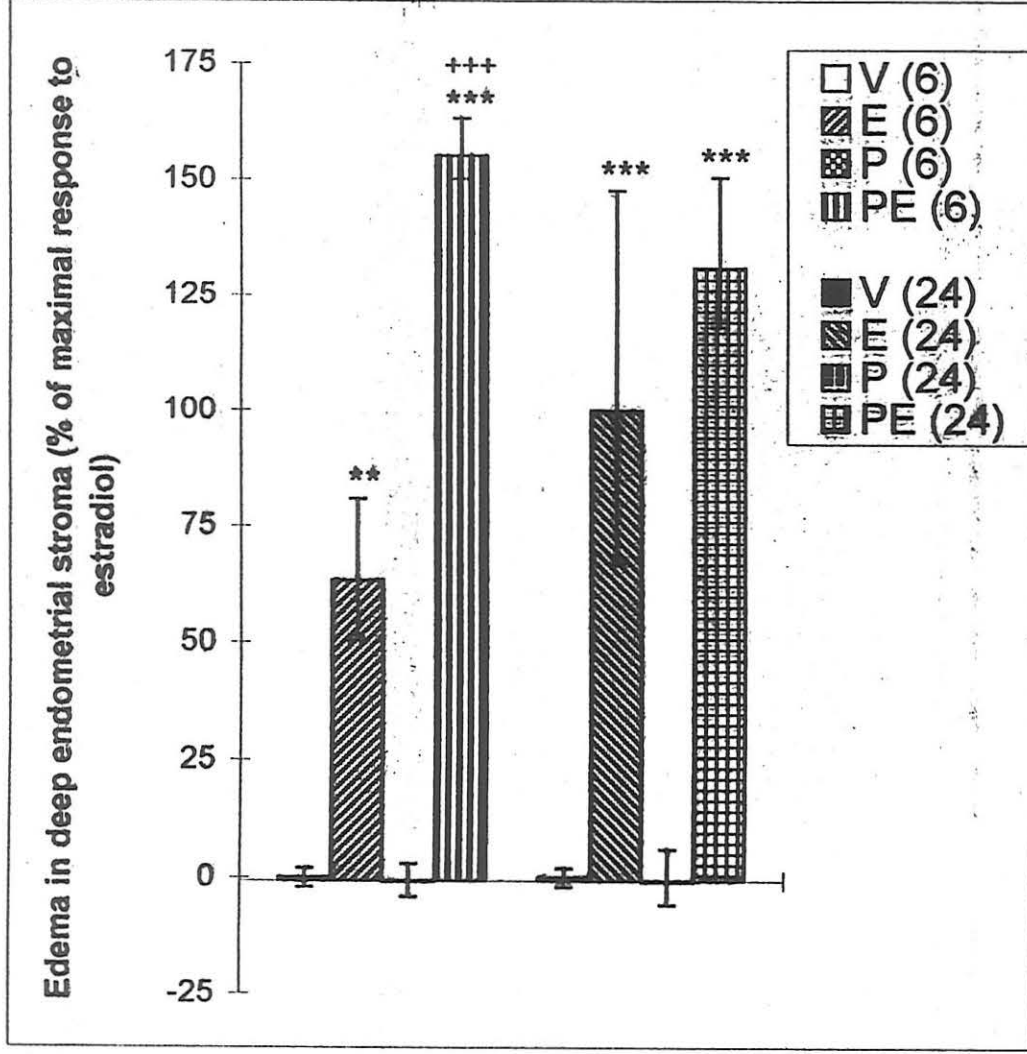
% población con manifestaciones neurológicas graves

% de población afectada

Niveles de plomo en sangre

Legend:
- 3 a 9
- 10 a 19
- 20 a 29
- 30 a 39
- 40 a 49
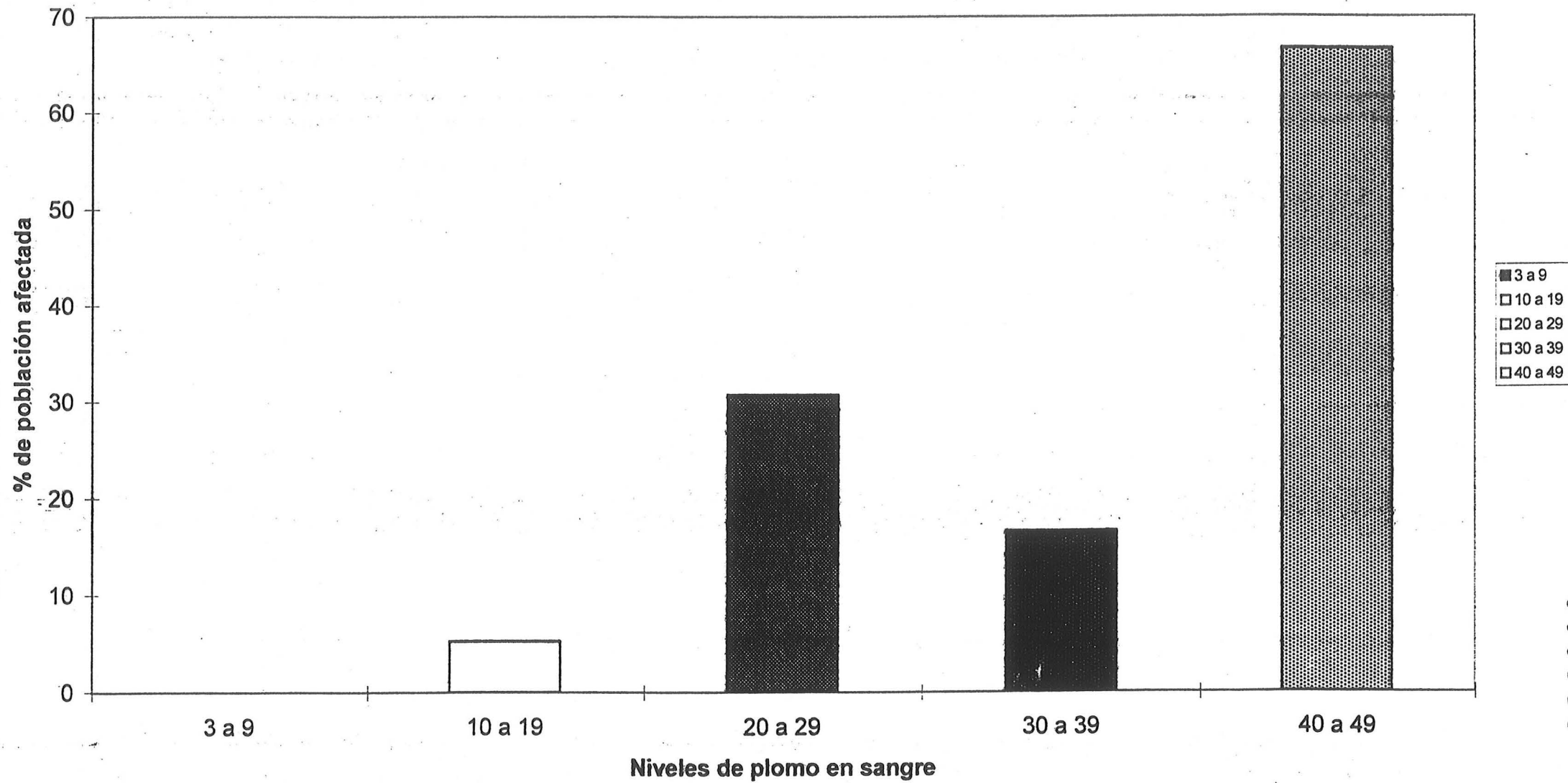
PATRICIA MATUS

De:	Roberto Martinez [rmartinez.rm@conama.cl]
Enviado:	Jueves 10 de Junio de 1999 03:23 PM
Para:	pmatus@conama.cl
Asunto:	datos de plomo

Importancia: Alta

000537

ATT00000.html   plomo.xls

Adjunto datos de plomo de todos los años muestreados con SFU (Paulo Artaxo),
estos son 95-96 y 98.
Roberto Martinez

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