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MALE REPRODUCTIVE EFFECTS OF LEAD, INCLUDING SPECIES EXTRAPOLATION FOR THE RABBIT MODEL

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Abstract — The effects of elevated blood lead on semen quality were evaluated in the rabbit model and compared to published effects in humans. Mature, male rabbits were given lead acetate by subcutaneous injection in the dose range of 0 to 3.85 mg/kg on a Monday-Wednesday-Friday basis. In each of eight treatment groups, a dosing regimen was developed to produce blood lead levels of 0, 20, 40, 50, 70, 80, 90, and 110 μg/dL. A 5-week pre-exposure period was followed by a 15-week exposure testing period allowing for response through six cycles of the seminiferous epithelium. Semen analyses revealed that increased blood lead levels were associated with adverse changes in the sperm count, ejaculate volume, percent motile sperm, swimming velocities, and morphology. Hormonal responses were minimal. Testicular pathology revealed a dose-dependent inhibition of spermiation. For six measures of semen quality, threshold estimates ranged from 16 to 24 μg/dL. Using the species extrapolation factor derived in this study, a rabbit dose would have to be divided by 1.56 to obtain the equivalent human dose for an equal percentage decrease in sperm concentration; however, rabbits are 3.75 more sensitive in terms of absolute decrease in sperm count for a given blood lead level. Published by Elsevier Science Inc.

Key Words: lead; rabbit model; semen quality; blood lead.

INTRODUCTION

The scientific process for the evaluation of reproductive risk from drugs and chemicals includes extrapolation from data obtained in animal models (1). The differences between species, however, may limit the appropriate and accurate application of toxic effects in human risk assessment. Important criteria for an animal model of reproduction in humans include the existence of documented, relatable physiology of the species and the *ability to analyze ejaculated semen*. These aspects permit studies in which toxic effects can be related to humans because the focus of medical evaluation of the male is on analysis of ejaculated whole semen, not just sperm cells.

The most common laboratory species, the rat, does have well-documented reproductive physiology. However, it does not readily permit analysis of ejaculated semen for longitudinal effects. A review of animal models by Amann (2) has emphasized that rabbits are the smallest and least expensive laboratory animals in which serial semen samples can easily be obtained for morphologic, biochemical, and fertility evaluation.

The purpose of this study was to evaluate the rabbit model for assessment of male reproductive responses to a model chemical, lead. A qualitative and quantitative comparison between the experimental responses in the rabbit and published epidemiologic responses in humans for lead would indicate the usefulness of the model for predicting human spermatotoxic responses to lead and other similarly acting chemical toxicants. Lead as a model reproductive toxicant offers a uniquely important perspective in the quantitative species comparison because human blood lead levels are generally reported along with the observed health effect. By focusing on differences as they relate to blood lead levels rather than environmental exposures, a far more efficient experimental approach can be used. The relationship between exposure and blood lead levels has been extensively studied. Therefore, the results of the present study are not route dependent.

Experimental evaluation of the effects of lead on reproductive function in rabbits has been reported for many years. In 1914, Cole and Bachuber (3) published data showing that offspring from Dutch male rabbits that

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were dosed with lead acetate showed increased mortality and decreased weights. In a more recently reported animal study using rabbits, Willems et al. (4) attempted to determine the effects of lead acetate administered by subcutaneous (s.c.) injection, but they failed to show effects on sperm morphology, sister chromatid exchanges, and on formation of micronuclei. The exposure was for 14 weeks; however, there were only five male rabbits in each treatment, and blood lead levels at the end of the Willems study were 6.6 μg/dL, 53.2 μg/dL, and 61 μg/dL. Sperm were obtained at sacrifice only. The design did not permit analysis of ejaculated semen, and the morphologic assessment reflected cells undergoing maturation at lower blood lead levels than those measured at the end of the study.

As a result of advantages offered by rabbits for reproductive toxicology, the National Toxicology Program agencies, National Institute of Environmental Health Sciences and National Institute for Occupational Safety and Health, have focused on research directed at statistical considerations of study designs (5) and a comparative evaluation of ethylene dibromide (6). The comparative study demonstrated that although the rabbit was not as sensitive as humans, four of seven semen parameters that were altered in the human study (6) were significantly changed in rabbits. An important consideration was that the ethylene dibromide dosing in the rabbit study was only for 5 d. The conclusion was that the rabbit appears to be an important model for evaluating male reproductive toxicity in humans and warrants further evaluation and "validating any animal species as a general model for human response requires evaluation of many different classes of chemicals" (6).

MATERIALS AND METHODS

Experimental design and schedule

The design described by Williams et al. (5) in which a 5-week pre-exposure (baseline testing) period is followed by a 10-week (exposure testing) period (Design 2) was used with an extension in the exposure testing period (15 weeks) to allow for the anticipated lead uptake (estimated to require 4 to 5 weeks to reach the target levels). This design allows for studying effects on semen quality through six cycles of the seminiferous epithelium (2). Male rabbits were sampled weekly through the entire 20-week period, thus permitting a comparison between baseline levels and the effects during the period of lead intoxication.

Two phases of experimentation were performed. The high-dose phase was conducted first, followed by the low-dose phase 9 months later. The high-dose phase tested five treatments with target blood lead concentrations of 0.0 μg/dL, 50 μg/dL, 70 μg/dL, 90 μg/dL, and 110 μg/dL. The low-dose phase used four treatments with target blood lead concentrations of 0.0 μg/dL, 20 μg/dL, 40 μg/dL, and 80 μg/dL. Each treatment group contained 7 animals in the high-dose phase and 15 animals in the later low-dose phase to increase statistical power. Except for the group size, the procedures used in the high-dose and low-dose phases were identical. All rabbits were ungrouped for the first 5 weeks of baseline testing to allow for culling of abnormally performing individuals. Following the 5 weeks of pretreatment baseline testing, rabbits that were deemed abnormal (azoospermic, urinated during ejaculation) were culled, and all remaining rabbits were randomly allocated to the treatments. Each treatment was coded such that individual animals were tested "blind."

Sexually mature male and female Dutch Belted rabbits approximately 6 to 7 months of age and approximately 2-kg body weight were used. The rabbits were obtained from Hazelton Research Products (Aberdeen, MD). They were maintained in an AAALAC-approved animal facility, kept in air-conditioned rooms (19 to 21°C), and caged as required by the current USDA guidelines. All rabbits were individually marked for identification and were observed daily. They received certified High Fiber Purina (#5325) rabbit chow (limited to 125 g/d), water ad libitum, and they were housed in stainless steel cages (25 × 20 × 16 in). A 12-h light/dark cycle was maintained. Trace analyses, including lead and pesticides, were conducted on food and water (analysis showed <0.5 ppm lead in feed and <0.003 ml/L in the water).

Administration of toxicant

Lead (Certified ACS Quality) was injected s.c. as a lead acetate solution in sterile 5% dextrose at doses of 0 to 3.85 mg/kg to achieve and maintain the target blood lead levels. This route circumvents the dietary effects on gastrointestinal absorption and allows greater control of blood lead concentrations. Control rabbits were given the 5% dextrose solution alone on a Monday-Wednesday-Friday (M-W-F) basis. Lead treated rabbits were given lead acetate by s.c. injection following the baseline testing as described in Table 1. Initial loading doses were used to increase lead uptake and adjustments (based on the percent of target blood level from the most recent analysis) were made during the plateau phase to maintain the targeted blood lead levels. The loading doses were administered on a M-W-F dosing schedule for the Study Weeks of 6 through 11. Maintenance dosing was followed through the last 10 weeks (Study Weeks 11 through 20). The doses were calculated from the data published by Falk and Zwennis (7) in which rabbits were dosed with lead acetate at 0.2 and 1.2 mg PbAc/kg on a

Table 1. Administered dosing for the target blood lead treatments

Treatments*	Loading doses, M-W-F (Weeks 6–10)	Maintenance doses, M-W-F (Weeks 11–20)
1a. Controls	0.00 mg/kg	0.00 mg/kg
2. 50 μg/dL	0.50 mg/kg	0.36 mg/kg
3. 70 μg/dL	1.30 mg/kg	0.60 mg/kg
4. 90 μg/dL	2.55 mg/kg	1.30 mg/kg
5. 110 μg/dL	3.85 mg/kg	2.00 mg/kg
1b. Controls	0.00 mg/kg	0.00 mg/kg
6. 20 μg/dL	0.20 mg/kg	0.13 mg/kg
7. 40 μg/dL	0.35 mg/kg	0.26 mg/kg
8. 80 μg/dL	1.30 mg/kg	0.60 mg/kg

*All treatment groups were tested without lead administration during Weeks 1 through 5.
For high-dose phase treatments, $n = 7$; for low-dose phase treatments, $n = 15$.
M-W-F, Monday-Wednesday-Friday.

M-W-F basis to achieve blood lead levels of 40 and 90 μg/dL, respectively.

Collection of blood and semen

Blood was collected by jugular phlebotomy. Five mL of whole blood was required for analysis of blood lead and serum hormones. Blood samples were taken every week for 20 weeks from each male rabbit. Semen was collected from sexually prepared males (three false mounts) on a regular weekly schedule for 20 weeks to maximize sperm output as generally described by Williams et al. (5). Each male ejaculated four times with a 20- to 30-min rest between collections. The collection periods were scheduled once weekly. The procedure for each collection involved introducing the female (teaser doe) into the male's cage. Following three attempted mounts, the female was held with an artificial vagina such that the ejaculated semen was collected in a small centrifuge tube.

Analysis of blood

The collected whole blood was analyzed for lead by graphite furnace atomic absorption spectroscopy with Zeeman background correction (GFAAS-Z). A Perkin-Elmer Zeeman/3030 equipped with an AS-60 auto computer-controlled sampler was used. The limit of detection was calculated to be 1.1 μg/dL.

Analysis of semen

Semen was analyzed for sperm concentration (number of cells per mL and total number of cells per ejaculate), motility (computer-assisted sperm analysis), morphometry, morphology, and volume. Sperm concentration, total sperm count, and ejaculate volume were measured from the four pooled ejaculates. The first ejaculate was analyzed for sperm count and motion analysis. All sperm were counted using a Makler chamber, and semen volumes were measured using a graduated pipette. The ejaculates were maintained at 37°C for no more than 10 min until dilution in Ham's F-10 tissue culture medium, then examined by microscopy and videotaped for computer analysis of the sperm motion parameters, which included curvilinear velocity (VCL), straight-line velocity (VSL), linearity, amplitude of lateral head displacement, and average path velocity (VAP). Percent motile sperm was determined from the summary data files. The motion analysis parameters were determined using Celltrack VP110 by Motion Analysis. Morphology was evaluated following staining of air-dried sperm with trypan blue, naphthol yellow, and eosin-Y. Two hundred sperm cells were evaluated for head shape and size, acrosome presence, and tail abnormalities. Morphometry was performed on 100 sperm from the same air-dried and stained samples. Area, perimeter, width, and length were determined for each sample using the Image Processor (model 1500) by Image Technology.

Hormone analyses

Serum was assayed for testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) by radioimmunoassay as generally described by Foote et al. (8). All analyses were performed on contract by the Oregon Regional Primate Research Center. All assays were performed in duplicate, the testosterone assays were performed with two different aliquots at 20 and 50 μL to make sure that each sample was monitored on different points of the curve. The overall intra- and interassay coefficients of variation (CV) for testosterone were 5.6 and 10%, respectively. The FSH assays were all within the 90% binding limit, and the intra-assay CV was 10.9%. For LH, values were obtained for both the 90% and the 95% binding limits to provide maximum sensitivity because rabbit values are so low. The intra-assay CV was 17.4%. The 95% binding limit data were analyzed. LH values below 0.1 ng/mL were not reported.

Species extrapolation

A regression of sperm count and blood lead levels for workers was developed and compared to that found in rabbits. Five studies reporting blood lead levels and sperm concentration of lead exposed workers were identified that presented comparable data (9–13). To determine the relationship of blood lead to sperm count over a wide range of exposures, a least square regression on the means was performed. The dose necessary to produce a given effect (e.g., 10%) in rabbits was divided by the dose needed to produce an equivalent effect in humans as proposed by Meistrich (1). Additionally, log transformed doses for both species were plotted to determine if constant relationships existed.

$$\text{Interspecies Extrapolation Factor (IEF)} = \frac{\text{Dose necessary to produce a given change in sperm measure in animal}}{\text{Dose necessary to produce an equivalent change in humans}}$$

Histopathology

At termination animals were weighed then sacrificed by overdose with a concentrated solution of sodium pentobarbital (approximately 75 mg/kg) via the marginal ear vein. The abdominal cavity was opened via a midline incision. One testis was perfused with formalin and the other was not. The accessory sex organs were dissected from the body as a single unit, weighed, and immersed in 10% buffered formalin. The rabbits were examined for gross evidence of intercurrent disease. Histopathology was performed on organs in which gross lesions were identified. The formalin-perfused testis was processed in glycol methacrylate resin. Sections approximately 2-μm thick were cut and stained with periodic acid Schiff's reagent and counterstained with hematoxylin (PAS/H). Epididymal tissue was processed via routine methods for paraffin embedding, sectioned to 6 μm, and stained with PAS/H. Testicular sections were examined histopathologically for evidence of altered spermatogenesis.

Statistical analysis

Dose–response modeling. Data analysis focused on the variable responses to increasing blood lead levels rather than tests for differences between treatments. Linear regression, nonlinear regression, and multivariate correlation analyses were applied to the data. The analyses were performed using SAS JMP and SAS/STAT 6.10 on a Pentium personal computer. The linear regression analyses were performed by the method of ordinary least squares and the nonlinear regression was performed using iterative algorithms: the Gauss-Newton method as described in Bard (14) or the Marquardt method (15). For each response variable, the dose–response model was fit to a set of data pairs (one pair for each animal) consisting of the measured blood level of lead and the measured response averaged for that animal over the last 5 weeks of the study. Each variable was tested graphically (using a normal probability plot) and/or numerically for normality; the variables representing proportions deviated from a normal distribution (as would be expected for proportion data), but all other variables appeared normally distributed. The models used have a single overall fitted intercept parameter and other parameters that are the same for all animals. An intercept term was included in all models to estimate the response level at zero administered dose (background level). A number of nonlinear models were considered, including a logistic curve, Weibull-type models, the power law, and Hill equation model. It was found that the power law model was best (the nonlinear iterative fit algorithm could always be made to converge for every data set unlike for the other models and it gave the same or better fit in terms of SSE, visual, or both). The nonlinear model used for the nonlinear regression is the power law, given as: $y = b + v\,x^n$, where y is the response, x is the dose, b is an intercept parameter, v is a scaling parameter, and n is the exponent parameter. When n is 1, the model is simply a linear model. Hence, whenever the estimate of the parameter n is found to be significantly different than 1, the estimated model would in fact be nonlinear. The size of the exponent would indicate the degree of nonlinearity of the model.

Threshold estimation. In the literature on toxicologic dose-response characterization, the dose–response behavior of a toxicant for which the fitted exponent parameter is found to be 1.5 or greater is considered to exhibit a threshold-like behavior (16,17). In this case, the dose–response curve displays small responses for low doses then takes a sharp bend upwards for the larger doses. Hence, the toxicant behaves as though there is a threshold dose that must be crossed before appreciable responses are demonstrated. In these analyses, if the linear regression showed that the relationship between blood lead level and the response variable was significant, then an attempt was made to fit the nonlinear model. If the nonlinear model was significant (at $P = 0.05$) that is, if the exponent parameter in the nonlinear model is larger than 1.5, then the exponent parameter can be examined to determine whether there is threshold-like behavior exhibited by the nonlinear fit. The estimate of approximate threshold was found by fitting two line segments to the data; the first line is fixed at zero slope and intercept equal to the mean of the control group response with the second line fitted to the remainder of the data by least squares. This "join point" is a function of the slope and intercept of the two lines, so it depends on the parameters of the best fitting second line in combination with the first. The dose coordinates of the join point is the estimate of the threshold. Essentially, it is the smallest dose at which a nonzero slope line becomes a better fit than just a flat line fixed at the background response level.

RESULTS

Blood lead levels

Figure 1 presents the blood lead levels over the 20 weeks of the study. The blood lead levels remained low

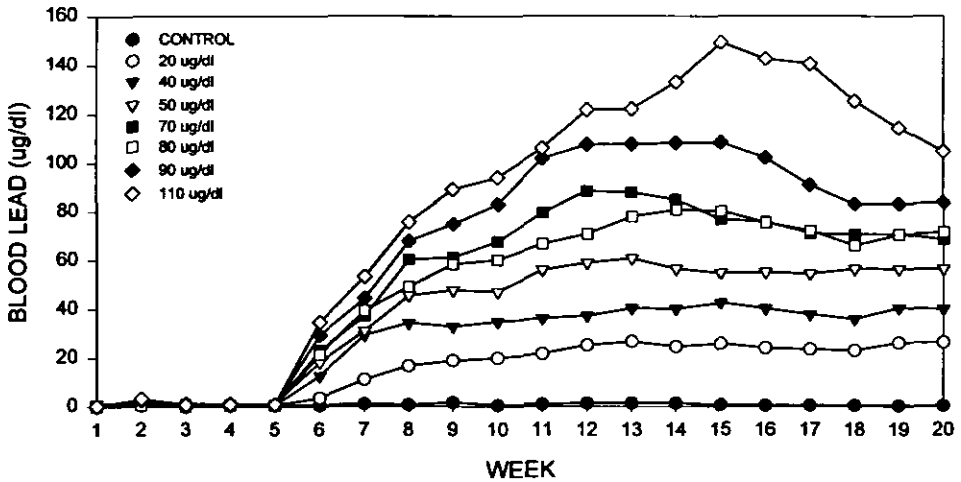

Fig. 1. Blood lead values as weekly treatment means throughout the study.

at the limit of detection (1.1 μg/dL) for the first 5 weeks for all treatments and throughout the 20 weeks for the controls. All values were plotted, including those below the limit of detection. Excursions above the target levels occurred in the high dose treatments. The average blood lead levels during the last 5 weeks were 0.47 μg/dL for the controls, 24.8 μg/dL for the 20-μg/dL target group, 39.6 μg/dL for the 40-μg/dL target group, 55.7 μg/dL for the 50-μg/dL target group, 72.2 μg/dL for the 70-μg/dL target group, 76 μg/dL for the 80-μg/dL group, 91.9 μg/dL for the 90-μg/dL target group, and 129.5 μg/dL for the 110-μg/dL target group. Clinically, all rabbits appeared in good health throughout the study. Rabbit weights were not significantly affected by exposure to lead ($P = 0.5810$).

Semen analysis

In general, sperm morphology and morphometric responses showed the strongest relationships to blood lead levels; sperm motility, sperm concentration, and volume showed moderate relationships; and hormonal effects showed weak or no relationship. The analysis showed that as blood lead increased, the proportion of cells with normal sperm morphology decreased, the proportion of motile cells decreased, sperm velocities decreased, sperm count and ejaculate volume decreased, and cell viability decreased. Also, for most of the variables that had a significant relationship with blood lead levels, this relationship was a nonlinear one and for some cases, a threshold-like dose–response relationship was apparent.

Sperm morphology response variables

The strongest and most significant relationships between blood lead level and response were observed among the variables measuring the proportions of sperm morphology deviations (Table 2). The variables "percent large heads" and "percent tapered heads" did not have exponent parameters that were significantly larger than 1.0 ($n = 1.094$ and $n = 1.255$, respectively); hence, these response variables appeared to have an approximately linear response relationship with blood lead levels. However, all the other morphology variables had fitted exponent parameters larger than 1.5, with all of these statistically significant to the 0.05 level or better. Figures 2 through 5 present the regression model projections for selected morphologic variables versus blood lead levels.

Sperm morphometric response variables

The group of variables with the next strongest relationship to blood lead levels was the morphometric group (Table 3). The variables are based on measurements of the sperm head and provide an objective computerized measurement of the sperm head. These data support the morphologic analyses conducted by visual discriminate analysis. Figures 6 through 8 present projections for sperm area, length, and perimeter versus blood lead levels.

Sperm motility response variables

Measures of the sperm motility showed a significant relationship to blood lead level (Table 4), but none showed a particularly strong relationship, according to R^2 values. The strongest effects observed are percent motile cells (PMOT) and straight line velocity (VSL). The variable amplitude of lateral head displacement (ALH) showed a weak but significant effect, but curvilinear velocity (VCL) and average path velocity (VAP) were not significant. Because many of the sperm cells in fact showed no motility (thus they were not included in

Table 2. Morphology variables group: significance of linear and nonlinear models

Variable name	R^2	Prob > |t|	Linear model significant at $P < 0.05$	Increase or Decrease	Nonlinear model significant at $P < 0.05$	Fitted exponent parameter	Threshold-like?
Percent amorphous form	0.4370	<0.0001	Y	I	NO COMP	1.880	NO COMP
Percent coiled tails	0.4439	<0.0001	Y	I	NO COMP	1.862	NO COMP
Percent double tails	0.2745	<0.0001	Y	I	Y	1.839	Y
Percent hooked heads	0.3882	<0.0001	Y	I	Y	2.575	Y
Percent isolated pieces	0.4866	<0.0001	Y	I	Y	2.144	Y
Percent large heads	0.3015	<0.0001	Y	I	N	1.094	–
Percent normal acrosome	0.6755	<0.0001	Y	D	Y	1.641	Y
Percent normal	0.6800	<0.0001	Y	D	Y	1.647	Y
Percent pin heads	0.3670	<0.0001	Y	I	NO FIT	–	–
Percent pyriform	0.5390	<0.0001	Y	I	Y	1.694	Y
Percent small heads	0.6362	<0.0001	Y	I	Y	1.533	Y
Percent tapered heads	0.4719	<0.0001	Y	I	N	1.255	–

NO FIT. Cannot fit a nonlinear model.
NO COMP. Cannot compute significance level for nonlinear model.

the velocity measurement), new variables were derived from VSL, VCL, and VAP by multiplying them with the percent motile cells to obtain increased sensitivity. These variables, VSL × PMOT, VCL × PMOT, and VAP × PMOT, all show a significant effect and showed much higher R^2 values. Also, these variables and their unmodified counterparts VSL, VCL, and VAP all had significant nonlinear fit exponent parameters; the exponent in each of these cases was around 2, which signifies that the fitted relationship is indeed threshold like. Figures 9 and 10 present regression model projections for the variables of percent motile cells and VSL.

Sperm counts and semen volume

Sperm concentration or volume variables showed decreasing responses to lead level, all of them except pooled sperm count were significant. Average volume of ejaculate borderlined significance ($P = 0.0557$), but showed a strong relationship graphically. The strongest relationship was total sperm count among the variables measuring sperm count or volume. Both total sperm count and average semen volume showed a significant nonlinear exponent large enough to be considered threshold like. In fact, the average ejaculate volume showed the largest exponent ($n = 2.777$) and thus the most nonlinear relationship of all the variables in this study. The regression data for sperm quantity variables are shown in Table 5. Figures 11 through 13 present the projections for total sperm count, sperm concentration, and average ejaculate volume versus blood lead levels.

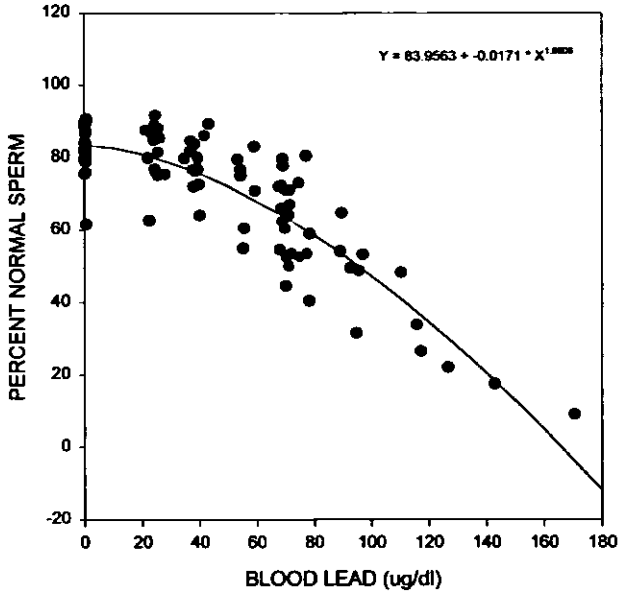

Fig. 2. Scatter diagram of percent normal sperm versus blood lead showing individual rabbit means for Weeks 16 through 20.

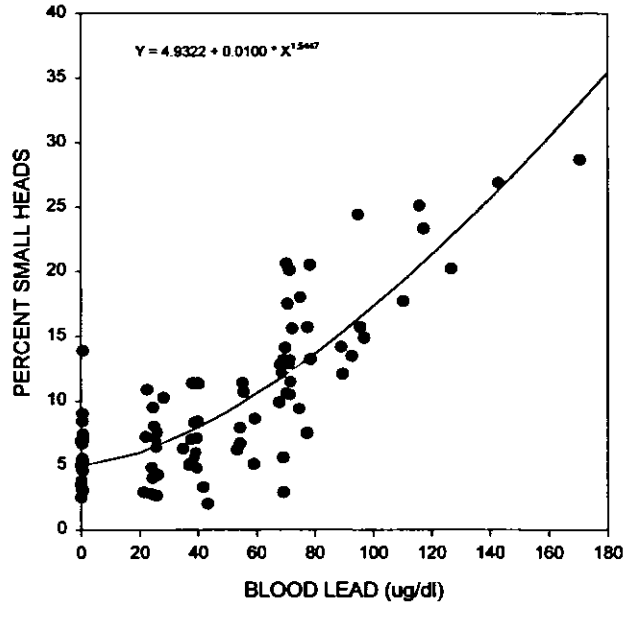

Fig. 3. Scatter diagram of percent small heads versus blood lead showing individual rabbit means for Weeks 16 through 20.

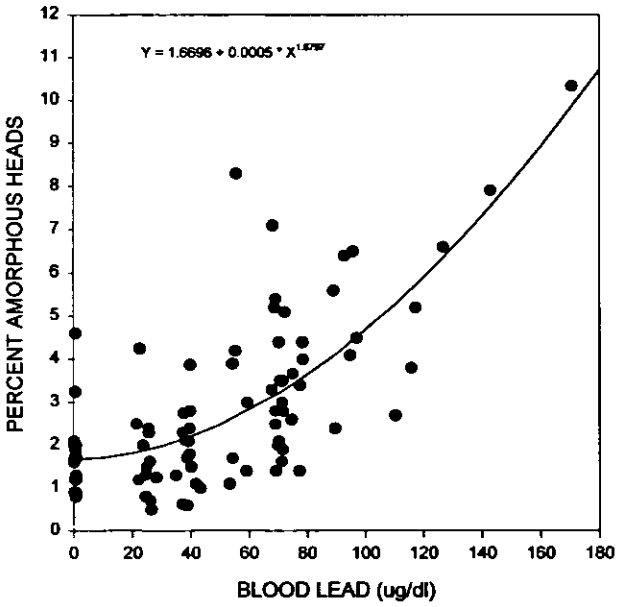

Fig. 4. Scatter diagram of percent amorphous heads versus blood lead showing individual rabbit means for Weeks 16 through 20.

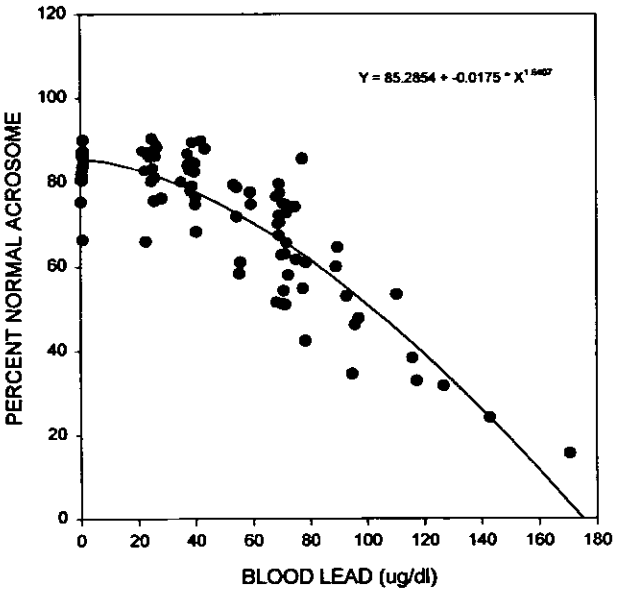

Fig. 5. Scatter diagram of percent normal acrosome versus blood lead showing individual rabbit means for Weeks 16 through 20.

Hormonal effects

The variables measuring hormonal effects were the levels of FSH, LH, and testosterone. Model projections showing values for LH, FSH, and testosterone are presented in Figures 14 through 16.

Threshold estimates

The six variables (percent normal cells, percent normal acrosomes, percent sperm mobility, VSL, total sperm count, and sperm head perimeter) with indicated thresholds are presented in Table 6. The blood lead threshold estimates range between 16 to 24 μg/dL.

Histopathology results

Lead treatment did not appear to affect testicular weight, minor tubular diameter, or the morphology of the germinal epithelium prior to the stage of spermatid release. However, a number of tubules contained cytoplasmic bodies (CBs), which were membrane bound and had one or more intracytoplasmic elongate spermatids. These CBs did not contain nuclei (other than the condensed nuclei of the contained spermatids) and were presumed to represent a coalescence of elongate spermatids with retained cytoplasmic droplets. These CBs were usually found in the tubular lumens, but occasionally they could be found within the germinal epithelium. They were also most frequently found in tubules that were at the stage of spermatid release. The percentage of tubules in the immediate postrelease stage that still contained elongate spermatids was also increased in treated animals, although this did occur to a lesser extent in control animals (Table 7). No treatment effect on either quantity or quality of Sertoli cells could be detected at the light microscopy level.

Figure 17 presents a light micrograph of a cross-section of a seminiferous tubule in a lead-dosed rabbit. Large, abnormal spermatids with cytoplasmic bodies containing retained cytoplasm and multiple spermatid heads can be seen at the luminal surface. These cells, which appear as membrane-bound cytoplasmic bodies containing one to several elongated spermatid nuclei, may be the result of the abnormal spermiation (process

Table 3. Morphometric variables group: Significance of linear and nonlinear models

Variable name	R^2	Prob > |t|	Linear model significant at $P < 0.05$	Increase or Decrease	Nonlinear model significant at $P < 0.05$	Fitted exponent parameter	Threshold-like?
Area of sperm head	0.3500	<0.0001	Y	D	Y	1.711	Y
Length of sperm head	0.4119	<0.0001	Y	D	N	1.081	N
Width of sperm head	0.1092	<0.0011	Y	D	Y	2.161	Y
Perimeter of sperm head	0.4357	<0.0001	Y	D	N	1.412	N

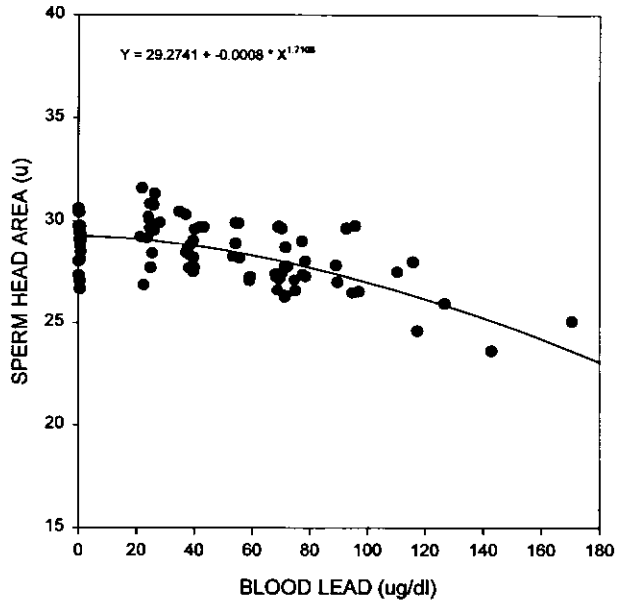

Fig. 6. Scatter diagram of sperm head area versus blood lead showing individual rabbit means for Weeks 16 through 20.

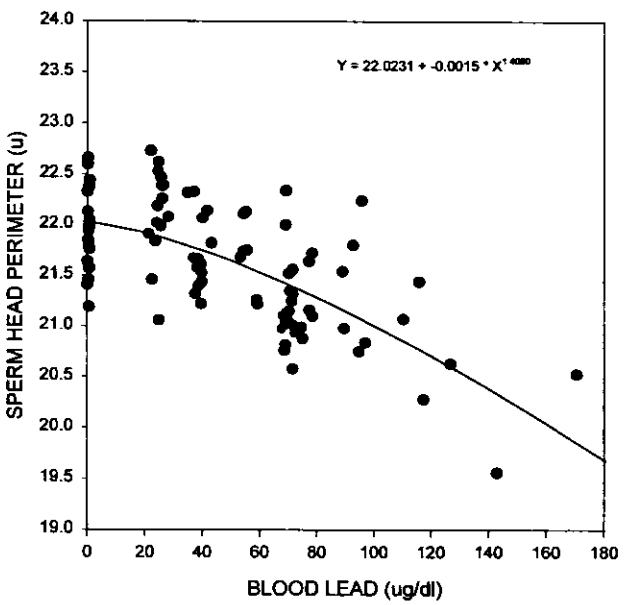

Fig. 8. Scatter diagram of sperm head perimeter versus blood lead showing individual rabbit means for Weeks 16 through 20.

of release of spermatids from the Sertoli cells). These abnormal cells occurred more frequently as blood lead levels increased and could be detected sporadically even at levels of 20 μg/dL. Notice that the germinal epithelium otherwise appears normal at the light microscopic level (all cell types present in appropriate proportions).

Major tissues, including brain, liver, gall bladder, kidney, and accessory sex organs, were evaluated histologically for evidence of other treatment effects and to ensure the study was not complicated by intercurrent disease. There were no lesions to indicate that infectious disease was present in any of the animals. In the animals that received high doses of lead treatment (blood leads over 100 mg/dL), the renal tubules at the corticomedullary junction were slightly dilated and were lined by squamous epithelial cells rather than the normal cuboidal cells (diagnosed as tubular degeneration).

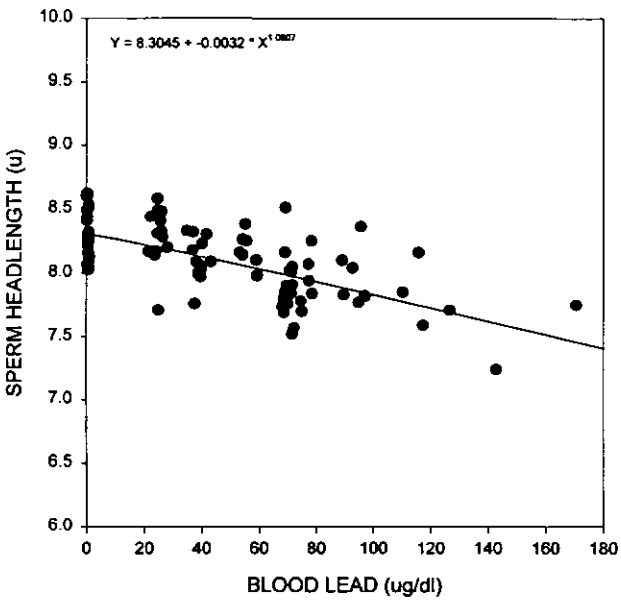

Fig. 7. Scatter diagram of sperm head length versus blood lead showing individual rabbit means for Weeks 16 through 20.

Species extrapolation

A regression of human data were calculated from five published articles (10–14) reporting blood lead and sperm concentration. The combined data from these articles revealed a decreasing linear relationship with a sperm concentration of 90.3 million/mL for a blood lead of zero and a decrease of 47 million/mL for an increase of 100 μg/dL in blood lead ($y = 90.3–0.47x$; $r^2 = 0.547$). The regression of the rabbit blood lead versus sperm concentration also resulted in a decreasing relationship with a sperm concentration of 527 million/mL for a blood lead of zero and a decrease of 175 million/mL for an increase of 100 μg/dL in blood lead ($y = 527.37–1.75x$; $r^2 = 0.507$). Figure 18 presents the plotted regressions of sperm concentrations for the rabbit and human. Rabbits demonstrated a steeper slope and sensitivity in terms of absolute sperm concentration. For calculation of the IEF, the percent change in sperm concentration for the rabbit and human at increasing blood lead levels was required. Figure 19 presents the regressions of percent change in sperm concentration

Table 4. Motility variables group: significance of linear and nonlinear models

Variable name	R^2	Prob > |t|	Linear model significant at $P < 0.05$	Increase or Decrease	Nonlinear model significant at $P < 0.05$	Fitted exponent parameter	Threshold-like?
Amplitude of lateral head displacement	0.0503	0.0299	Y	I	NO FIT	–	–
Linearity (VSL/VCL)	0.1211	0.0006	Y	D	N	1.711	–
Percent of motile cells (PMOT)	0.2300	<0.0001	Y	D	Y	2.039	Y
Average path velocity (VAP)	0.0115	0.3031	–	0	–	–	–
Curvilinear velocity (VCL)	0.0138	0.2597	–	0	–	–	–
Straight line velocity (VSL)	0.1668	<0.0001	Y	D	Y	1.990	Y
VAP × PMOT/100	0.1898	<0.0001	Y	D	Y	2.140	Y
VCL × PMOT/100	0.1606	<0.0001	Y	D	Y	2.278	Y
VSL × PMOT/100	0.2621	<0.0001	Y	D	Y	1.845	Y

–, Not significant/not calculated.
NO FIT, cannot fit a nonlinear model due to insufficient data.

with increasing blood lead for rabbits and humans. From these data, we calculated that a blood lead of 30.2 μg/dL in rabbits would produce a 10% decrease in sperm concentration and a blood lead level of 19.4 μg/dL would produce a 10% decrease in sperm concentration in men. The IEF of 1.56 was determined by dividing 19.2 into 30.2. Using this IEF, the rabbit dose would have to be divided by 1.56 to obtain the equivalent human dose for a 10% decrease in sperm concentration. Additionally, we plotted sperm concentrations vs log transformed doses for both rabbits and humans. A constant relationship of 3.75× exists between the dose–response curves. Linear responses were compared for both species as based on a recently published paper by Crawford and Wilson (18). They argue that low-dose linearity of the dose–response curve might be the rule rather than the exception and present the mathematical basis for their recommendation of using linear dose–response curves even for noncarcinogenic endpoints.

DISCUSSION

The longitudinal design of this study was essential for studying spermatogenic endpoints; however, modeling of the longitudinal effects has presented many difficulties. The duration of spermatogenesis in the rabbit is 48 d; therefore, sperm produced before 48 d of the onset of exposure may not reflect the full effect of the toxicant. In fact, it is recommended that studies of male reproductive toxicants extend at least through six cycles of the seminiferous epithelium to allow for toxicant

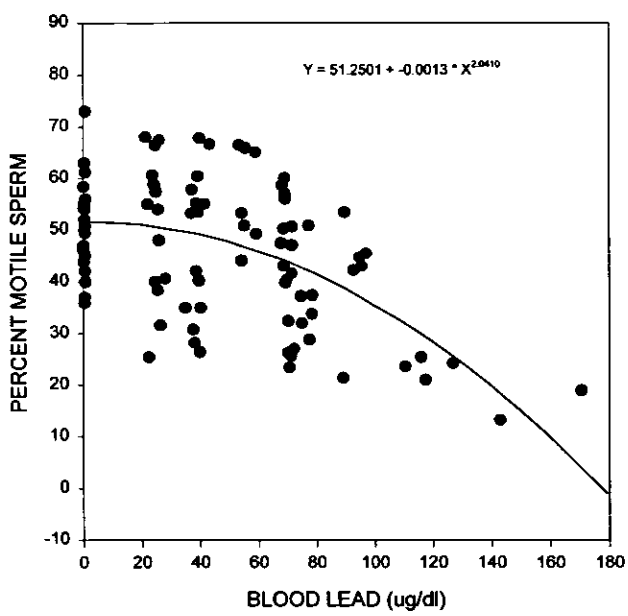

Fig. 9. Scatter diagram for percent motile sperm versus blood lead showing individual rabbit means for Weeks 16 through 20.

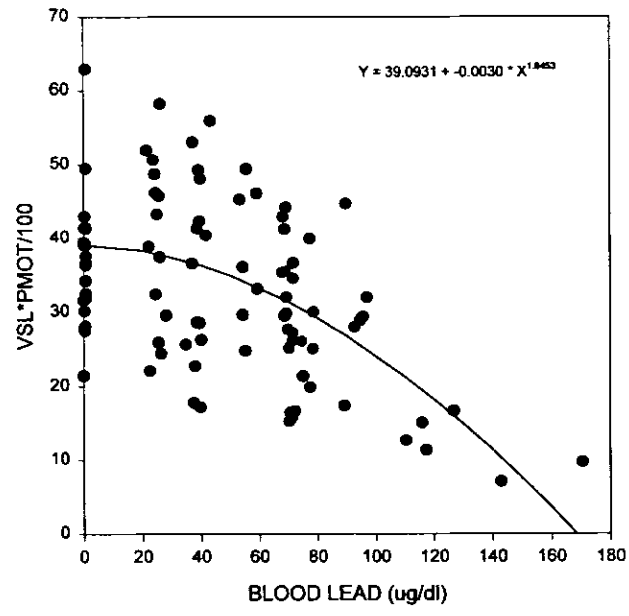

Fig. 10. Scatter diagram of straight line velocity × percent motile cells versus blood lead showing individual rabbit means for Weeks 16 through 20.

Table 5. Sperm count and sperm volume variables: significance of linear and nonlinear models

Variable name	R^2	Prob > |t|	Linear model significant at $P < 0.05$	Increase or Decrease	Nonlinear model significant at $P < 0.05$	Fitted exponent parameter	Threshold-like?
Pooled sperm count	0.0176	0.2021	–		–	–	–
First ejaculate sperm count	0.0863	0.0040	Y	D	N	2.155	–
Total sperm count	0.0800	0.0057	Y	D	Y	2.384	Y
First ejaculate volume	0.0437	0.0432	Y	D	N	2.219	–
Average ejaculate volume	0.0392	0.0557	Y (see text)	D	Y	2.777	Y

–, Not significant/not calculated.

uptake and full effects on spermatogenesis (2). Even after 6 weeks of exposure, the ejaculate contains sperm produced before exposure to the toxicant. Additionally, if the data used to establish the regression model for effects over time included all 15 weeks of exposure, including 5 weeks to establish the blood lead plateau, then the dose–response slope would be biased (reduced) because the full effect of the established blood lead plateau would not be reflected in the semen until about the 15th week because the data would include values reflecting sperm produced prior to the target lead dose (5-week pre-exposure + 6 cycles = 64 d or 9.1 weeks = 14 weeks). Therefore, we opted to model the lead-induced effects across all treatments averaged for the last 5 weeks of the study.

A comparison of sperm concentration data from human epidemiologic reports and the current rabbit data revealed that the rabbit is a qualitatively and quantitatively useful animal model. Lancranjan et al. (19) reported decreased sperm concentrations in workers with blood lead mean levels of 41 μg/dL (range of 29 to 54); Assennato et al. (9) reported decreased sperm concentration in workers with blood lead group means of 61 ± 20 μg/dL; Lerda (10) reported decreased sperm concentrations in workers with blood lead group means of 48.6 (range of 40.5 to 55); Telisman et al. (11) reported sperm concentration decreases in workers with blood lead group means of 37.1 (11.9 to 104). In the present study with rabbits, a projected decrease in sperm concentration of 11.4 million/mL was obtained at 40-μg/dL blood lead. The 50-μg/dL target blood lead treatment averaged a decrease in sperm concentration of 19.3 million/mL. Collectively, from comparing the data from the five reported epidemiologic studies we used in the regression of blood lead and sperm concentration, the rabbit is more sensitive than the human in terms of absolute decrease in sperm concentration for a given blood lead. The rabbit has a normal sperm concentration of about 527 million/mL (mean sperm concentration for the first ejaculate of all rabbits during the baseline period). This value compared to the mean sperm concentration for humans

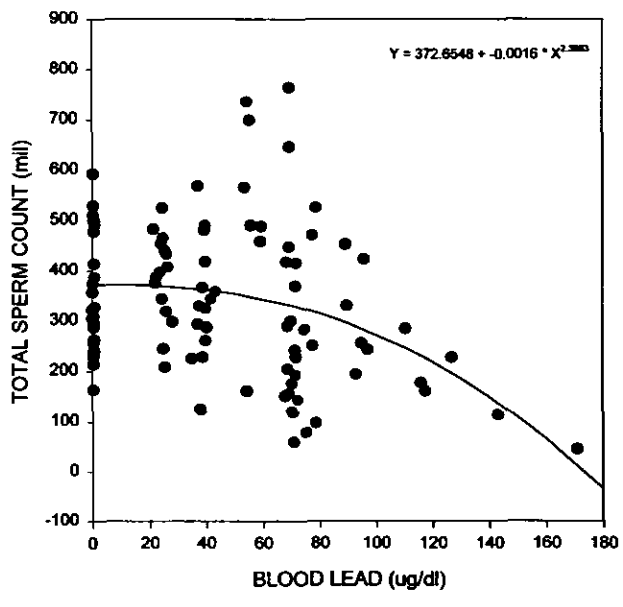

Fig. 11. Scatter diagram of total sperm count versus blood lead showing individual rabbit means for Weeks 16 through 20.

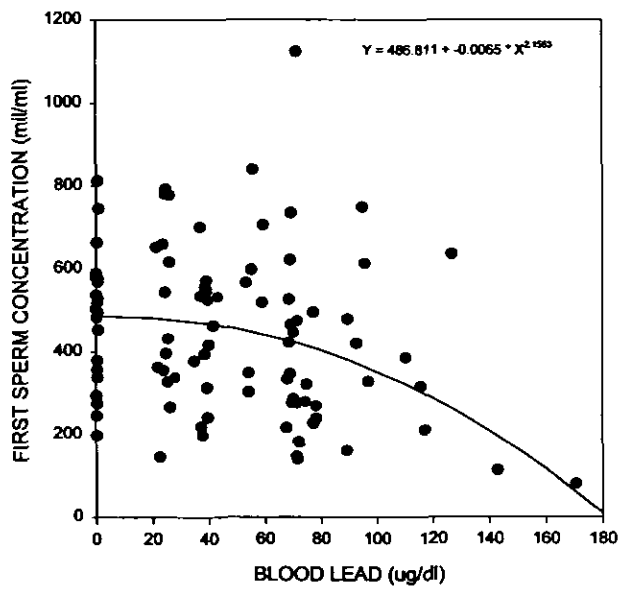

Fig. 12. Scatter diagram of first ejaculate sperm concentration versus blood lead showing individual rabbit means for Weeks 16 through 20.

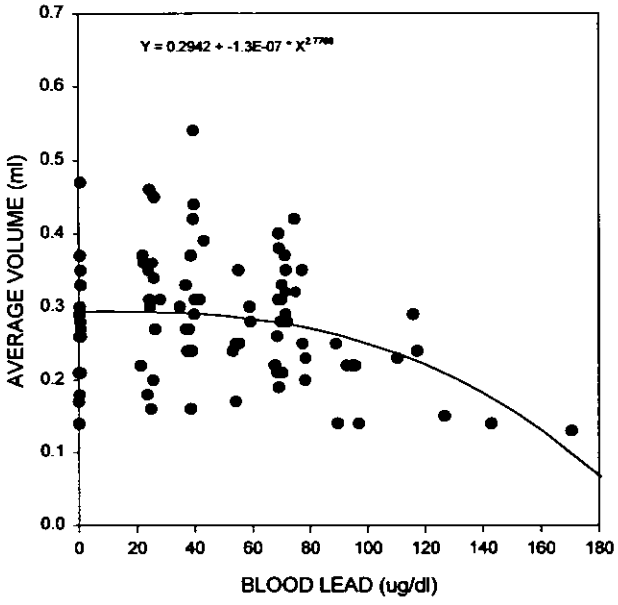

Fig. 13. Scatter diagram of average ejaculate volume versus blood lead showing individual rabbit means for Weeks 16 through 20.

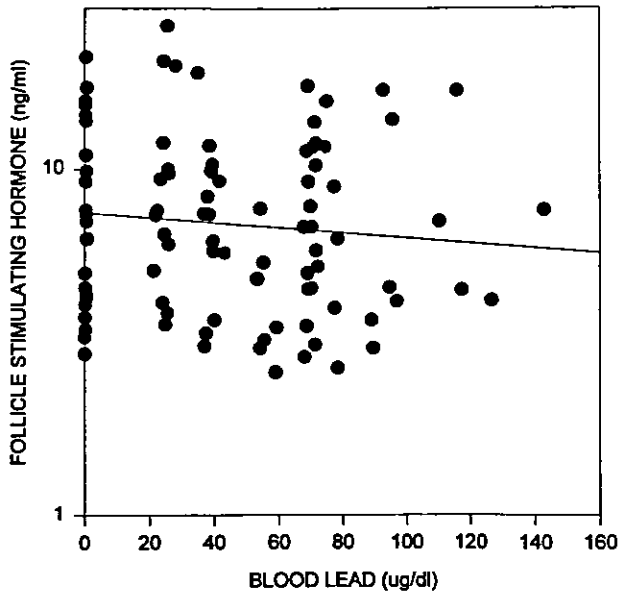

Fig. 15. Scatter diagram of follicle-stimulating hormone versus blood lead showing individual rabbit means for Weeks 16 through 20.

of 90 million/mL (from the five published reports used in the regression of blood lead and sperm concentration) reveals a fivefold lower sperm concentration for humans. The greater sensitivity (dose–response curve slope) in the rabbit probably results in part from the lower normal sperm concentration in humans. Although rabbits are more sensitive in terms of absolute decrease in sperm concentration, the impact would be greater in men because the percent change in sperm concentration would be greater. For equal doses, the reduction in sperm concentration for a blood lead of 100 μg/dL would be 52% in men (47/90 million/mL) and 33% in rabbits (175/527 million/mL).

A comparison of ejaculate volume data in human studies and the rabbit model indicates that the rabbit may

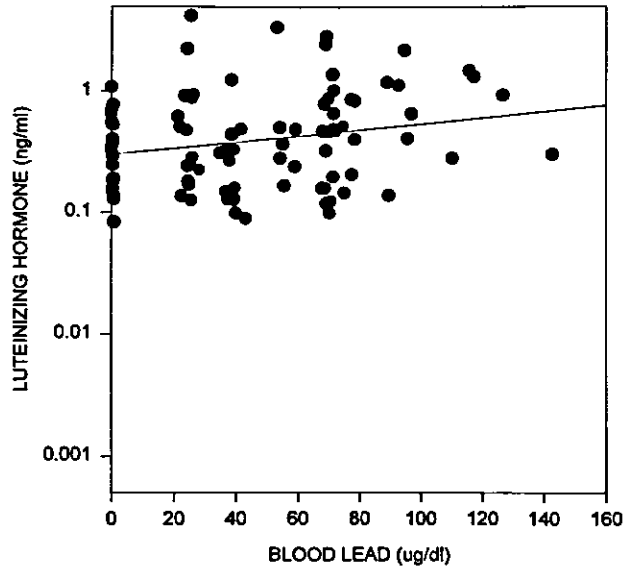

Fig. 14. Scatter diagram of luteinizing hormone versus blood lead showing individual rabbit means for Weeks 16 through 20.

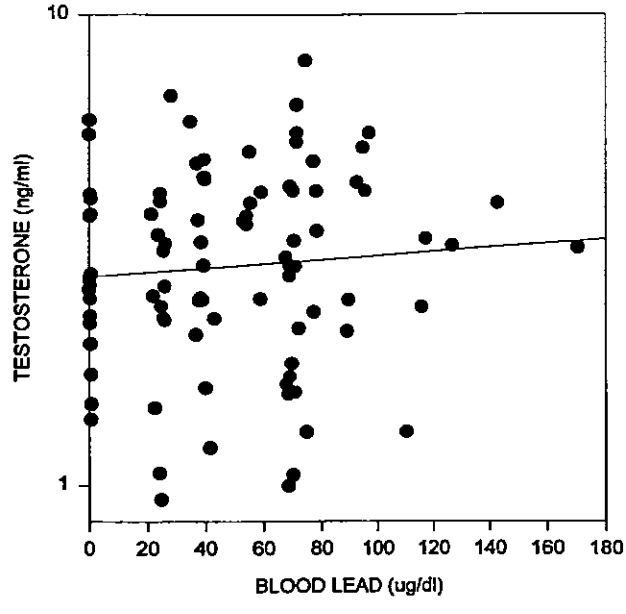

Fig. 16. Scatter diagram of testosterone versus blood lead showing individual rabbit means for Weeks 16 through 20.

Table 6. Fitted model exponent parameters and the estimated threshold

Variable	Fitted exponent parameter	Significance of nonlinear model over linear model (P)	Approximate threshold (dose in μg/dL)
Percent normal cells	1.6607	<0.001	16.2
Percent normal acrosomes	1.6469	<0.001	17.1
Percent sperm motility	2.0412	<0.05	21.3
Sperm velocity (straight line)	1.9645	<0.005	22.1
Total sperm count	2.3885	<0.005	23.7
Sperm head perimeter	1.4090	<0.05	16.3

be slightly less sensitive. Lerda (10) reported a decrease of 0.2 mL (4.1 to 3.9 mL or 4.8%) in the worker group with a blood lead mean of 48.6 μg/dL. Telisman et al. (11) reported a nonsignificant decrease of 0.1 mL (2.6 to 2.5 mL or 3.8%), in workers with average blood lead levels of 37.1 μg/dL. In the present study, a projected decrease of 0.017 mL (0.496 to 0.479 mL or 3.4%) was observed for the 50-μg/dL blood lead treatment.

A comparison of the effects of lead on sperm motility in human data and in the rabbit model show interesting similarities. A significant decrease in the percentage of motile cells was reported by Lerda (11) and Lancranjan et al. (19). A nonsignificant effect on the percent of motile sperm was reported by Braunstein et al. (12). The present study found a decrease in the percentage of motile cells of 7.5% projected at 50 μg/dL. Increased motility or velocity has been reported by Wildt and Berlin (20) at group mean blood lead levels of 46.1 μg/dL and more recently by Osorio et al. (21). Osorio reported a positive trend (increased velocities) in mean group levels of 0 to 24, 25 to 39, and >40 μg/dL. While we cannot directly compare the actual data, the rabbits in the present study also showed increased velocities until we adjusted for a correlated effect, the decrease in the distribution of motile cells. Our adjusted data revealed a significant decrease in velocities with increasing blood lead levels.

Table 7. Animals with spermatid defects

Treatments (μg/dL)	Control	20	40	50	70	80	90	110
No. animals with abnormal spermatids	0	1	5	3	3	7	1	7
No. animals observed	22	15	15	7	7	15	7	7
Percent animals with lesions	0	7	33	43	43	47	14	100

A positive for spermatid defects was defined as the presence of cytoplasmic bodies containing one to several elongate spermatids (or portions of elongate spermatids) in any tubule.

Fig. 17. Light micrograph of a cross-section of a seminiferous tubule in Stage 8, just prior to the time of spermatid release. Many large, abnormal cytoplasmic bodies containing retained cytoplasm and multiple spermatid heads can be seen at the luminal surface (arrow). Periodic acid Schiff/hematoxyin stain. Twenty weeks of exposure, 90 μg/dL treatment. Magnification, ×160.

Comparing the sperm morphology data in human and rabbit resulting from lead exposure also shows similarity. Lancranjan et al. (19) reported that, "The most frequent observed pathologic aspect revealed by semen analysis was teratospermia." Lerda (10) reported that the number of abnormal sperm significantly increased from 33.4% in the control group to 72.2% in the 48.6-μg/dL blood lead group. Davis et al. (22) reported that the width and percent normal (morphology) were decreased in lead battery workers with blood lead levels of 21 to 39 μg/dL. The rabbits in the present study demonstrated a decrease in the number of normal cells characterized by an increase in the number of small heads and a decrease in the number of normal acrosomes. Abnormal sperm morphology was our most sensitive measure of effect on semen quality; effects were seen at levels as low as 20 μg/dL in the present study. A lead industry sponsored report claims a threshold for spermatogenic effects in lead exposed workers of 50 to 60 μg/dL (23).

The reported effects of lead on human hormones are inconsistent. Assennato et al. (19) found sperm count suppression without endocrine dysfunction in storage battery workers with mean blood lead levels of 61 μg/dL. They concluded that lead had a direct toxic effect on the testis that reduced sperm production. McGregor and Mason (24) reported that moderate lead exposure (blood lead levels of 17 to 77 μg/dL, mean value of 47 μg/dL) resulted in increased FSH levels, decreased LH

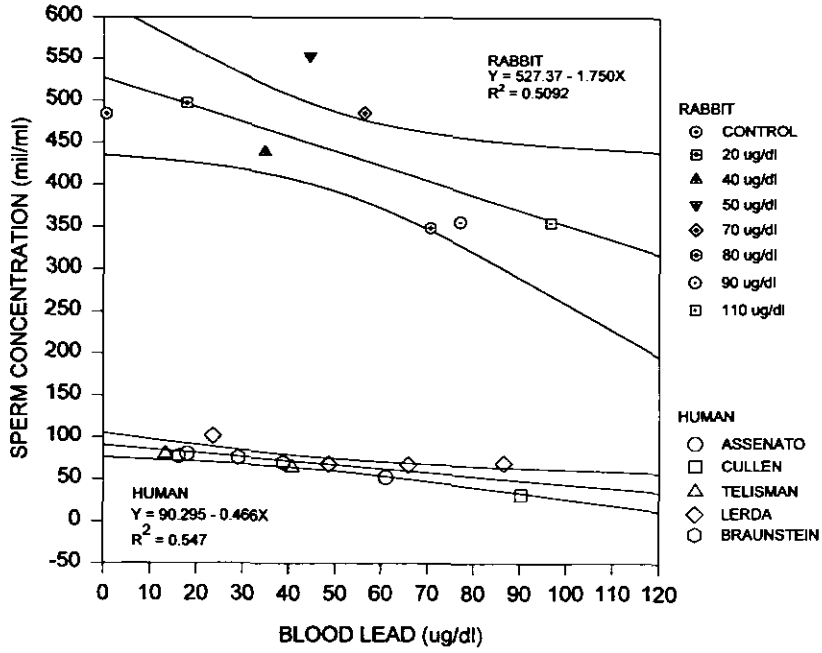

Fig. 18. Scatter diagram showing a regression between sperm concentration and blood lead for both human and rabbit. Rabbit data are represented by group means, whereas the human data are from published reports.

levels, and no significant change in testosterone levels. Ng et al. (25) found male endocrine changes in 122 men (battery production workers) with mean blood leads of 35 μg/dL (range 9.6 to 77.4). Specifically, LH and FSH were significantly elevated, although testosterone was not changed. They concluded that both primary and secondary effects occur in a dose-related fashion. Rodamilans et al. (26) found an increase in testosterone and LH in men with mean blood leads of 76 μg/dL. The rabbits in the present study (combined data) did not demonstrate significant changes. During the high-dose phase, however, increases in FSH (P = 0.001) and borderline (P = 0.055) decreases in testosterone were found.

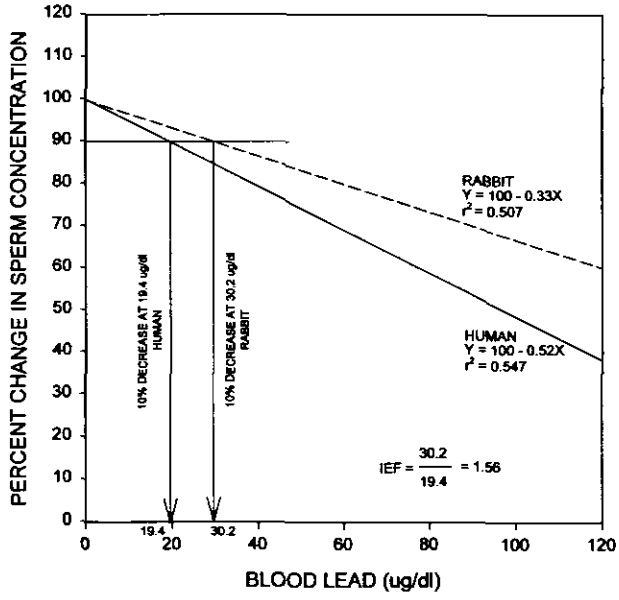

Fig. 19. Plot representing the percent change in sperm concentration for rabbits and humans. The Interspecies Extrapolation Factor (IEF) is represented by the dose at which there is a 10% decrease in sperm concentration in the rabbit (30.4 μg/dL) over the dose at which there is a 10% decrease in sperm concentration in the human (19.4 μg/dL).

While this report presents threshold estimates, we recognize the controversial issues associated with thresholds. However, it is generally assumed that thresholds exist for noncarcinogenic toxicants because of the known physiologic reserve and biologic repair capacities (27). Certainly spermatogenesis has known reserve capacity and repair. Therefore, we evaluated thresholds estimates for the six variables with the strongest dose–response relationships. The obvious nonlinear dose–response curves for many of the variables motivated us to calculate thresholds. On the basis of suggestions in the literature on toxicologic dose–response characterization (16,17), a toxicant for which the fitted exponent parameter is found to be 1.5 or greater can be considered to exhibit a threshold-like behavior. We therefore presented the threshold estimates for those variables with high exponent values. It was encouraging that the thresholds had such a small range (16 to 24 μg/dL) among the most responsive variables. The nonlinear dose–response for the rabbit sperm count data, along with the presentation

of thresholds, complicates the computation of interspecies extrapolation factors. If however, it is accepted that the slope of the nonlinear dose–response curve is steeper for high doses and less steep for low doses, then the linear dose response can be accepted as an average and applicable for overall species extrapolation.

While other protocols and species may be useful in screening chemicals for purposes of basic categorization as reproductive toxicants, their data and endpoints are not as useful to guide human hazard evaluations in the field of risk assessment. Here quantitative, mechanistic, preclinical measures such as sperm count, motility, and morphology are most useful and needed. On the basis of findings in this study and a comparison to lead induced effects in men, the rabbit is a quantitatively predictive model especially useful in evaluating the spermatogenic effects of toxicants.

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