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Effect of chronic developmental lead exposure on cell-mediated immune functions

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SUMMARY

Studies were performed to investigate the effects of chronic, low level pre- and post-natal lead exposure on cell-mediated immune function in rats. Weanling female rats were exposed to lead (as lead acetate) in their drinking water at 0, 25, and 50 ppm for 7 weeks. At the end of 7 weeks they were mated with untreated males and continued on the same dosage throughout gestation and lactation. The offspring of these females were weaned at 21 days of age and continued on the same lead exposure regimen as their mothers. These offspring were used in immune surveillance procedures between 35 and 45 days of age.

Lead exposure at the levels employed had no statistically significant effect on growth and did not result in overt signs of toxicity. Thymic weights were significantly decreased in both males and females of the two lead dosage groups. Furthermore, lead exposure resulted in suppression of responsiveness of lymphocytes to mitogen stimulation and in reduced delayed hypersensitivity responsiveness. Results indicate that chronic low-level lead exposure causes suppression of cell-mediated immune function.

INTRODUCTION

There has been increasing interest in the effect of environmental contaminants on immune function in recent years. A number of compounds of environmental concern have now been shown to produce immunosuppression or reduce host resistance to infectious agents. The list of these compounds includes the chlorinated hydrocarbons, polychlorinated biphenyls and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (Faith & Moore, 1977; Friend & Trainer, 1970; Koller & Thigpen, 1973; Street & Sharma, 1975; Thigpen *et al.*, 1975; Vos & De Roij, 1972; Vos & van Genderen, 1973; Vos, Moore & Zinkl, 1973; Vos & Moore, 1974), organometallics like triphenyltin and methyl mercury (Ohi *et al.*, 1976; Spyker, 1975; Verschuuren *et al.*, 1970), and inorganic metals such as lead, cadmium and mercury (Hemphill, Kaeberle & Buck, 1971; Jones, Williams & Jones, 1971; Koller, 1973).

Lead-related diseases have been recognized for more than 2000 years (Chislom, 1971). Lead causes both acute and chronic poisoning depending on exposure length and level (Chislom, 1971) and toxicity is often overtly manifested in the form of various gastrointestinal and neurological symptoms. Immunosuppression appears to be a more subtle effect of exposure to heavy metals. In this respect, lead has been shown to suppress antibody responses in adult mice and rabbits (Koller, 1973; Koller & Kovacic, 1974; Koller, Exon & Roan, 1976), to reduce resistance to bacterial challenge in mice (Hemphill *et al.*, 1971)

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and to reduce the phagocytic function of the reticuloendothelial system in rats (Trejo *et al.*, 1972). Such immunosuppressive effects, however, were mostly investigated at toxic, high-level lead exposures in the above studies. This leads one to wonder if more moderate, low-level lead exposures may produce immune response deficits in otherwise apparently 'asymptomatic' lead-exposed animals.

Immunosuppression induced by low-level lead exposure was suggested by earlier observations (Grant *et al.*, 1978), which lead to initiation of the study reported here. Using the same dosage regimen as that employed here, rats chronically exposed pre- and post-natally to low levels of lead appeared to be more vulnerable to various infectious diseases (Grant *et al.*, 1978). The studies reported here were, therefore, undertaken to investigate more systematically the effects of low-level pre- and post-natal lead exposure on cell-mediated immune function. For many agents, the pre-natal organism is more sensitive to chemical injury than the corresponding adult (McLachlan & Dixon, 1976). For example, offspring from mice exposed to methyl mercury while pregnant have been shown to be immunosuppressed (Spyker, 1975). The results of the studies reported here showed mitogen responsiveness of lymphocytes and delayed hypersensitivity responses to be reduced in rats exposed to lead early in development.

MATERIALS AND METHODS

Animals. Weanling female Sprague Dawley (CD) rats were purchased from Charles River Breeding Laboratories (Wilmington, Massachusetts) at 21 days of age. The animals were housed individually in hanging wire-mesh cages and handled as described below.

Lead dosage and breeding. The weanling females were exposed to lead as previously described (Kimmel *et al.*, 1978) by incorporating lead acetate in the drinking water at 0, 25, and 50 ppm as lead acetate. This water source was provided *ad libitum*. The rats were fed a semi-purified diet (zinc control diet, Teklad Test Diets) to avoid uncontrolled lead exposure as a result of variable lead contamination found in commercial laboratory animal diets.

The female rats were maintained on the above dosage regimen for 7 weeks. They were then mated with untreated males of the same strain and continued on the same dosage regimen throughout gestation and lactation. At 21 days of age, offspring of these females were weaned onto the same dose of lead as their mothers. Offspring (25–30 animals per dose group) were used in immune surveillance procedures between 35 and 45 days of age.

Mitogens. The mitogens employed in this study were phytohaemagglutin-p (PHA) and Concanavalin A (Con A) (Difco Laboratories, Detroit, Mighican). PHA was employed at the level of 50 μmg/culture and Con A was employed at the level of 0.1 λ/culture.

Cell cultures. Animals used for *in vitro* lymphocyte cultures were killed with CO_2 and their spleens and thymuses removed aspetically. Thymuses were carefully trimmed of associated lymph nodes. Cell suspensions were prepared as previously described (Faith & Moore, 1977). Cell counts were determined with a Coulter Counter (Model ZB, Coulter Electronics, Inc., Hialeah, Florida). The lymphoid cells (mixed populations) obtained from individual spleens or thymuses were cultured as previously described (Faith & Moore, 1977). The rate of cellular DNA systhesis was measured by the incorporation of ^{3}H-thymidine into acid-insoluble material as previously described (Faith & Moore, 1977).

The degree of mitogen stimulation was determined by calculating a stimulation index; this was the mean counts per min (cpm) of the triplicate mitogen-stimulated cultures divided by the mean cpm of the non-stimulated cultures.

Delayed hypersensitivity reactions. Delayed hypersensitivity reactions were performed using the method of Lefford (1974). Briefly, the animals were sensitized by i.p. injection of 0.1 ml of Freund's complete adjuvant (HR37A, Difco Laboratories, Detroit, Michigan). Seven days after sensitization the animals were injected i.p. with ^{3}H-thymidine ((methyl-^{3}H) thymidine, specific activity 6.7 Ci/mM, New England Nuclear, Boston, Massachusetts); 1μCi/gm body weight. Twenty-four hr later, one ear was injected with 10λ purified protein derivative (PPD) (Aplisol, Parke-Davis, Detroit, Michigan); 10 mg/ml and the other ear injected with 10λ phosphate-buffered normal saline (PBS). Twenty-four hr after this challenge, the animals were killed with CO_2 and a 6 mm diameter plug taken from each ear. The tissue plugs were solubilized with NCS (Amersham/Searle Corp., Arlington Heights, Illinois) in standard scintillation vials, and 10 ml of scintillation fluid (Aquasol, New England Nuclear, Boston, Massachusetts) was added. The samples were counted in a Packard Tri-Carb Liquid Scintillation Spectrometer, Model 2450. An index of reactivity (DHR index) was calculated by dividing the cpm of the challenged ear by the cpm of the control ear.

Histopathology. Tissues selected for histopathological examination were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 6 μm, and routinely stained with haematoxylin and eosin.

Statistical analyses. Analysis of variance procedures (Hollander & Wolfe, 1973) were employed to assess the significance of treatment effects, replicate differences, and replicate X treatment interactions. In some cases the logarithmic transformation was employed to equalize the variancies. Pairwise comparisons were made by Fisher's LSD test (Hollander & Wolfe, 1973). The significance of dose-response trends was determined by Jonckheere's test (Snedecor & Cochran, 1967).

RESULTS

Effects of pre- and post-natal lead exposure on body weight and selected organs

Table 1 summarizes the body weight and selected organ effects of pre- and post-natal lead exposure. Lead exposure at the levels employed had no significant effect on body weights in either males or females Actual thymus weights were reduced in both exposure groups in male and female animals, as were relative thymus weights. Also among lead-exposed males there was an increase in actual ($P<0{\cdot}10$) and relative ($P<0{\cdot}01$) spleen weights in the 50 ppm exposure group.

Histologically there was no difference in spleens or thymuses in lead-exposed animals and non-exposed animals.

In vitro responsiveness of lymphocytes to mitogen stimulation

Table 2 and Fig. 1 illustrate the reactivity of thymic and splenic lymphocytes from lead-exposed rats to mitogen stimulation by the mitogens Con A and PHA. Both treatment groups showed deviation from normal. In the 25 ppm dosage group thymic cells were significantly reduced in responsiveness to PHA and splenic lymphocytes were significantly reduced in responsiveness to both PHA and Con A. In the 50 ppm dosage group thymic lymphocytes did not differ significantly from controls in their response to either Con A or PHA while splenic lymphocytes were significantly reduced in their response to both Con A and PHA. The differences in responsiveness of thymic or splenic lymphocytes from the 25 ppm dosage group and the 50 ppm dosage group were not statistically significant.

Although all pairwise comparisons for thymic cells stimulated with Con A were not significant, the overall dose-response trend was significant at the $P<0{\cdot}05$ level.

Delayed hypersensitivity reactions

Fig. 2 illustrates the response of sensitized rats to challenge with PPD. Rats exposed to lead pre- and post-natally either at 25 ppm or 50 ppm were suppressed in their ability to mount delayed hypersensitivity reactions to PPD ($P<0{\cdot}10$).

TABLE 1. Selected weight effect in lead exposed rats

	Dosage group*		
	0 ppm lead	25 ppm lead	50 ppm lead
Males			
Body wt (gms)	113.33 ± 20·63†	117·99 ± 22·84	113·48 ± 20·26
Thymus wt (gms)	0.510 ± 0.079	0·405 ± 0·047‡§	0·446 ± 0·078¶
Thymus /body wt ($X10^{-3}$)	4·61 ± 1.02	3·56 ± 0·86**	3·99 ± 0·74
Spleen wt (gms)	0·423 ± 0·127	0·420 ± 0.090	0·562 ± 0·194¶
Spleen/body wt ($X\ 10^{-3}$)	3·71 ± 0·65	3·55 ± 0.20	4·84 ± 1·01§
Females			
Body wt (gms)	103·38 ± 18·46	104·99 ± 24·22	92·79 ± 17·47
Thymus wt (gms)	0·529 ± 0·066	0·422 ± 0·078§	0·416 ± 0·051§
Thymus/body wt ($X10^{-3}$)	5·19 ± 0·65	4·19 ± 0·98§	4·56 ± 0·58¶
Spleen wt (gms)	0·441 ± 0·138	0·388 ± 0·124	0·391 ± 0·131
Spleen/body wt ($X10^{-3}$)	4·21 ± 0.80	3·64 ± 0·51	4·14 ± 0·75

* At least ten males and ten females were observed in each dosage group.

† The results are recorded as the mean ± s.d.

‡All levels of statistical significance are for indicated dosage group versus control group.

§$P< 0.01$

¶$P< 0{\cdot}10$

**$P< 0.05$

TABLE 2. Proliferative response of splenic and thymic lymphocyte cultures to PHA and Con A following *in vivo* exposure to lead acetate

Stimulus	Treatment	Thymidine incorporation* Unstimulated cultures (C) ±s.d. dpm	Stimulated cultures (E) ±s.d. dpm	Stimulation index (E/C) ±s.d.
Splenic lymphocytes				
PHA	None†	302.2±140·3	2546·0±845·8	8·8±2l3
	25 ppm Pb‡	301·8±139·9	1139·9±704·3§	3·8±1·6§
	50 ppm Pb‡	288·3±82·4	1251·4±823·6§	4·0±0·9§
Con A	None		7144·5±7205·8	19·6±13·0
	25 ppm Pb		2185·9±1071·7¶	7·3±2·7¶
	50 ppm Pb		233·84±1844·6¶	8·4±8·6¶
Thymic lymphocytes				
PHA	None	239·5±77·7	905·1±427·3	3·9±1·8
	25 ppm Pb	290·2±113·8	593·4±310·2§	2·1±0·9§
	50 ppm Pb	220·4±43·7	684·7±257·5	3·2±1·2
Con A	None		12970·5±5765.6	54·5±24·8
	25 ppm Pb		15169·6±6919·2	51·9±20·4
	50 ppm Pb		10375·0±4297·0	43·7±15·8

* The values given represent the mean dpm±standard deviation.
† There were ten animals in the non-treated or control group.
‡ There were nine animals in both of the lead-exposed groups.
§ Indicates a significant difference from control at the $P< 0·05$ level.
¶ Indicates a significant difference from control at the $P< 0·01$ level.

DISCUSSION

Because of its many useful properties, lead has been mined and worked by man since antiquity. Lead ingestion results in toxicity, and lead-related diseases have long been recognized (Chisolm, 1971). In recent years, it has become clear that low level exposure to toxic chemicals may result in subtle effects without evidence of overt toxicity. Examples of this include behavioural effects of low-level pre-natal exposure of mice to methyl mercury (Spyker, 1975), and reduced resistance to bacterial infection by low-level exposure of mice to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (Thigpen, *et al.*, 1975) or lead (Hemphill *et al.*, 1971). The studies reported here were undertaken to determine if chronic low-level pre- and post-natal lead exposure affects immune function. The exposure regimen employed in this study resulted in fetal and neonatal lead exposure as lead has been shown to cross placenta in small quantities (Kimmel *et al.*, 1978) and to be readily excreted in milk (Pentschew & Garrow, 1966).

Offspring from females dosed with 25 and 50 ppm of lead acetate in their drinking water showed no inhibition of growth exhibited by body weight gains. In addition, the animals showed no other overt signs of toxicity. When selected lymphoid organs were examined, it was found that actual thymic weights were decreased in males and females of both dosage groups (25 and 50 ppm). Relative thymic weights were also reduced in all groups. This observed reduction in actual and relative thymic weight is similar to findings in studies with other toxic compounds (Faith & Moore, 1977, Gupta *et al.*, 1973; Harris *et al.*, 1973; Seinen & Williams, 1976; Vos *et al.*, 1973, Vos & Moore, 1974). In male offspring of the 50 ppm dosage group, there was also an increase in splenic weight and relative spleen weight, which may be due to increased extramedullary haematopoiesis occurring in the spleens of these animals. The reason for increased splenic haematopoiesis in these animals was not clear but did not appear to be due to lead-induced anaemia.

In vitro culture techniques were utilized to assess the response of thymic and splenic lymphocytes to

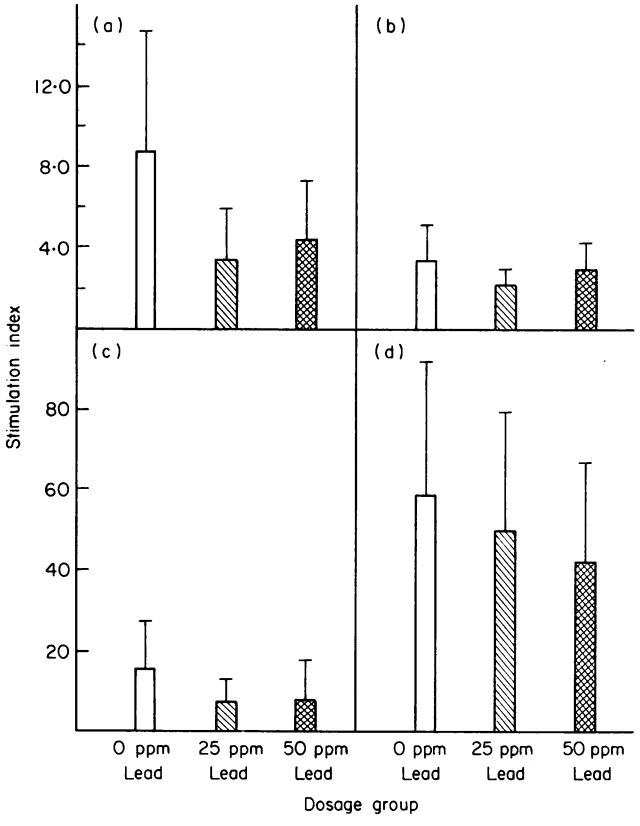

FIG. 1. Effect of pre- and post-natal lead exposure on the responsiveness of thymic and splenic lymphocytes to mitogen stimulation. The response of splenic lymphocytes to PHA, (a) spleen and (b) thymus, was suppressed significantly in both lead dosage groups ($P< 0·05$) The response of thymic lymphocytes to PHA was significantly suppressed in the 25 ppm dosage group ($P< 0·05$). The response of splenic lymphocytes to Con A, (c) spleen and (d) thymus, was significantly suppressed in both dosage groups ($P< 0·01$). The response of thymic lymphocytes was not significantly suppressed in either dosage group, but the overall dose-response tend was significant at the $P< 0·05$ level. The results are reported as mean $\pm$ s.d.

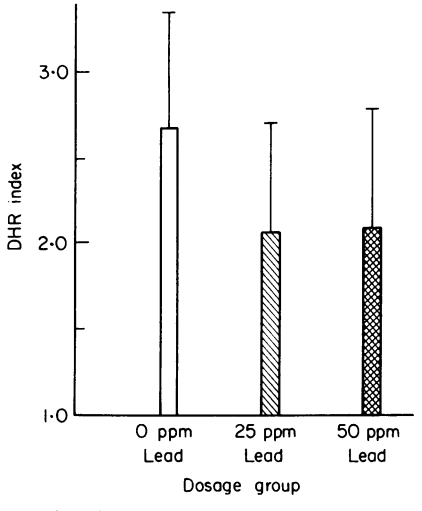

FIG. 2. Effect of pre- and post-natal lead exposure on delayed hypersensitivity responsiveness. Both lead exposure groups were significantly syppressed in their delayed hypersensitivity responses ($P< 0·10$). The results are reported as mean $\pm$ s.d.

the T-cell mitogens PHA and Con A. Thymic lymphocytes tended to be somewhat reduced in their response to PHA with the reduction being significant in animals from the 25 ppm lead dosage group; however, the difference between the two lead dosage groups was not significant. The response of thymic cells to Con A tended to be reduced in both the 25 ppm and 50 ppm dosage groups but this reduction in response was not statistically significant. In contrast the response of splenic lymphocytes from animals of both dosage groups was significantly reduced to either PHA or Con A.

Two possible explanations for the observed differences in mitogen responsiveness of thymic and splenic lymphocytes are: (1) an alteration in the normal circulatory or homing patterns of lymphocytes in lead-exposed animals; or (2) lymphocyte functional properties are affected by lead exposure. It is impossible, from the results of these studies, to say whether one or both of these possibilities is correct. If lymphocyte function is altered by lead exposure it would appear that the function of peripheral lymphocytes is affected to a greater degree than lymphocytes in central organs such as thymus. Suppression of lymphocyte responsiveness to mitogen stimulation was greater in spleen (a peripheral lymphoid organ) than in thymus (a central lymphoid organ), in fact responsiveness of thymic lymphocytes to Con A was not significantly affected in either dosage group.

An alteration in the normal circulatory or homing patterns of T cells from thymus to spleen, whereby mitogen responsive T cells did not find their way to spleen in normal numbers, would result in the same observation (greater suppression of mitogen responsiveness in spleen than in thymus). It is well documented that lymphocytes have specific traffic or ciculatory patterns (Cantor & Weissman, 1976; Guy-Grand *et al.*, 1971; Linna, Back & Hemmingsson, 1971; Niewenhuis & Ford, 1976; Weissman, 1967). A number of studies have shown that treatment of lymphocytes, *in vitro* or *in vivo*, with various agents will interfere with normal circulatory patterns: (1) *in vitro* treatment of lymphocytes with the enzymes glucosidase, neuraminidase, and trypsin alters homing patterns of the treated cells (Gesner & Ginsburg, 1964; Morse & Asofsky, 1974; van den Broek, 1971; Woodruff & Gesner, 1967 & 1968); (2) *in vivo* treatments with anti-lymphocyte serum have been shown to alter homing patterns of murine lymphocytes (Morse & Asofsky, 1974); (3) injections of cortisone have been shown to alter lymphocyte traffic patterns in rabbits (van den Broek, 1971); and (4) lymphocytes from animals exposed developmentally to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) have altered homing patterns (Faith & Luster, 1978). It is conceivable that lead exposure may alter lymphocyte traffic patterns either directly or indirectly through an adrenal hormone mechanism.

In addition to affecting lymphocyte mitogen responsiveness, pre- and post-natal lead exposure suppressed delayed hypersensitivity responsiveness (DHR). While this suppression was only statistically significant at $P<0{\cdot}10$, one would expect that it would alter the manner in which a lead-exposed animal would respond to infectious challenge, as was observed earlier (Grant *et al.*, 1978). It is possible that interference with macrophage function could result in suppression of DHR seen in these experiments.

TABLE 3. Blood lead levels in rats following chronic developmental lead exposure

Dosage group*	Blood lead level (μg/100b)†
0 ppm	5·5± 1·0‡
25 ppm	29·3± 14·1
50 ppm	52·8± 10·0

* Animals exposed pre- and post-natally to lead acetate in the drinking water.

† Blood lead levels were determined by atomic adsorption spectrometry as described previously (Kimmel *et al*, 1978).

‡ Results reported as mean blood lead level± standard error.

However, this appears unlikely since it has been reported that lead exposure in mice as high as 1300 ppm in the drinking water resulted in slightly enhanced machrophage function instead of suppression of machrophage function (Koller & Roan, 1977). This finding is in contrast to the findings of Trejo *et al.*, (1972) but the lead dosages were given by injection in their study instead of *per os*.

The results reported here have shown chronic low level pre- and post-natal lead exposure to be immunosuppressive. Additional significance is lent to these results when viewed in light of the blood lead levels obtained with the lead exposure regimens employed in this and other studies (Grant *et al.*, 1978; Kimmel *et al.*, 1978). Blood lead determinations indicate that the levels seen with the 25 and 50 ppm doses employed here (see Table 3) are comparable to blood lead levels found in many children in urban areas (Caprio, Margulis & Joselow, 1974; Committee on Biologic Effects of Atmospheric Pollutants, 1971). For example, 34·9% of 5226 children between 1 and 5 years old tested in Newark, New Jersey had blood lead levels greater than 50 μg/100 ml (Caprio, Margulis & Joselow, 1974). In light of these facts, low level lead exposure may be more undesirable than previously thought, since it is conceivable that it leads to immunosuppression in humans as well as in rats as demonstrated here.

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REFERENCES

Cantor, H. & Weissman, I. (1976) Development and function of subpopulations of thymocytes and T lymphocytes. *Prog. Allergy* **20**, 1.

Caprio, R.J., Margulis, H.L. & Joselow, M.M. (1974) Lead adsorption in children and its relationship to urban traffic densities. *Arch. Environ. Health*, **28**, 195.

Chislom, J.J., Jr. (1971) Lead posoning. *Sci. Amer.* **224**, No. 2, 15.

Committee on Biologic Effects of Atmospheric Pollutants, Division of Medical Science, National Research Council. (1971) *Lead Airborne lead in perspective*, p. 71. National Academy of Sciences, Washington, D.C.

Faith, R.E. & Luster, M.I. (1978) Investigations on the effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on Parameters of Various Immune Functions. *Ann. N.Y. Acad. Sci.* (In press.)

Faith, R.E. & Moore, J.A. (1977) Impairment of thymus dependent immune functions by exposure of the developing immune system to 2, 3, 7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *J. Toxicol. Environ. Health*, **3**, 451.

Friend, M. & Trainer, D.O. (1970) Polychorinated biphenyl: Interaction with duck hepatitis virus. *Science*, **170**, 1314.

Gesner, B.M. & Ginsburg, V. (1964) Effect of glycosidases on the fate of transfused lymphocytes. *Proc. nat. Acad. Sci.* (*Wash.*) **52**, 750.

Grant, L.D., Kimmel, C.A., West, G.L., Matinez-Vargas, C.M. & Howard, J.L. (1978) Chronic low-level lead toxicity in the rat. II. Effects on postnatal physical and behavioral development. *Toxicol. Appl. Pharmacol.* (Submitted.)

Gupta, B.N., Vos, J.G., Moore, J.A., Zinkl, J.G. & Bullock, B.C. (1973) Pathologic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in laboratory animals. *Environ. Health Perspect.* **5**, 125.

Guy-Grand, D., Griscelli, C., Vassalli, P. & McCluskey, R. (1971) Migration, recirculation and transformation of large dividing lymph node cells in syngenic recipient rats following intravenous injection. *Adv. exp. Med. Biol.* **12**, 641.

Harris, M.W., Moore, J.A. Vos, J.G. & Gupta, B.N. (1973) General biological effects of TCDD in laboratory animals. *Environ. Health Perspect.* **5**, 101.

Hemphill, F.E., Kaeberle, M.L. & Buck, W.B. (1971) Lead suppression of mouse resistance to *Salmonella typhimurium*. *Science*, **172**, 1031.

Hollander, M. & Wolfe, D.A. (1973) *Nonparametric Statistical Methods*. Hohn Wiley & Sons, New York.

Jones, R.H., Williams, R.L. & Jones, A.M. (1971) Effects of heavy metals on the immune response. Preliminary findings for cadmium in rats. *Proc. Soc. exp. Biol.* (*N.Y.*), **137**, 1231.

Kimmel, C.S., Grant, L.D., Sloan, C.S. & Gladen, B.C. (1978) Chronic low-level lead toxicity in the rat. I. Maternal toxicity and prenatal effects. *Toxicol. Appl. Pharmacol.* (Submitted.)

Koller, L.D. (1973) Immunosuppression produced by lead, cadmium and mercury. *Am. J. Vet. Res.* **34**, 1457.

Koller, L.D., Exon, J.H. & Roan, J.G. (1976) Humoral antibody response in mice after single dose exposure to lead or cadmium. *Proc. Soc. exp. Biol.* (*N.Y.*), **151**, 399.

Koller, L.D. & Kovacic, S. (1974) Decreased antibody formation in mice exposed to lead. *Nature* (*Lond.*), **250**, 148.

Koller, L.D. & Roan, J.G. (1977) Effects of lead and cadmium on mouse peritoneal macrophages. *J. Reticuloendothel. Soc.* **21**, 7.

Koller, L.D. & Thigpen, J.E. (1973) Reduction of antibody to pseudorabies virus in polychlorinated biphenyl-exposed rabbits. *Am. J. Vet. Res.* **34**, 1605.

Lefford, M.J. (1974) The measurement of tuberculin hypersensitivity in rats. *Int. Arch. Allergy appl. Immunol.* **47**, 570.

Linna, T.J. Back, R. & Hemmingsson, E. (1971) Lymphoid cell migration. *Adv. exp. Med. Biol.* **12**, 149.

McLachlan, J.A. & Dixon, R.L. (1976) Transplacental toxicity of diethylstilbesterol: A special problem in safety evaluation. *Advances in Modern Toxicology* (ed. by M. Mehlman, R. E. Shapiro and H. Blumenthal), p. 423. Hemisphere Publishing Corp., New York.

Morse, H.C., III & Asofsky, R. (1974) *In vivo* effects of anti-thymocyte serum on the homing patterns and graft-versus-host reactivity of murine splenic lymphocytes. *Cell. Immunol.* **11**, 19.

NIEUWENHUIS, P. & FORD, W.L. (1976) Comparative migration of B- and T-lymphocytes in the rat spleen and lymph nodes. *Cell. Immunol.* **23**, 254.

OHI, G., FUKUDA, M., SITO, H. & YAGYU, H. (1976). Effect of methyl-mercury on humoral immune response in mice under conditions simulated to practical situations. *Bull. Environ. Contam. Toxicol.* **15**, 175.

PENTSCHEW, A. and GARROW, F. (1966) Lead encephalomyelopathy of the suckling rats and its implications on the porphyrinopathic nervous diseases. With special reference to the permeability disorders of the nervous system. *Acta Neuropathol.* **6**, 266.

SEINEN, W. & WILLIAMS, M. I. (1976) Toxicity of organotin compounds. I. Atrophy of thymus and thymus-dependent lymphoid tissue in rats fed di-n-octyltindichloride. *Toxicol. Appl. Pharmacol.* **35**, 63.

SNEDECOR, G.W. & COCHRAN, W.G. (1967) *Statistical Methods.* Iowa State University Press, Ames, Iowa.

SPYKER, J.M. (1975) Assessing the impact of low level chemicals on development: Behavioral and latent effects. *Fed. Proc.* **34**, 1835.

STREET, J.C. & SHARMA, R.P. (1975) Alteration of induced cellular and humoral immune responses by pesticides and chemicals of environmental concern: Quantitative studies of immunosuppression by DDT, Aroclor 1254, carbaryl, carbafuran, and methylparathion. *Toxicol. Appl. Pharmacol.* **32**, 587.

THIGPEN, J.E., FAITH, R.E., MCCONNELL, E.E. & MOORE, J.A. (1975) Increased susceptibility to bacterial infection as a sequela of exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Infect. Immunity*, **12**, 1319.

TREJO, R.A., DI LUZIO, N.R., LOOSE, L.D. & HOOFMAN, E. (1972) Reticuloendothelial and hepatic functional alterations following lead acetate administration. *Exp. Mol. Pathol.* **17**, 145.

VAN DEN BROCK, A.A. (1971) Immunological effects of cortisone caused by interference with lymphoid cell traffic. *Adv. Exp. Med. Biol.* **12**, 667.

VERSCHUUREN, H.B., RUITENBERG, E.J., PERTOOM, F., HELLEMAN, P.W. & VAN ESCH, G.J. (1970) Influence of triphenyltin acetate on lymphatic tissue and immune responses in guinea pigs. *Toxicol. Pharmacol.* **16**, 400.

VOS, J.G. & DE ROIJ, T. (1972) Immunosuppressive activity of a polychlorinated biphenyl preparation on the humoral immune response in guinea pigs. *Toxicol. Appl. Pharmacol.* **21**, 549 .

VOS, J.G. & VAN GENDEREN, H. (1973) Toxicological aspects of immunosuppression. *Pesticides and the Environment: A Continuing Controversy* (ed. by W. B. Deichmann), p. 527. Symposia Specialists, Miami.

VOS, J.G., MOORE, J.A. & ZINKL, J.G. (1973) Effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on the immune system of laboratory animals. *Environ. Health Perspect.* **5**, 149.

VOS, J.G. & MOORE, J.A. (1974) Suppression of cellular immunity in rats and mice by maternal treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Int. Arch. Allergy appl. Immunol.* **47**, 777.

WEISSMAN, I.L. (1967) Thymus cell migration *J. exp. Med.* **126**, 291.

WOODRUFF, J.J. & GESNER, B.M. (1967) The effect of. neuraminidase on the fate of transfused lymphocytes *J. clin. Invest.* **46**, 1134,

WOODRUFF, J.J. & GESNER, B.M. (1968) Lymphocytes: Circulation altered by trypsin. *Science*, **161**, 176.