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Air Quality Criteria for Lead (First External Review Draft)

Volume II of II

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Air Quality Criteria for Lead

Volume II

National Center for Environmental Assessment-RTP Office
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, NC

DISCLAIMER

This document is a first external review draft being released for review purposes only and does not constitute U.S. Environmental Protection Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

PREFACE

National Ambient Air Quality Standards (NAAQS) are promulgated by the United States Environmental Protection Agency (EPA) to meet requirements set forth in Sections 108 and 109 of the U.S. Clean Air Act. Sections 108 and 109 of the Clean Air Act require the EPA Administrator (1) to list widespread air pollutants that reasonably may be expected to endanger public health or welfare; (2) to issue air quality criteria for them that assess the latest available scientific information on nature and effects of ambient exposure to them; (3) to set "primary" NAAQS to protect human health with adequate margin of safety and to set "secondary" NAAQS to protect against welfare effects (e.g., effects on vegetation, ecosystems, visibility, climate, manmade materials, etc); and (5) to periodically review and revise, as appropriate, the criteria and NAAQS for a given listed pollutant or class of pollutants.

Lead was first listed in the mid-1970's as a "criteria air pollutant" requiring NAAQS regulation. The scientific information pertinent to Lead NAAQS development that was available at the time was assessed in the EPA document *Air Quality Criteria for Lead*; published in 1977. Based on the scientific assessments contained in that 1977 lead air quality criteria document (1977 Lead AQCD), EPA established a 1.5 $\mu g/m^3$ (90-day average) Lead NAAQS in 1978.

To meet Clean Air Act requirements noted above for periodic review of criteria and NAAQS, newly available scientific information published since the 1977 Lead AQCD was assessed and discussed in a revised Lead AQCD and Addendum published in 1986 and in a Supplement to the 1986 AQCD/Addendum published by EPA in 1990. A 1990 Lead Staff Paper, prepared by EPA's Office of Air Quality Planning and Standards (OPQPS), drew upon key findings and conclusions from the 1986 Lead AQCD/Addendum and 1990 Supplement (as well as other OAQPS-sponsored lead exposure/risk analyses) in posing options for the EPA

Administrator to consider with regard to possible revision of the Lead NAAQS. However, EPA decided not to revise the lead NAAQS at that time.

The purpose of this revised Lead AQCD is to critically evaluate and assess the latest scientific information that has become available since the literature assessed in the above 1986 Lead AQCD/Addendum and 1990 Supplement, with the main focus being on pertinent new information useful in evaluating health and environmental effects of ambient air lead exposures. This includes discussion in this document of information regarding: the nature, sources, distribution, measurement, and concentrations of lead in the environment; multimedia lead exposure (via air, food, water, etc.) and biokinetic modeling of contributions of such exposures to concentrations of lead in brain, kidney, and other tissues (e.g., blood and bone concentrations, as key indices of lead exposure).; characterization of lead health effects and associated exposure-response relationships; and delineation of environmental (ecological) effects of lead. This First External Review Draft of the revised Lead AQCD mainly assesses pertinent literature published or accepted for publication through June, 2004.

The present First External Review Draft (dated December 2005) of the revised Lead AQCD is being released for public comment and review by the Clean Air Scientific Advisory Committee (CASAC) to obtain comments on the organization and structure of the document, the issues addressed, the approaches employed in assessing and interpreting the newly available information on lead exposures and effects, and the key findings and conclusions arrived at as a consequence of this assessment. Public comments and CASAC recommendations will be taken into account in making appropriate further revisions to this document for incorporation into a Second External Review Draft of the document to be released in early 2006 for further public comment and CASAC review. Public comments and CASAC advice received on the Second External Review Draft materials will then be taken into account in incorporating further revisions into the final version of this Lead AQCD, which is to be completed and issued by October 1, 2006. Evaluations contained in the present document will be drawn on to provide inputs to associated Lead Staff Paper prepared by EPA's Office of Air Quality Planning and Standards (OAQPS), which will pose options for consideration by the EPA Administrator with regard to proposal and, ultimately, promulgation of decisions on potential retention or revision, as appropriate, of the current Lead NAAQS.

Preparation of this document was coordinated by staff of EPA's National Center for Environmental Assessment in Research Triangle Park (NCEA-RTP). NCEA-RTP scientific staff, together with experts from and academia, contributed to writing of document chapters. Earlier drafts of document materials were reviewed by scientists from other EPA/units and by non-EPA experts in several public peer consultation workshops held by EPA in July/August 2005.

NCEA acknowledges the valuable contributions provided by authors, contributors, and reviewers and the diligence of its staff and contractors in the preparation of this draft document.

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Abbreviations and Acronyms

αFGF	α-fibroblast growth factor
AA	arachidonic acid
AAS	atomic absorption spectroscopy
ACBP	Achenbach Child Behavior Profile
ACE	angiotensin converting enzyme
ACh	acetylcholine
AChE	acetylcholine esterase
ADP	adenosine dinucleotide phosphate
AE	anion exchange
AFC	antibody forming cells
AI	angiotensin I
ALA	δ-aminolevulinic acid
ALAD	δ-aminolevulinic acid dehydratase
ALAS	aminolevulinic acid synthetase
ALAU	urinary δ-aminolevulinic acid
ALD	aldosterone
ALS	amyotrophic lateral sclerosis
ALT	alanine aminotransferase
AMEM	Alpha Minimal Essential Medium
AMP	adenosine monophosphate
ANCOVA	analysis of covariance
ANF	atrial natriuretic factor
Ang II	angiotensin II
ANOVA	analysis of variance
ANP	atrial natriuretic peptide
AP	alkaline phosphatase
AP-1	activated protein-1
APDC	ammonium pyrrolidine dithiocarbamate
ApoE	apolipoprotein E
AQCD	Air Quality Criteria Document
AS52	cells derived from the CHO cell line
AST	aspartate aminotransferase
ASV	anode stripping voltammetry
3AT	3-amino triazole; 3-amino triazide

ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
ATSDR	Agency for Toxic Substances and Disease Research
AVCD	atrioventricular conduction deficit
β	beta-coefficient; slope of an equation
βFGF	β-fibroblast growth factor
17β–HS	17β-hydroxysteriod
3β-HSD	3β-hydroxysteriod dehydrogenase
17β-HSDH	17β-hydroxysteriod dehydrogenase
6β-OH-cortisol	6-β-hydroxycortisol
$Ba(NO_3)_2$	barium nitrate
BAEP	brainstem auditory-evoked potentials
BAER	brainstem auditory-evoked responses
B cell	B lymphocyte
BCS	bovine calf serum
BEI	biological exposure index
BFU-E	blood erythroid progenitor
BLL	blood lead level
BM	basement membrane
BMI	body mass index
BOTMP	Bruinicks-Oseretsky Test of Motor Proficiency
BP	blood pressure
BSA	bovine serum albumin
BSI	Brief Symptom Inventory
BTQ	Boston Teacher Questionnaire
BUN	blood urea nitrogen
bw	body weight
C3H10T/12	mouse embryo cell line
CA3	cornu ammonis 3 region of hippocampus
Ca-ATPase	calcium-dependent adenosine triphosphatase
CaEDTA	calcium disodium ethylenediaminetetraacetic acid
CAL	calcitonin
cAMP	cyclic adenosinemonophosphate
CANTAB	Cambridge Neuropsychological Testing Automated Battery
CAT	catalase; Cognitive Abilities Test

CBCL	Achenbach Child Behavior Checklist
CBCL-T	Total Behavior Problem Score
CCB	cytochalasin B
CCS	cosmic calf serum
C-CV_{RSA}	coefficient of component variance of respiratory sinus arrhythmia
^{109}Cd	cadmium-109 radionuclide
CDC	Centers for Disease Control and Prevention
CESD, CES-D	Center for Epidemiologic Studies Depression (scale)
CFR-GM	colony forming unit-granulocyte/macrophage progenitor
CFU-E	colony forming unit blood-erythroid progenitor
CFU-GEMM	colony forming unit blood-pluripotent progenitor
cGMP	cyclic guanosine-3',5'-monophosphate
ChAT	choline acetyltransferase
CHD	coronary heart disease
CHO	Chinese hamster ovary cell line
CI	confidence interval
CLS	Cincinnati Lead Study
CMI	cell-mediated immunity
CNS	central nervous system
^{57}Co	cobalt-57 radionuclide
ConA	concanavalin A
COR	cortisol
CoRx	(co-reaction?)
COX-2	cyclooxygenase-2
CP	coproporphryn
CPT	current perception threshold
cr	creatinine
cre	creatinine
CREB	cyclic AMP-response element binding protein
CRI	chronic renal insufficiency
CSF	cerebrospinal fluid
CSF-1	colony-stimulating factor-1
CuZn-SOD	copper and zinc-dependent superoxide dismutase
CV	conduction velocity
CVD	cardiovascular disease

CVLT	California Verbal Learning Test
CV_{R-R}	coefficient of variation of the R-R interval
CYP1A1	cytochrome P-450 1A1
CYp3a11	cytochrome P-450 3a11
CYP450	cytocrome P-450
D	D-statistic
dbcAMP	dibutyryl cyclic adenosine-3',5'-monophosphate
DCV	distribution of conduction velocities
DDT	dithiothreitol
DFS	decayed or filled surfaces, permanent teeth
dfs	covariate-adjusted number of caries
DG	dentate gyrus
DMEM	Dulbecco's Minimal Essential Medium
DMEM/F12	Dulbecco's Minimal Essential Medium/Ham's F12
DMFS	decayed, missing, or filled surfaces, permanent teeth
DMSA	2,3-dimercaptosuccinic acid
DMTU	dimethylthiourea
DNA	deoxyribonucleic acid
DO	distraction osteogenesis
DOPAc	3,4-dihydroxyphenylacetic acid
dp/dt	rate of left ventricular isovolumetric pressure
DTH	delayed type hypersensitivity
DTT	dithiothreitol
E	embryonic day
E_2	estradiol
EBE	early biological effect
EBV	Epstein-Barr virus
ECG	electrocardiogram
EDTA	ethylenediaminetetraacetic acid
EEG	electroencephalogram
EKG	electrocardiogram
electro	electrophysiological stimulation
EMEM	Eagle's Minimal Essential Medium
eNOS	endothelial nitric oxide synthase
EP	erythrocyte protoporphyrin

EPO	serum erythropoietin
EPSC	excitatory postsynaptic currents
ERG	electroretinogram; electroretinographic
EROD	ethoxyresorufin-O-deethylase
ERT	estrogen replacement therapy
ESP	electrostatic precipitator
ESRD	end-stage renal disease
EST	estradiol
ET	endothelein; essential tremor
ET-ASS	electro-thermal atomic absorption spectrometry
F	F-statistic
FBS	fetal bovine serum
FCS	fetal calf serum
FEF	forced expiratory flow
FEP	free erythrocyte protoporphyrin
FEV	forced expiratory volume
FI	fixed interval
FMLP	N-formyl-L-methionyl-L-leucyl-L-phenylalanine
fMRI	functional magnetic resonance imaging
FSH	follicle stimulating hormone
FT3	free triiodothyronine
FT4	free thyroxine
FTES	free testosterone
FTII	Fagan Test of Infant Intelligence
FVC	forced vital capacity
γ-GT	γ-glutamyl transferase
G12 CHV79	cells derived from the V79 cell line
G6PD, G6PDH	glucose-6-phosphate dehydrogenase
GABA	gamma aminobutyric acid
GAG	glycosaminoglycan
GCI	General Cognitive Index
GD	gestational day
GDP	guanosine diphosphate
GEE	generalized estimating equations

GFAAS	graphite furnace atomic absorption spectroscopy
GFR	glomerular filtration rate
GI	Gastrointestinal
GLU	glutamate
GM	geometric mean
GMP	guanosine monophosphate
GnRH	gonadotropin releasing hormone
GnRH	gonadotropin releasing hormone
GPEI	glutathione S-transferase P enhancer element
GPx	glutathione peroxidase
GRP78	glucose-regulated protein 78
GSD	geometric standard deviation
GSH	reduced glutathione
GSSG	glutathione disulfide
GST	glutathione-S-transferase
GTP	guanosine triphosphate
H_2O_2	hydrogen peroxide
H_2SO_4	sulfuric acid
Hb	hemoglobin
HBSS	Hank's Balanced Salt Solution
HCG; hCG	human chorionic gonadotropin
$HClO_4$	perchloric acid
Hct	hematocrit
HDL	high-density lipoprotein (cholesterol)
HET	Binghamton heterogeneous stock
HFE	hemochromatosis (gene)
HFF	human foreskin fibroblasts
Hgb	hemoglobin
H-H	high-high
HHANES	Hispanic Health and Nutrition Examination Survey
H-L	high-low
HNO_3	nitric acid
HOME	Home Observation for Measurement of Environment
HPRT	hypoxanthine phosphoribosyltransferase (gene)
HR	heart rate

HSPG	heparan sulfate proteoglycan
HSPGx	(heparan sulfate proteoglycan peroxide?)
hTERT	catalytic subunit of human telomerase
HTN	hypertension
i.p., IP	intraperitoneal
i.v., IV	intravenous
IBL	integrated blood lead index
IBL × WRAT-R	integrated blood lead index × Wide Range Achievement Test-Revised (interaction)
ICP-AES	inductively coupled plasma atomic emission spectroscopy
ICP-MS	inductively coupled plasma mass spectrometry
IDMS	isotope dilution mass spectrometry
IFN	interferon (e.g., IFN-γ)
Ig	immunoglobulin (e.g., IgA, IgE, IgG, IgM)
IGF_1	insulin-like growth factor 1
IL	interleukin (e.g., IL-1, IL-1β, IL-4, IL-6, IL-12)
immuno	immunohistochemical staining
IMP	inosine monophosphate
iNOS	inducible nitric oxide synthase
IPSC	inhibitory postsynaptic currents
IQ	intelligence quotient
ISI	interstimulus interval
IVCD	intraventricular conduction deficit
KABC	(Kaufman Assessment Battery for Children?)
KTEA	Kaufman Test of Educational Achievement
KXRF, K-XRF	K-shell X-ray fluorescence
LC_{50}	lethal concentration (at which 50% of exposed animals die)
LC_{74}	lethal concentration (at which 74% of exposed animals die)
LDH	lactate dehydrogenase
LDL	low-density lipoprotein (cholesterol)
L-dopa	3,4-dihydroxyphenylalanine (precursor of dopamine)
LET	linear energy transfer (radiation)
LH	luteinizing hormone
LHRH	luteinizing hormone releasing hormone
L-NAME	L-N^G-nitroarginine methyl ester

LOWESS	locally weighted scatter plot smoother
LPO	lipoperoxide
LPP	lipid peroxidation potential
LPS	lipopolysaccharide
LTP	long term potentiation
LVH	left ventricular hypertrophy
LXRF	L-shell X-ray fluorescence
MA-10	mouse Leydig tumor cell line
MANCOVA	multivariate analysis of covariance
MAO	monoamine oxidase
MCH	mean corpuscular hemoglobin
MDA	malondialdehyde
MDCK	kidney epithelial cell line
MDI	Mental Development Index (score)
MDRD	Modification of Diet in Renal Disease (study)
MEM	Minimal Essential Medium
Mg-ATPase	magnesium-dependent adenosine triphosphatase
MIBK	methyl isobutyl ketone
MLR	mixed lymphocyte response
MMSE	Mini-Mental State Examination
MMTV	murine mammary tumor virus
MN	micronuclei formation
MND	motor neuron disease
MNNG	N-methyl-N'-nitro-N-nitrosoguanidine
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
MROD	methoxyresorufin-O-demethylase
MRS	magnetic resonance spectroscopy
MS	mass spectrometry
MSCA	McCarthy Scales of Children's Abiltities
MVV	maximum voluntary ventilation
MW	molecular weight (e.g., high-MW, low-MW)
N, n	number of observations
N/A	not available
NAc	nucleus accumbens

NAD	nicotinamide adenine dinucleotide
NADH	reduced nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
NAD(P)H	reduced nicotinamide adenine dinucleotide phosphate
NADS	nicotinamide adenine dinucleotide synthase
NAG	N-acetyl-β-D-glucosaminidase
Na-K-ATPase	sodium-potassium-dependent adenosine triphosphatase
NBT	nitro blue tetrazolium
NCD	nuclear chromatin decondensation (rate)
NCS	newborn calf serum
NCTB	Neurobehavioral Core Test Battery
NDI	nuclear divison index
NE	norepinephrine
NES	Neurobehavioral Evaluation System
NF-κB	nuclear transcription factor kappa B; nuclear transcription factor-κB
NHANES	National Health and Nutrition Examination Survey
NIOSH	National Institute for Occupational Safety and Health
NK	natural killer
NMDA	N-methyl-D-aspartate
NMDAR	N-methyl-D-aspartate receptor
NO	nitric oxide
NO_2	nitrogen dixide
NO_3	nitrate
NOR	nuclear organizing regions
NOS	nitric oxide synthase
NO_x	nitrogen oxides
NR	not reported
NS	nonsignificant
NSAID	non-steroidal anti-inflammatory agent
NTA	nitrilotriacetic acid
NTx	N-telopeptides
O_2	oxygen
OH	hydroxyl
7-OH-coumarin	7-hydroxy-coumarin
1,25-OH-D	1,25-dihydroxyvitamin D

25-OH-D	25-hydroxyvitamin D
8-OHdG	8-hydroxy-2'-deoxyguanosine
OR	odds ratio
p	probability value
p.o., PO	per os (oral administration)
P450 1A2	cytochrome P-450 1A2
P450 CYp3a11	cytochrome P-450 3a11
PAD	peripheral arterial disease
PAI-1	plasminogen activator inhibitor-1
PAR	population attributable risk
Pb	lead
^{203}Pb	lead-203 radionuclide
^{206}Pb	stable isotope of lead-206
$PbCl_2$	lead chloride
$Pb(Ac)_2$	lead acetate
$Pb(ClO_4)_2$	lead chlorate
$Pb(NO_3)_2$	lead nitrate
PbB	blood lead concentration
PBG-S	porphobilinogen synthase
PBMC	peripheral blood mononuclear cells
PbO	lead oxides (or litharge)
PBP	progressive bulbar paresis
Pct	percentile
PCV	packed cell volume
PDE	phosphodiesterase
PDGF	platelet-derived growth factor
PDI	Psychomotor Development Index
PEF	expiratory peak flow
PG	prostaglandin (e.g., PGE_2, PGF_2)
PHA	phytohemagglutinin A
Pi	inorganic phosphate
PKC	protein kinase C
PM	particulate matter
PM_{10}	combination of coarse and fine particulate matter

PMA	progressive muscular atrophy
PMN	polymorphonuclear leucocyte
PMR	proportionate mortality ratio
PN	postnatal (day)
P5N	pyrimidine 5'-nucleotidase
PND	postnatal day
POMS	Profile of Mood States
ppm	parts per million
PPVT-R	Peabody Picture Vocabulary Test-Revised
PRA	plasma renin activity
PRL	prolactin
PRR	prevalence rate ratio
PTH	parathyroid hormone
PVC	polyvinyl chloride
PWM	pokeweed mitogen
r	Pearson correlation coefficient
R^2	multiple correlation coefficient
r^2	correlation coefficient
R/ALAD	ratio of ALAD activity, before and after reactivation
RAVLT	Rey Auditory Verbal Learning Test
RBC	red blood cell; erythrocyte
RBF	renal blood flow
RBP	retinol binding protein
RCPM	Ravens Colored Progressive Matrices
REL	rat epithelial (cells)
RNA	ribonucleic acid
ROS	reactive oxygen species
RPMI 1640	Roswell Park Memorial Institute basic cell culture medium
RR	relative risk; rate ratio
RT	reaction time
Rx	(reaction?)
SA7	simian adenovirus
SBIS-4	Stanford-Binet Intelligence Scale-4th edition
s.c., SC	subcutaneous
SCAN	Test for Auditory Processing Disorders

SCE	sister chromatid exchange
SD	Spraque-Dawley (rat); standard deviation
SDH	succinic acid dehydrogenase
SDS	Symbol Digit Substitution
SE	standard error; standard estimation
SEM	standard error of the mean
SES	socioeconomic status
SGC	soluble guanylate cyclase
SHBG	sex hormone binding globulin
SHE	Syrian hamster embryo cell line
SIR	standardized incidence ratio
SMR	standardized mortality ratio
SNP	sodium nitroprusside
SOD	superoxide dismutase
SOD	superoxide dismutase
SOPR	sperm-oocyte penetration rate
SRIF	somatostatin
SRT	simple reaction time
SSADMF	Social Security Administration Death Master File
SSB	single-strand breaks
SSEP	somatosensory-evoked potential
StAR	steroidogenic acute regulatory protein
SVC	sensory conduction velocity
SVRT	simple visual reaction time
T	testosterone
TBPS	Total Behavior Problem Score
TCDD	methionine-choline-deficient diet
T cell	T lymphocyte
TEL	tetraethyl lead
TES	testosterone
TG	6-thioguanine
TH	tyrosine hydroxylase
Th1	T-derived lymphocyte helper 1
TIMS	thermal ionization mass spectrometry
TLC	treatment of lead-exposed children

TNF	tumor necrosis factor (e.g., TNF-α)
tPA	plasminogen activator
TRH	thyroid releasing hormone
TSH	thyroid stimulating hormone
TT3	total triiodothyronine
TT4	serum total thyroxine
TTES	total testosterone
TTR	transthyretin
TWA	time-weighted average
TX	tromboxane (e.g., TXB_2)
U	uriniary
UCP	urinary coproporphyrin
UDP	uridine diphosphate
Ur	urinary
UV	ultraviolet
V79	Chinese Hamster lung cell line
VC	vital capacity; vitamin C
VDR	vitamin D receptor
VE	vitamin E
VEP	visual-evoked potential
vit C	vitamin C
vit E	vitamin E
VMA	vanilmandelic acid
VMI	Visual-Motor Integration
VSMC	vascular smooth muscle cells
WAIS	Wechsler Adult Intelligence Scale
WHO	World Health Organization
WISC	Wechsler Intelligence Scale for Children
WISC-R	Wechsler Intelligence Scale for Children-Revised
WRAT-R	Wide Range Achievement Test-Revised
WTHBF-6	human liver cell line
XRF	X-ray fluorescence
yr	year
ZPP	zinc protoporphyrin

CHAPTER 5 ANNEX

ANNEX TABLES AX5-2

Table AX5-2.1. Effect of Lead on Erythrocyte Morphology, Mobility, and Other Miscellaneous Parameters

Dose & Route of Exposure	Duration	Species	Blood lead	Effect	Authors
100 µg/dL lead, 10 mg/dL, lead, In vitro	1 h	Human erythrocytes	—	Plasma lead uptake was at the rate of 0.17 µ moles/h.	Sugawara et al. (1990)
	24 h			Uptake comparable in erythrocyte ghosts and in intact cells. No association of lead with membranes at 24 h.	
2 µM lead acetate 2 µM lead	0- 1 h	MDCK Kidney epithelial cell line, In vitro	—	Anion exchange (AE) plays a critical role in regulating intracellular pH in erythrocytes and epithelial cells and facilitates Pb uptake.	Bannon et al. (2000)
	0-2 h	Human erythrocytes, in vitro			
10 or 20 mM lead acetate, i.p. once a week (100 or 200 µmoles)/ kg b.wt.	5 weeks	Albino rats	Control - 1-12 µg/100 mL Exposed - 100-800 µg/dL	Exposure to lead significantly decreased the erythrocyte mobility. The decreases in mobility were either simultaneous or prior to the decreases in hemoglobin (Hb) or hematocrit (Ht). In exposed rats, a significant negative correlation was found between mobility and blood lead levels. Decreases in ALAD (δ-aminolevulinic acid), was also apparent in exposed animals.	Terayama et al. (1986)
20 mM lead acetate, i.p. once a week (200 µmoles/kg b.wt)	5 weeks	Male Wistar Albino rats	Control - 1-12 µg/100 mL Exposed - 100-800 µg/dL	Exposure to lead significantly decreased RBC membrane sialic acid content, erythrocyte survival, hemoglobin, and hematocrit. This was evident to a minor extent below blood lead levels 100 µg/100 mL and was generally present from 100 µg/100 mL and higher.	Terayma and Muratsuga (1988)
200 µM of lead acetate, i.p.	Once a week for 5 weeks	Rat	0-600 µg/dL	Lead exposure significantly decreases RBC count, Hb values, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin, decreases erythrocyte mobility, membrane sialic acid content and deformability.	Terayama et al. (1993)
Lead, i.p. 20 mg/ kg b.wt.	14 consecutive days	Male Albino rat		Acetyl choline esterase (AchE), NADH dehydrogenase, and Na^+-K^+ ATPase activities in rat erythrocyte membranes were inhibited by lead exposure. Erythrocyte membrane sialic acid, hexose, hexosamine were inhibited by lead exposure. Membrane phospholipids and cholesterol were increased.	Jehang and Motlag (1995)

Table AX5-2.1 (cont'd). Effect of Lead on Erythrocyte Morphology, Mobility, and Other Miscellaneous Parameters

Dose & Route of Exposure	Duration	Species	Blood lead	Effect	Authors
1 µM lead nitrate	1 h	Erythrocytes from lead exposed healthy humans	Controls - 8.3 µg/dL Exposed 70.5 µg/dL	Lead exposure in healthy human RBC membranes resulted in increased levels of arachidonic acid (AA). The increase in AA correlated in a dose dependent manner with elevation in lead and with serum iron. On the other hand, a negative correlation was found between Aa and serum calcium. It is inferred that substitution of lead to calcium, which is essential for the release of phospholipase A2 for AA release may be the reason for increased RBC membrane AA.	Osterode and Ulberth (2000)
1 µM lead nitrate, In vitro	1 h	Erythrocytes from healthy human volunteers	—	Lead inhibits Gordos effect in human erythrocytes; electron spin labeling studies indicated cell shrinkage and decreased volume.	Eriksson and Beving (1993)
	6 and 12 mo	Erythrocytes from lead exposed rats		Cation-osmotic hemolysis (COH) in 12 month lead-exposed rats was lower in the areas of lower ionic strength on erythrocyte membranes.	Mojzis and Nistiar (2001)
0.1-200 µM, lead nitrate in the reaction buffer	1-6 h	In vitro, human erythrocytes	—	Lead crosses the erythrocyte membrane by the anion exchanger and can also leave erythrocytes by a vanadate – sensitive pathway, identified with the calcium pump. The high ratio of erythrocyte to plasma Pb seen in vivo appeared to be due to the presence of a labile Pb2+- binding component present in erythrocyte cytoplasm.	Simons (1993)
0.1-10 µM lead ions from 10 mM Pb(NO3)2 solution, In vitro	24 h	Erythrocytes from healthy human volunteers	—	Pb activates erythrocyte K^+ channels, Ca^{2+} sensitive erythrocyte Scramblase, triggers Phosphatidyl serine receptors and result in cell shrinkage and decreased life span.	Kempe et al. (2005)
20 µM lead ion, In vitro	2 min- 2 h	Erythrocytes from human umbilical cord	—	Pb attenuates prolytic effect on neonatal erythrocytes in iso-or hypotonic low ionic strength media.	Serrani et al. (1997)
				Hemolytic activity of Organo leads increases with their hydrophobicity: triethyllead chloride < tri-n-propyllead chloride < tributyl tin chloride.	Kleszcynska et al. (1997)
20 µM lead ions, In vitro	1 h	Human umbilical cord erythrocytes	—	Lead ions increase the resistance to lysis in media of diminishing tonicity. These changes might be mediated by changes in membrane structure.	Corchs et al. (2000)
0.1 mM lead final concentration, In vitro	1 h	Erythrocytes from healthy humans	—	Lead particles adhere to the external and internal surfaces of the human erythrocyte membrane and disturb the lamellar organization of lipid bilayers	Suwalsky et al. (2003)

Table AX5-2.1 (cont'd). Effect of Lead on Erythrocyte Morphology, Mobility, and Other Miscellaneous Parameters

Dose & Route of Exposure	Duration	Species	Blood lead	Effect	Authors
1-10 μM lead acetate, In vitro	3 h	Erythrocytes from healthy humans	—	Low concentrations of lead alter the physico chemical properties of proteins and lipids in erythrocyte membranes.	Slobozhanina et al. (2005)
0-1200 nM lead, In vitro	1 h	Erythrocytes from healthy humans	—	Significant increase in the phosphorylation of membrane cytoskeletal proteins in lead treated human erythrocytes at concentrations above 100 nM mediated by enhanced PKC activity.	Belloni- Olivi et al. (1996)

RBC—Red blood cells; Hb—Hemoglobin; NADH—Nicotinamide adenine dinucleotide dehydrogenase;
PKC—Protein kinase C; Ache—Acetyl choline esterase

Table AX5-2.2. Lead, Erythrocyte Heme Enzymes, and Other Parameters

Dose & Route of Exposure	Duration	Species	Blood lead	Effect	Authors
Dietary, 0-100µg/g dry wt. of the diet	35 days	Adult male Zebra finches	0-1.5 µg/mL	Significant negative correlation was observed between blood-Pb concentration and log ALAD activity. RBC ALAD activity ratio is a sensitive indicator of dietary lead concentration regardless of the mode of exposure.	Scheuhammer et al. (1987)
Lead acetate, oral gavage, 1.5 mg/kg b.wt, lead acetate	3 or 11 weeks	Red-tailed Hawks	0.195-0.752 µg/dL	Erythrocyte phorphobilinogen synthetase was depressed significantly with in the 1st wk of treatment. Rapid but brief increase in free protoporphyrin. Hematocrit, erythrocyte count, Hb were all decreased and blood viscosity increased in exposed group.	Redig et al. (1991)
20 µg/mL as lead acetate in drinking water	5 weeks	Female Wistar Albino rats	37.8 µg/dL	Lead exposure decreases hematocrit, hemoglobin, and the number of erythrocytes and enhances blood viscosity	Toplan (2004)
17µM Me/kg lead acetate, Per OS	5 days	Female Rabbits	—	Lead causes a significant decrease in blood ALAD activity, increases free erythrocyte protoporphyrins, increases aminolevulinic acid and coporphyrin excretion in urine.	Zareba and Chmelnicka (1992)
1.5 mg lead/ kg body wt, oral dose	8 yrs.	Cynomolgus Monkey, in vitro	—	Kinetic analyses of erythrocyte δ- aminolevulinic acid revealed differences in P^H optimum and Michaelis constants with lead exposure. The ALAD enzyme kinetics of lead exposed monkeys and humans are similar.	Dorward and Yagminas (1994)
		Dogs from urban and rural areas of Greece.	326, 97-68 µg/L	Significant negative correlation existed between blood-lead levels and ALAD activity. 807-992 µmol/PBG/LRBC/h is established as the normal erythrocyte ALAD range for dogs	Polizopoulou et al. (1994)
Occupational exposure	11-22 yrs	Human erythrocytes from exposed populations.	1.39-1.42 µ mol/l	Liquid chromatography with inductively coupled plasma spectrometry had revealed ALAD to be the principle lead binding protein. The percentage of lead bound to ALAD was influenced by a common polymorphism in the ALAD gene.	Bergdahl et al. (1997)
0-20 mg Pb liter −1	29 days	Juvenile Rainbow trout erythrocytes	—	Significant decreases in the erythrocyte ALAD activity after a 29-day exposure to 121 and 201 mg Pb liter −1	Burden et al. (1998)

Table AX5-2.2 (cont'd). Lead, Erythrocyte Heme Enzymes, and Other Parameters

Dose & Route of Exposure	Duration	Species	Blood lead	Effect	Authors
Lead acetate 160 mg/L in water	8 weeks	Wistar rats	≥20– ≥40 µg/dL	Lead increases blood and liver lead, erythrocyte porphyrin content, hypoactivity of both hepatocytic and erythrocytic ALAD	Santos et al. (1999)
	—	Fish from regions close to the smelters and down stream	—	Smelter site fish had elevated lead concentrations, decreased ALAD activity and species differences in this inhibitory activity were apparent that could be attributed to Zn levels.	Schmitt et al. (2002)
1.46 µmol/liter In vitro	48 h	Human whole blood erythrocyte hemolysates, normal and lead intoxicated individuals	—	The effects of various divalent cations on erythrocyte porphobilinogen are concentration and PH dependent. Zn restores the lead inhibited activity.	Farant and Wigfield (1987)
Lead 0.34 µM/L-1.17 µM/L, subcutaneous injection	1 h	Male albino New Zealand rabbits	—	Lead causes the most inhibition and Zn activation of rabbit Erythrocyte porphobilinogen activity.Cu^{2+}, Cd^{2+}, and Hg^{2+} are intermediary. Each divalent ion has a characteristic effect on the PH- activity relationship of PBG-S.	Farant and Wigfield (1990)
0-60 pM lead ion, in vitro	20 min	Human erythrocyte lysates	—	Human erythrocyte lysate porphobilinogen activity is increased by Zn^{2+} with a Km of 1.6 pM and inhibited by lead with a Ki of 0.07 pM, lead reduced the affinity for the substrate 5- aminolevulinate, non-competitively.	Simons et al. (1995)
200-500 ppm lead in drinking water	14 or 30 days	Male ddY mice	24-51 µg/100 mL	Lead inhibits erythrocyte and bone marrow P5'N activity. Erythrocyte ALAD activity was inhibited by 90%. Elevation of Urinary excretion of ALA with no change in erythrocyte protoporphyrin and urinary co porphyrin as against in the lead exposed humans indicates that protoporphyrin metabolism might be more resistant to lead in mice than humans.	Tomokuni et al. (1989)
0.1-100 µM lead ion, In vitro	5 min	Human erythrocyte ghosts	—	Under normal incubation conditions lead inhibits, Ca^{2+} -Mg^{2+} ATPase with an IC50 of 6.0µM. Lead inhibits Ca^{2+}- Mg^{2+} ATPase related to sulphahydryl groups above 1.0 µM lead and by direct action of lead upon Calmodulin below 1.0 µM.	Mas- Oliva (1989)
20-5 µg/kg body wt 1 mg/ kg body wt.	Pregnancy through lactation	Erythrocytes from Sprague-Dawley rats	—	Na^{2+}- K^{+}- ATPase and Ca^{2+}- Mg^{+}- ATPase of erythrocyte membranes from lead-depleted animals did not change in P0 generation as compared to 1 mg/kg b.wt lead animals, where as in F1 generation lead depleted rats showed reduced activity.	Eder et al. (1990)

Table AX5-2.2 (cont'd). Lead, Erythrocyte Heme Enzymes, and Other Parameters

Dose & Route of Exposure	Duration	Species	Blood lead	Effect	Authors
20 mg Pb acetate/ Kg b.wt, i.p, In vivo	14 days	Male Albino rats erythrocytes	—	Lead significantly decreases erythrocyte membrane acetyl choline esterase, NADH dehydrogenase, membrane sialic acid, hexose, and hexosamine.	Jehang and Motlag (1995)
				Lead ions inhibit aerobic glycolysis and diminish ATP level in human erythrocytes in vitro. Magnesium partly abolishes these effects by stimulating Magnesium dependent enzymes. Effect is seen both by direct addition of lead acetate to erythrocyte ghosts as well as in the ghosts obtained after preincubation of erythrocytes with lead acetate. Ca^{2+}, Mg^{2+} ATPase is less sensitive and Mg ATPase is practically insensitive to lead under these conditions.	Grabowska and Guminska (1996)
10-200 µg/dL lead ions (lead acetate) In vitro	20 h	Human umbilical cord erythrocytes	—	Lead significantly decreased the concentration of ATP, ADP, AMP, adenosine, GTP, GDP, GMP, Guanosine, IMP, inosine, hypoxanthine, NAD and NADP concentrations.	Bosiacka and Hlynczak (2003)
Lead acetate through water or i.p. 1 or 2 mg/Kg b.wt.	Every 4^{th} day for 1 month	Wistar rats	1.51-35.31 µg/dL	The concentrations of adenosine tri phosphate (ATP), Guanosine triphosphate (GTP),Nicotinamide adenine dinucleotide NAD^{+}, nicotinamide adenine dinucleotide phosphate $NADP^{+}$ adenylate and Guanylate(AEC and GEC) were significantly reduced in erythrocytes of exposed animals. Results indicate lead ions disrupt erythrocyte energy pathway.	Bosiacka and Hlynczak (2004)

ALAD — Aminolevulinic acid; Cu^{2+}—Copper; Cd^{2+}—Cadmium; Hg^{2+}—Mercury; PBG-S Porphobilinogen synthetase; Zn—Zinc; ATP—Adenosine triphosphate—ADP—Adenosine diphosphate; AMP-Adenosine monophosphate;
GTP—Guanosine tri phosphate; GDP—Guanosine diphosphate; GMP—Guanosine monophosphate; IMP—Inosine monophosphate;
NAD—Nicotinamide adenine dinucleotide; NADP—Nicotinamide adenine dinucleotide phosphate

Table AX5-2.3. Lead Binding and Transport in Human Erythrocytes

Dose & Route of Exposure	Duration	Species	Blood lead	Effect	Authors
0-60 pM lead ion, In vitro	20 minutes	Human erythrocyte lysates	—	Human erythrocyte lysate porphobilinogen activity is increased by Zn^{2+} with a Km of 1.6 pM and inhibited by lead with a Ki of 0.07 pM, lead reduced the affinity for the substrate 5- aminolevulinate, non-competitively.	Simons et al. (1995)

Zn—Zinc

Table AX5-2.4. Lead Effects on Hematological Parameters

Dose & Route of Exposure	Duration	Species	Blood lead	Effect	Authors
4-6 mg/Kg b.wt, i.p., daily	15 and 30 days	Intact and splenctamized rats	—	Lead increases urinary δ-amino levulinic acid (ALA) excretion, depletion in RBC hemoglobin content, and more number of reticulocytes in peripheral blood, and results in accumulation of immature erythrocytes both in intact and splenctomized rats.	Gautam and Roy Chowdhury (1987)
0.82 mg lead / kg b.wt./day, oral gavage	3 or 11 weeks	Red-tailed hawks erythrocytes	0.195-0.375 mg/mL	Activity of porphobilinogen synthase/ ALAD was depressed significantly in lead exposed rats and did not return to normal values until 5 weeks after the termination of the treatment. A rapid and relatively brief increase in erythrocyte free proto porphyrin and a slower, prolonged increase in Zn complex.	Redig et al. (1991)
17 µM Me/Kg b.wt lead acetate or 3.5 mg of Pb/kg body wt, i.p	5 days	Female Rabbits	17.5 µg/dL	Lead causes a significant inhibition of ALAD in the blood , increases free erythrocyte protoporphyrin, and urinary excretion of Aminolevulinic acid and coporphyrin	Zareba and Chmielnicka (1992)
17 µM Me/Kg b.wt lead acetate or 3.5 mg of Pb/ kg body wt, i.p. or per OS 17.5 mg/kg b.wt single injection	5 days i.p.	Female Rabbits	—	Lead induced ALAS activity in liver and kidney, both after i.p and p.o. administration. i.p. administration of lead also induced kidney heme oxygen levels.	Chmielnicka et al. (1994)
Cu deficient 1 mg Cu/Kg	4 weeks	Rat	—	Moderately high Zn in the diet reduces plasma copper but not plasma ceruloplasmin,	Panemangalore and Bebe (1996)
Marginal deficient 2 mg/kg				Does not affect the recovery of plasma Cu or activity after oral copper sulphate in Cu deficient diets.	
Control 5 mg Cu/Kg				Does not influence RBC Super oxide dismutase activity.	
High Zn 60 mg/kg.					
0.02 - 40 ppm Pb, dietary	90 days	Male and female Swiss mice	0.7-13.0 µg/dL	Increased RBC number and increased hemoglobin and decreased hematocrit up on lead exposure.	Iavicoli et al. (2002)
20 µg/mL, lead acetate in drinking water	5 weeks	Female Wistar Albino rats	37.8 µg/dL	Erythrocyte count, hematocrit and hemoglobin were all decreased and blood viscosity increased in lead exposed workers	Toplan et al. (2004)

ALA—Aminolevulinic acid; ALAS—Aminolevulinic acid synthetase; ALAD—Aminolevulinic acid dehydratase, RBC—Red blood cells

Table AX5-2.5. Lead Interactions with Calcium Potassium in Erythrocytes

Dose & Route of exposure	Duration	Species	Blood lead	Effect	Authors
0-325 µM, lead nitrate, In vitro	0-60 min	In vitro	—	Pb modifies the threshold sensitivity of individual K^+channels to Ca^{2+} with a biphasic time course. The increase of Pb concentration increased the extent of the initial inhibition and decreased the duration. The inhibitory effect was not observed when addition of Calcium preceded the addition of Pb. Pb decreased the rate of uptake of ^{86}Rb	Alveraz et al. (1986)
0 µM- 5 mM lead, In vitro	0-100 min	Human erythrocyte hemolysates	—	Lead and Ca transport was carried out by a passive transport system with two kinetic components (Michaelis- Menten and Hill) Pb and Ca were capable of inhibiting the transport of the other metals in a non-competitive way.	Salinas et al. (1999)
1-4 µM lead acetate, In vitro	0-30 min	Rabbit reticulocytes	—	Pb at low concentrations inhibits the uptake of Fe (II) into all three (heme, cytosolic and stromal) fractions. The saturable components were inhibited at lower concentrations of Pb than the non- saturable components.	Qian and Morgan et al. (1990)
1-50 µM lead ion, In vitro	20 min	Marine fish erythrocytes	—	Lead activates Ca^{2+} activated potassium channels. Treatment of erythrocytes with 1-2 µM lead led to a minor intra cellular K loss and at Pb concentrations of 20-50 µM 70% of potassium was lost.	Silkin (2001)

Pb—Lead; K^+—Potassium; Na^{2+}- K^+ ATPase—sodium potassium ATPase; Ca^{2+}- Mg^{2+} ATPase—Calcium, Magnesium ATPase.

Table AX5-2.6. Lead, Heme and Cytochrome P–450

Dose & Route of exposure	Duration	Species	Blood lead	Effect	Authors
0-75 mg of Pb^{2+}/Kg b. wt. i.p., Single injection	0-30 h	C57 BL/6 male mice	—	Lead causes an increase in δ- amino levulinic acid levels in plasma and a decrease in the heme saturation of hepatic tryptophan -2,3 dioxygenase. P-450- dependent activities, EROD and O-dealkylation of alkoxyresorufins decreased progressively. Lead exposure decreased mRNA levels of the P450 CYp3a11. The decrease in P450 transcription was a mechanism dependent on heme by inhibition of heme synthesis and also by a mechanism independent of heme in which lead decreases P-450 transcription.	Jover et al. (1996)

EROD—Ethoxy resorufin - O- dealkylase.
CYp3a11—Cytochrome P-450 3a11.

Table AX5-2.7. Lead, Erythrocyte Lipid Peroxidation, and Antioxidant Defense

Dose & Route of exposure	Duration	Species	Blood lead	Effect	Authors
7.5 mg of lead acetate or 4.09 mg of lead Kg^{-1} b.wt, oral	28 days, multiple analyses at day 7,14, 21 and 28	Erythrocytes from male Calves	0.1-1.6 ppm	Lead exposure significantly reduced erythrocyte super oxide dismutase activity until day 21 followed by a marginal increase by day 28. Total, protein-bound and non protein- bound –SH content of erythrocytes declined.	Patra and Swarup et al. (2000)
5.46 mg lead as lead acetate, oral	14 days, multiple analyses at day 0, 7 and 14	Erythrocytes from female goats	0.09-1.12 ppm	Lead exposure caused a significant increase of erythrocytic GPx, SOD and CAT activities, total thiol groups and total antioxidant status.	Mousa et al. (2002)
10 mg/kg b.wt lead acetate, intra muscular, daily Pre treatment with melatonin	7 days	Rat	—	Lead significantly decreased heme synthesis, decreased Hb, decreased liver δ- ALAS and erythrocyte ALAD. Markedly elevates hepatic lipid peroxidation, reduced anti oxidant enzymes such as total sulphahydryl groups and Glutathione. Pre Treatment with melatonin reduced the inhibitory effect of lead on both enzymatic and non enzymatic antioxidants and reduced the iron deficiency caused by lead.	El- Missiry (2000)
A. ALA 40 mg/kg b.wt every other day and /or B. Melatonin 10 mg/kg	Every other day 3 times daily for 2 weeks	Male Sprague- Dwaley rats	—	Melatonin effectively protects nuclear DNA and lipids in rat lung and spleen against the oxidative damage caused by the carcinogen ALA,	Karbownik et al. (2000)
Lead acetate 0.2%, in drinking water, followed by individual or combined treatment of lipoic acid (25 mg/Kg b.wt and DMSA 20 mg/kg b.wt, i.p.)	5 weeks	Male Albino rats	97.5 µg/dL	Lead exposure results in decreased blood hemoglobin, hematocrit, enhanced erythrocyte membrane lipid peroxidation, decline in the activities of erythrocyte membrane Na^{+}-K^{+} ATPase, Ca^{2+} ATPase, and Mg^{2+} ATPase. Treatment with lipoic acid and/or DMSA reduced the lead induced adverse changes in the biochemical parameters	Siva Prasad et al. (2003)
δ-Aminolevulinic acid, 1-5 mM, In vitro	10 days	CHO cells	—	δ- Aminolevulinic acid treatment induces oxidative stress in Chinese hamster ovary cells by inducing Glutathione, Glutathione disulphide, Malandialdehyde equivalents, and Catalase. N-acetyl cysteine administration reverses the decrease in cell survival and colony formation induced by δ- ALA.	Neal et al. (1997)

SOD—Super oxide dismutase; CAT—Catalase; ALAS—Aminolevulinic acid synthatase, ALAD—Aminolevulinic acid dehydratase; ALA—Aminolevulinic acid; GP_x—Glutathione peroxidase

CHAPTER 5 ANNEX

ANNEX TABLES AX5-3

Table AX5-3.1. Summary of Key Studies on Neurochemical Alterations

Subject	Exposure Protocol	Peak Blood Pb or [Pb] used	Observed Effects	Reference
rat PND 16-18	Hippocampal cultures	0.1 & 1.0 µM $PbCl_2$	Pb blockage of IPSCs were partially reversible while EPSCs were not	Braga et al. (2004)
rat PND 50	1500 ppm $Pb(Ac)_2$ chow 10 d before breeding & maintained to sacrifice	31.9 µg/dL	Decreases the NR1 subunit splice variant mRNA in hippocampus	Guilarte and McGlothan (2003)
rat PND 7, 14, 21, 28 & 50	1500 ppm $Pb(Ac)_2$ chow 10 d before breeding & maintained to sacrifice	—	Alters NMDAR subtypes & reduces CREB phosphorylation	Toscano et al. (2002)
rat PND 21	750 ppm $Pb(Ac)_2$ chow from gestational day 0 to PND 21	46.5 µg/dL	Increased expression of nicotinic receptors	Jett et al. (2002)
	Cultured PC12 cells	0.03-10 µM $Pb(NO_3)_2$	Pb acts as a high affinity substitute for calcium in catecholamine release	Westerink and Vijverberg (2002)
adult rat	water - 0.1-1.0% $Pb(Ac)_2$ from gestational day 15 to adult	61.8 µg/100 mL	Hippocampal GLU & GABA release exhibits biphasic effects from chronic Pb	Lasley and Gilbert (2002)
adult rat	water - 0.1-1.0% $Pb(Ac)_2$ from gestational day 15 to adult	117.6 µg/100 mL	NMDA receptor function is upregulated	Lasley et al. (2001)
	Cultured PC12 cells	0.53 µM $Pb(Ac)_2$	PKC is involved in TH upregulation but not downregulation of ChAT	Tian et al. (2000)
embryonic rat	hippocampal neurons	100 fM-100 nM	Decreases $[Ca^{2+}]i$ & increases Ca^{2+} efflux by a calmodulin-dependent mechanism	Ferguson et al. (2000)
rat	750 or 1500 ppm $Pb(Ac)_2$ chow from 10 d pre-mating to PND 14, 21, & 28	61.1 µg/dL	Dose-response effect between level of Pb and expression of NR1 gene	Guilarte et al. (2000)
	Cultured PC 12 cells	5-20 µM $Pb(Ac)_2$	Induces expression of immediate early genes but requires PKC	Kim et al. (2000)

Table AX5-3.1 (cont'd). Summary of Key Studies on Neurochemical Alterations

Subject	Exposure Protocol	Peak Blood Pb or [Pb] used	Observed Effects	Reference
rat PND 50	750 or 1500 ppm $Pb(Ac)_2$ chow from 10 d pre-mating to PND 50	31.9 µg/dL	Reductions in NMDAR receptors result in deficits in LTP and spatial learning	Nihei et al. (2000)
	calcineurin in mixture	10 - 2000 pM $Pb(NO_3)_2$	Has a stimulatory (low) and inhibitory (high) effect on calcineurin	Kern and Audesirk (2000)
adult rat	cerebrocortical membranes	0.01-4 µM free $Pb(Ac)_2$	Pb binds to the NMDA receptor channel in a site different from zinc	Lasley and Gilbert (1999)
adult rat PND 2	0.2% $Pb(Ac)_2$ in water and chow	52.9 µg/100 mL	GLU & GABA release are inhibited independent of Pb exposure period	Lasley et al. (1999)
rat	Cultured hippocampal neurons	0.01-10 µM Pb Cl_2	Inhibits glutamatergic and GABAergic transmission via calcium channel	Braga et al. (1999a)
rat PND 17	Cultured hippocampal neurons	0.1-10 µM Pb Cl_2	Increases tetrodotoxin-insensitive spontaneous release of GLU & GABA	Braga et al. (1999b)
rat PND 7, 14, 21, 28	750 ppm $Pb(Ac)_2$ chow from 14 d pre-mating to experimental use	59.87 µg/dL	NMDAR-2A subunit protein expression is reduced in the hippocampus	Nihei and Guilarte (1999)
rat PND 7, 14, 21, 28	750 ppm $Pb(Ac)_2$ chow from 14 d pre-mating to experimental use	59.87 µg/dL	Alters the levels of NMDA receptor subunits mRNA in hippocampus	Guilarte and McGlothan (1998)
rat PND 22-adult	water - 0.2% $Pb(Ac)_2$ from gestational day 16 to PND 21	—	Induces loss of septohippocampal cholinergic projection neurons in neonates lasting into young adulthood	Bourjeily and Suszkiw (1997)
rat PND 28 56, 112	water - 1000 ppm $Pb(Ac)_2$ from gestational day 4-use	39.6 µg/dL	Significant increase in $[^3H]$MK-801 binding after chronic exposure	Ma et al. (1997)

Table AX5-3.1 (cont'd). Summary of Key Studies on Neurochemical Alterations

Subject	Exposure Protocol	Peak Blood Pb or [Pb] used	Observed Effects	Reference
rat PND 21-adult	50 or 150 ppm $Pb(Ac)_2$ water for 2 weeks - 8 mo	28.0 µg/dL	Differential effects in [^{3}H]MK-801 binding with dopamine & D_1 agonists	Cory-Slechta et al. (1997)
adult rat	water - 0.2% $Pb(Ac)_2$ from PND 0 - adult	37.2 µg/100 mL	Presynaptic glutamatergic function in dentate gyrus is diminished	Lasley and Gilbert (1996)
rat - 4 mo	water - 0.2% $Pb(Ac)_2$ from gestational day 16 to PND 28	22.0 µg/dL	Developmental Pb results in long-lasting hippocampal cholinergic deficit	Bielarczyk et al. (1996)
rat PND 111	water at 50 ppm $Pb(Ac)_2$ for 90 d; start at PND 21	18 µg/dL	Decreases in vivo release of dopamine in the nucleus accumbens	Kala and Jadhav (1995)
	Cultured bovine chromaffin cells	variable kind & concentration	Exerts dual stimulatory and inhibitory effects on adrenal PKC	Tomsig and Suszkiw (1995)
rat	Homogenized cortex	ranging $Pb(Ac)_2$	Pb activates PKC in the range of 10^{-11} to 10^{-8} M	Long et al. (1994)
	Cultured bovine chromaffin cells	variable kind & concentration	Pb and calcium activate the exocytotic release of norepinephrine	Tomsig and Suszkiw (1993)
			Review paper discussing Pb-calcium interactions in Pb toxicity	Simons (1993)
			Review paper exploring Pb as a calcium substitute	Goldstein (1993)
rats PND 14 or 56	neuronal membranes	chow containing 750 ppm $Pb(Ac)_2$	Inhibitory effect on [^{3}H]MK-801 binding & loss of binding sites in neonates	Guilarte and Miceli (1992)
rat	cortical synaptosomes	1-50 nM free Pb or 1 µM $Pb(NO_3)_2$	Triggers acetylcholine release more effectively than calcium	Shao and Suszkiw (1991)

Table AX5-3.1 (cont'd). Summary of Key Studies on Neurochemical Alterations

Subject	Exposure Protocol	Peak Blood Pb or [Pb] used	Observed Effects	Reference
rat	hippocampal neurons	2.5-50 μM Pb Cl_2	Pb has a blocking effect on the NMDA subtype of glutamate receptors	Alkondon et al. (1990)
rat	brain protein kinase C	10^{-10} M Pb salts	Stimulates brain protein kinase C and diacylglycerol-activated calcium	Markovac and Goldstein (1988)

Table AX5-3.2. Summary of Key Studies on Neurophysiological Assessments

Subject	Exposure Protocol	Peak Blood Pb or [Pb] used	Observed Effects	Reference
rat PND 22	250 ppm $Pb(Ac)_2$ 3-6 weeks (electro) or 7-13 weeks (immuno)	30.8 µg/dL	Reduces midbrain dopamine impulse flow & decreases dopamine D_1 receptor sensitivity in nucleus accumbens	Tavakoli-Nezhad and Pitts (2005)
rat PND 42-64	100, 250, or 500 ppm $Pb(Ac)_2$ in chow for 3-6 w	54.0 µg/dL	Decrease in number of spontaneously active midbrain dopamine neurons	Tavakoli-Nezhad et al. (2001)
			Review paper examining glutamatergic components contributing to impairments in synaptic plasticity	Lasley and Gilbert (2000)
rat PND 130-210	0.2% $Pb(Ac)_2$ in water	75.4 µg/dL	Deficits in synaptic plasticity in the dentate gyrus from early exposure	Gilbert et al. (1999a)
adult rat	water - 0.1-1.0% $Pb(Ac)_2$ from gestational day 16 to adult	117.6 µg/dL	Biphasic dose-dependent inhibition of hippocampal LTP	Gilbert et al. (1999b)
adult rat	0.2% $Pb(Ac)_2$ in water	30.1 µg/dL	Chronic Pb exposure significantly decreases range of synaptic plasticity	Zhao et al. (1999)
adult rat	0.2% $Pb(Ac)_2$ in water PND 0-21	30.1 µg/dL	Impairments in LTP and paired-pulse facilitation in the hippocampal DG	Ruan et al. (1998)
rat PND 90-130	750 ppm $Pb(Ac)_2$ chow from 50 d pre-mating to experimental use	16.04 µg/100 mL	NMDA-dependent forms of synaptic plasticity are more vulnerable than NMDA-independent potentiation or paired pulse-facilitation	Gutowski et al. (1998)
rat 7-18 mo	water - 0.2% $Pb(Ac)_2$ from gestational day 16 to experimental use	—	Impairs ability to maintain LTP over time in the dentate gyrus	Gilbert and Mack (1998)
rat PND 13-140	750 ppm $Pb(Ac)_2$ chow from 50 d pre-mating to experimental use	28.5 µg/dL	Paired-pulse stimulation of CA3 region shows inhibitory mechanisms	Gutowski et al. (1997)
adult rat	water - 0.2% $Pb(Ac)_2$ from PND 0 - adult	—	Chronic Pb increases the threshold for LTP in dentate gyrus in vivo	Gilbert et al. (1996)
rat PND 4-30	Hippocampal neurons	1-100 µM Pb Cl_2	Identified the nicotinic acetylcholine receptor as a target for Pb	Ishihara et al. (1995)
rat	750 ppm $Pb(Ac)_2$ chow from 50 d pre-mating to experimental use	16.2 µg/100 mL	LTP and learning are impaired if exposed to Pb in the immature brain	Altmann et al. (1993)

Table AX5-3.3. Summary of Key Studies on Changes in Sensory Function

Subject	Exposure Protocol	Peak Blood Pb or [Pb] used	Observed Effects	Reference
mice PND 7-90	0.15 % $Pb(Ac)_2$ in dams water from PND 0-21	26 µg/dL	Produces a rod photoreceptor-selective apoptosis inhibited by Bcl-xl overexpression	He et al. (2003)
	rat retinas	0.01-10 µM $Pb Cl_2$	Pb & calcium produce rod photoreceptor cell apoptosis via mitochondria	He et al. (2000)
rat PND 21 or 90	0.02% & 0.2% $Pb(Ac)_2$ in dams water PND 0-21 & 3 weeks as adult	59.0 µg/dL	Functional alterations and apoptotic cell death in the retina	Fox et al. (1997)
monkey 13 years	2 mg/kg/day $Pb(Ac)_2$ in capsule for 13 y	168.0 µg/dL	Mild increase in detection of pure tones outside of threshold	Rice (1997)
monkey	350 or 600 mg $Pb(Ac)_2$ for 9.75 years	55 µg/dL	Consistent prolongations of latencies on the brain stem auditory evoked potential	Lilienthal and Winneke (1996)
	bovine retinas	50 pM-100 nM $Pb(Ac)_2$	Direct inhibition of purified rod cGMP PDE, magnesium can reverse effect	Srivastava et al. (1995)
	rat retinas	10^{-9} to 10^{-4} M	Alters several physiological & biochemical properties of rod photoreceptors	Fox et al. (1994)
			Review paper examining effects upon auditory and visual function	Otto and Fox (1993)
adult rat	0.02% & 0.2% $Pb(Ac)_2$ in dams water PND 0-21	59.4 µg/dL	Inhibits adult rat retinal, but not kidney, Na^+, K^+-ATPase	Fox et al. (1991)
monkey 6 yr	glycerine capsule with 25 or 2000 µg/kg/day $Pb(Ac)_2$	220 µg/dL	Morphological damage in the visual cortical area V1 and V2	Reuhl et al. (1989)
rat PND 90	0.2% $Pb(Ac)_2$ in dams water PND 0-21	0.59 ppm	Long-term selective deficits in rod photoreceptor function and biochemistry	Fox and Farber (1988)

Table AX5-3.4. Summary of Key Studies on Neurobehavioral Toxicity

Subject	Exposure Protocol	Peak Blood Pb or [Pb] used	Observed Effects	Reference
adult rat	16 mg $Pb(Ac)_2$ via gavage 30 d pre- pregnancy to PND 21	83.2 µg/dL	Developmental Pb exposure results in enhanced acquisition of cocaine self-administration	Rocha et al. (2005)
rat PND 70	16 mg $Pb(Ac)_2$ via gavage 30 d pre-pregnancy to PND 21	53.24 µg/dL	Increased sensitivity to cocaine in rats perinatally exposed to Pb	Nation et al. (2004)
rat PND 120	16 mg $Pb(Ac)_2$ via gavage 30 d pre-pregnancy to PND 21	38.0 µg/dL	Self-administering rats perinatally exposed to Pb demonstrate and increased sensitivity to the relapse phase of cocaine abuse	Nation et al. (2003)
			Review paper examining impairments in complex cognitive function	Cory-Slechta (2003)
rat PND 53	300 or 600 ppm $Pb(Ac)_2$ in drinking water	158 µg/dL	1) Impairment of sustained attention, response initiation, & reactivity to errors 2) Specific deficits in associative ability showing damage to the amygdala or Na_c	Morgan et al. (2001) Garavan et al. (2000)
rat PND 60	8 or 16 mg $Pb(Ac)_2$	6.8 µg/dL	Long-lasting changes in drug responsiveness to cocaine and related drugs	Miller et al. (2001)
adult rat	75 or 300 ppm $Pb(Ac)_2$	36 µg/dL	Impaired learning of a visual discrimination task	Morgan et al. (2000)
rat PND 80	400 mg/l Pb Cl_2 in dam water PND 1-30	—	Postnatal Pb results in neuroplastic deficits related to long-term memory	Murphy and Regan (1999)
rat 14-22weeks	75 or 300 ppm $Pb(Ac)_2$ in water gestation day 0-experimental use	51 µg/dL	Impairment of reversal learning as an associative deficit	Hilson and Strupp (1997)

Table AX5-3.4 (cont'd). Summary of Key Studies on Neurobehavioral Toxicity

Subject	Exposure Protocol	Peak Blood Pb or [Pb] used	Observed Effects	Reference
adult rat	100 or 350 ppm $Pb(Ac)_2$ in water PND 21-use	35.0 µg/dL	Post-washout decrease in sensitivity to MK-801	Cory-Slechta (1997)
adult rat	50 or 150 ppm $Pb(Ac)_2$ in water PND 21-use	30.6 µg/dL	1) Enhances the stimulus properties of NMDA via a possible dopaminergic path 2) Low level Pb exposure is associated with D_1 subsensitivity.	Cory Slechta et al. (1996a and 1996b)
adult rat	500 ppm $Pb(Ac)_2$ in water for adult rats	28.91 µg/dL	Decreases sensitization to the locomotor-stimulating effects of cocaine	Nation et al. (1996)
			Review examining the similarities between experimental & epidemiological data	Rice (1996)
rat 22 weeks	75 or 300 ppm $Pb(Ac)_2$ in water PND 25 - use	39 µg/dL	Significantly impaired on the alteration task with variable intertrial delays	Alber and Strupp (1996)
rat	50 or 150 ppm $Pb(Ac)_2$ in water PND 21-use	—	Altered cholinergic sensitivity due to Pb and several agonists	Cory-Slechta and Pokora (1995)
rat	50 or 150 ppm $Pb(Ac)_2$ in water PND 21-use	35.7 µg/dL	Postweaning lead exposure resulted in a MK-801 subsensitivity	Cory Slechta (1995)
rat	50 or 250 ppm $Pb(Ac)_2$ in water PND 21-use	73.5 µg/dL	1) Potentiates the effects of NMDA on repeated learning 2) Learning impairments are not caused by changes in dopaminergic function	Cohn and Cory-Slechta (1994 a,b)
adult rat	500 ppm $Pb(Ac)_2$ in water for adult rats	20.9 µg/dL	Chronic Pb exposure attenuated the reinforcing effect of brain stimulation	Burkey and Nation (1994)
monkeys	mother's blood Pb from gestation week 5-birth	21 to 79 µg/dL	Reduced sensitivity to changes in reinforcement contingencies during behavioral transitions and in steady state	Newland et al. (1994)

Table AX5-3.4 (cont'd). Summary of Key Studies on Neurobehavioral Toxicity

Subject	Exposure Protocol	Peak Blood Pb or [Pb] used	Observed Effects	Reference
rat	50 or 250 ppm $Pb(Ac)_2$ in water PND 21-use	73.5 µg/dL	1) Impairs selective learning that are not a result of non-specific performance or motivational efforts	Cohn et al. (1993);
			2) Subsensitivity to the accuracy-impairing & rate-altering effects of MK-801 on a multiple schedule of repeated learning and performance	Cohn and Cory-Slechta (1993)
adult rat	500 ppm $Pb(Ac)_2$ in chow for 105 days	28 µg/dL	Chronic Pb exposure attenuates cocaine-induced behavioral activation	Grover et al. (1993)
young rat	100 or 350 ppm $Pb(Ac)_2$ in dams water PND 0-21	34 µg/dL	Induces functional D_2-D_3 supersensitivity to the stimulus properties of agonists	Cory-Slechta et al. (1992)
monkeys 3 or 7 yr	1500 µg/kg/day $Pb(Ac)_2$ in glycerine capsule	36 µg/dL	Pb exposure during different developmental periods produce different effects on FI performance in juveniles versus adults	Rice (1992a)
monkeys 8-9 yr	1500 µg/kg/day $Pb(Ac)_2$ in glycerine capsule	36 µg/dL	Impairment on concurrent discrimination tasks	Rice (1992b)
monkeys 0.5 or 3 yr	2000 µg/kg/day $Pb(Ac)_2$ in glycerine capsule	115 µg/dL	Decreased interresponse times & a greater ratio of responses per reinforcement on the differential reinforcement of low rate schedule	Rice (1992c)
rat	50 or 250 ppm $Pb(Ac)_2$ in water PND 21-use	73.2 µg/dL	Increased sensitivity to the stimulus properties of dopamine D_1 & D_2 agonists	Cory-Slechta and Widowski (1991)
monkey 7-8 yr	1500 µg/kg/day $Pb(Ac)_2$ in glycerine capsule	36 µg/dL	Pb exposure in only infancy impairs spatial discrimination reversal tasks	Rice (1990)
monkey 6-7 yr	1500 µg/kg/day $Pb(Ac)_2$ in glycerine capsule	36 µg/dL	There is not an early critical period for impairment on spatial delayed alternation tasks	Rice and Gilbert (1990a)
monkey 5-6 yr	1500 µg/kg/day $Pb(Ac)_2$ in glycerine capsule	36 µg/dL	Post-infancy exposure impairs nonspatial discrimination reversal while exposure during infancy exacerbates the effect.	Rice and Gilbert (1990b)

Table AX5-3.5. Summary of Key Studies on Cell Morphology and Metal Disposition

Subject	Exposure Protocol	Peak Blood Pb or [Pb] used	Observed Effects	Reference
rat PND 110	water - 0.2% $Pb(Ac)_2$ from gestational day 16 to PND 21 or use	—	Reduction in hippocampal neurogenesis with no spatial learning impairments	Gilbert et al. (2005)
	Rat C6 glioma cells & human astrocytoma cells	5-10 µM $Pb(Ac)_2$	Directly targets GRP78 & induces its compartmentalized redistribution GRP78 plays a protective role in Pb neurotoxicity	Qian et al. (2005)
rat PND 60	1500 ppm $Pb(Ac)_2$ for 30-35 days	20.0 µg/dL	Significant deleterious effects on progenitor cell proliferation	Schneider et al. (2005)
rat embryos	Cultured neurospheres	0.1-100 µM $Pb(Ac)_2$	Differentially affects proliferation & differentiation of embryonic neural stem cells originating from different brain regions	Huang and Schneider (2004)
	Cultured oligodendrite progenitor cells- PND 2	1 µM $Pb(Ac)_2$	Pb inhibition of proliferation & differentiation of oligodendrocyte cells requires PKC	Deng and Poretz (2002)
	Cultured oligodendrite progenitor cells- PND 2	0.1-100 µM $Pb(Ac)_2$	Interferes with maturation of oligodendrocyte progenitor cells	Deng et al. (2001)
	Cultured cerebellar granule neurons	5-50 µM $Pb(NO_3)_2$ or $Pb(ClO_4)_2$	Specific transport systems carry Pb into neurons	Mazzolini et al. (2001)
rat	Cultured C6 glioma cells	1 µM $Pb(Ac)_2$	Induces GRP78 protein expression and GRP78 is a strong Pb chelator	Qian et al. (2000)
rat and human	Cultured rat astroglial, human neuroblastoma	1 µM $Pb(Ac)_2$	Immature astroglia vs. neuronal cells are most likely to bind Pb in the brain	Lindahl et al. (1999)
rat and human	PKC isozymes	10^{-12} to 10^{-4} M $Pb(Ac)_2$	Concentration dependent differential effects of Pb upon PKC isozymes	Sun et al. (1999)
	Cultured GH3, C6, and HEK293 cells	1-10 µM $Pb(NO_3)_2$	Cellular uptake of lead is activated by depletion of intracellular calcium	Kerper and Hinkle (1997)

Table AX5-3.5 (cont'd). Summary of Key Studies on Cell Morphology and Metal Disposition

Subject	Exposure Protocol	Peak Blood Pb or [Pb] used	Observed Effects	Reference
frog tadpoles	Elvax implantation for 6 weeks	10^{-10} to 10^{-6} M $PbCl_2$	Stunted neuronal growth from low Pb levels are reversible with chelator	Cline et al. (1996)
rat	Cultured hippocampal neurons	100 nM $PbCl_2$	Possible neurite development inhibition via hyperphosphorylation	Kern and Audesirk (1995)
adult rat	Pb binding protein of kidney and brain	0.1-1.6 µM	Attenuation of Pb inhibition of ALAD involves sequestration of Pb and a donation of zinc to the enzyme	Goering et al. (1986)

CHAPTER 5 ANNEX

ANNEX TABLES AX5-4

Table AX5-4.1. Effect of Lead on Reproduction and Development in Mammals* Effects on Offspring

Citation	Species/ Strain/Age	Dose/Route/ Form/Duration	Endpoint	Blood Lead Concentration (PbB)
al-Hakkak et al. (1998)	Mouse/BALB/c, weaning	0, 25, 50 mg lead monoxide alloy/kg in chow for 35–70 days	Reduced number of spermatogenia and spermatocytes in the 50 mg group after 70 days; reduced number of implantations after mating (after 35 days exposure).	PbB not reported
Appleton (1991)	Rat/Long-Evans hooded, adult	Lead acetate single dose by i.v. at 30 mg/kg	Increase in serum calcium and phosphorous; SEM analysis revealed 'lead line' in tooth that was composed of hypomineralized interglobular dentine.	PbB not reported
Bataineh et al. (1998)	Rat/Sprague-Dawley, adult	1000 ppm lead acetate in drinking water for 12 weeks	Fertility was reduced; total number of resorptions was increased in female rats impregnated by males.	PbB not reported
Berry et al. (2002)	Rat/Sprague-Dawley, 21 days old	Lead nitrate (1000 ppm lead) in drinking water for 6 weeks	Mean plasma growth hormone levels decreased by 44.6%; reduced mean growth hormone amplitude by 37.5%, mean nadir concentration by 60%, and growth hormone peak area by 35%; findings are consistent with decreased hypothalamic growth hormone-releasing factor secretion or reduced somatotrope responsiveness; exogenous growth hormone in lead-treated and control rats, this response was blunted by the lead treatment; plasma IGF1 concentration was not significantly affected by lead treatment.	PbB 37.40±3.60 µg/dL
Bogden et al. (1995)	Rat/Sprague-Dawley, 12 weeks old	250 mg/L of lead acetate in drinking water from GD 1 until after 1 week after weaning	Dam and pup hemoglobin concentrations, hematocrit, and body weights and lengths were reduced.	PbB <15 µg/dL
Camoratto et al. (1993)	Rat/Sprague-Dawley, adult	0.02% lead nitrate in drinking water from gestation day 5 of dams until PND 4 of offspring	Female pups exposed to lead beginning in utero were smaller, no corresponding effect in males; pituitary responsiveness to a hypothalamic stimulus.	PbB 17–43 µg/dL
Corpas (2002a)	Rat/Wistar, adult	Lead acetate 0 or 300 mg/L in drinking water during gestation and lactation	Alterations in hepatic system of neonates (PND 12) and pups (PND 21); reductions in hemoglobin, iron, alkaline and acid phosphatase levels, and hepatic glycogen, and elevated blood glucose.	PbB ~22 µg/dL
Corpas (2002b)	Rat/Albino (NOS), adult	Lead acetate 0 or 300 mg/L in drinking water during gestation and lactation	Effects energy metabolism; decrease in testis and seminal vesicle weights, and an increase in DNA and RNA levels on PN day 21; protein was significantly decreased, alkaline and acid phosphatase levels of the gonads were reduced; reduction of the thickness of the epithelium and seminiferous tubule diameter.	PbB 54–143 µg/dL

Table AX5-4.1 (cont'd). Effect of Lead on Reproduction and Development in Mammals* Effects on Offspring

Citation	Species/ Strain/Age	Dose/Route/ Form/Duration	Endpoint	Blood Lead Concentration (PbB)
Cory-Slechta et al. (2004†)	Rat/Long-Evans, adult	Lead acetate in drinking water (150 ppm); 2 months before breeding until the end of lactation 14 rats no maternal stress lead exposure, 15 rats no maternal stress with lead exposure, 18 rats maternal stress without lead exposure, 23 rats maternal stress and lead exposure	Pb alone (in male) ($p<0.05$) and Pb plus stress (in females) ($p<0.05$) permanently elevated corticosterene levels in offspring.	PbB 30–40 µg/dL
Dey et al. (2001)	Mouse/ Albino (NOS), ~100 g	lead citrate 5 µg/kg-d p.o. from early pregnancy (NOS) until birth	Perforations, tissue damage, cell deformity, disordered organization of collagen bundles found in offspring; reduction in the symmetry of sulphate group of skin pups of mice exposed to lead citrate (5 µg/kg-d) throughout gestation exhibited a variety of skin anomalies, including perforations, tissue damage, cell deformity, and disordered collagen bundles lead was found to affect initial genomic expression in embryos fathered by male rats.	PbB not reported
Flora and Tandon (1987)	Rat/Albino (NOS), adult	Lead nitrate dissolved in water 2–20 mg/kg-d i.v. on day 9, 10, 11 of gestation; 6 rats in each group (0, 5, 10, 20, 40 mg/kg lead)	Dose-dependant increase in external malformations at all doses ($p<0.001$), particularly tail defects; dose dependant decrease in number of live births at 20 and 400 mg/kg ($p<0.001$); dose-dependent increase in number of resorptions per dam at ≤10 mg/kg ($p<0.01$).	PbB 13–45 µg/dL
Fox et al. (1991)	Rat/Long-Evans hooded, adult	Lactation exposure via dams exposed to 0.02 or 0.2% lead in drinking water from PND 1 through weaning (PND 21) 8 female pups per litter (number of litter unspecified) control pups, 8 pups for litter (number of litter unspecified) low level exposure pups, 8 pups per litter (number of litter unspecified) moderate level exposure pups	Long-term, dose-dependent decreases retinal Na/K ATPase activity in the female offspring (only female pups were used) (-11%; -26%) ($p<0.05$).	PbB 18.8 or 59.4 µg/dL at weaning

Table AX5-4.1 (cont'd). Effect of Lead on Reproduction and Development in Mammals* Effects on Offspring

Citation	Species/ Strain/Age	Dose/Route/ Form/Duration	Endpoint	Blood Lead Concentration (PbB)
Fox et al. (1997†)	Rat/Long-Evans hooded, adult	0.02 or 0.2% lead acetate in drinking water from PND 0-PND 21; 8 female pups per litter control pups; 8 pups per litter low level exposure; 8 pups per litter moderate level exposure (number of litters per dose unspecified)	Developmental and adult lead exposure for 6 weeks produced age and dose-dependent retinal degeneration such that rods and bipolar cells were selectively lost; at the ultrastructural level, all dying cells exhibit the classical morphological features of apoptotic cell death; decrease in the number of rods was correlated with the loss of rhodopsin content per eye confirming that rods were directly affected by lead ($p<0.05$); single-flash rod ERGs and cone ERGs obtained from lead-exposed rats demonstrated that there were age- and dose-dependent decreases in the rod a-wave and b-wave sensitivity and maximum amplitudes without any effect on cones; in adult rats exposed to lead for three weeks, qualitatively similar ERG changes occurred in the absence of cell loss or decrease in rhodopsin content ($p<0.05$); developmental and adult lead exposure for three and six weeks produced age- and dose-dependent decreases in retinal cGMP phosphodiesterase (PDE) activity resulting in increased CGMP levels ($p<0.05$); retinas of developing and adult rats exposed to lead exhibit qualitatively similar rod mediated ERG alterations as well as rod and bipolar apoptotic cell death ($p<0.05$); similar biochemical mechanism such as the inhibition of rod and bipolar cell cGMP PDE, varying only in degree and duration, underlies both the lead-induced ERG rod-mediated deficits and the rod and bipolar apoptotic cell death ($p<0.05$).	PbB weanlings 19±3 (low exposure) or 59±8 µg/dL (moderate exposure), adult 7±2 µg/dL (at PND 90)
Gandley et al. (1999)	Rat/Sprague-Dawley, adult	Male rats exposed to 25 or 250 ppm acetate lead in drinking water for at least 35 days prior to breeding	Fertility was reduced in males with PbB in range 27–60 µg/dL, lead was found to affect initial genomic expression in embryos fathered by male rats with blood lead levels as low as 15–23 µg/dL; dose-dependant increases were seen in an unidentified set of proteins with a relative molecular weight of approximately 70 kDa.	PbB 27–60 µg/dL (fathers) 15–23 µg/dL (offspring)
Govoni et al. (1984)	Rat/Sprague-Dawley, adult	2.5 mg/mL lead acetate in drinking water from GD 16 to postnatal week 8	Decreased sulpiride binding in the pituitary is consistent with the elevated serum PRL concentrations previously described in lead-exposed rats; DOPAc concentrations were reduced by 21% in lead-treated rats.	PbB 71±8 µg/dL
Hamilton et al. (1994)	Rat/Sprague-Dawley, 25 days old	Lead acetate in drinking water at 250, 500 or 1000 ppm; 8 weeks prior to mating through GD 21	Altered growth rates; reduced early postnatal growth; decreased fetal body weight.	PbB 40–100 µg/dL
Han et al. (2000)	Rat/Sprague-Dawley, 5 weeks old	250 mg/mL lead acetate in drinking water for 5 weeks followed by 4 week no exposure (mated at end of 4-week no exposure period)	Pups born to lead-exposed dams had significantly ($p<0.0001$) lower mean birth weights and birth lengths.	PbB 10–70 µg/dL
Hannah et al. (1997)	Mouse/Swiss ICR preimplantation embryos	In vitro incubation of two- and four-cell embryos with 0.05-200 µM lead acetate for 72 hours (time required for blastocyst formation)	Exposure of embryos to lead was only toxic at 200 µM, which reduced cell proliferation and blastocyst formation.	PbB not reported

Table AX5-4.1 (cont'd). Effect of Lead on Reproduction and Development in Mammals* Effects on Offspring

Citation	Species/ Strain/Age	Dose/Route/ Form/Duration	Endpoint	Blood Lead Concentration (PbB)
Iavicoli et al. (2003)	Mouse/Swiss, adult	Lead acetate in food (0.02, 0.06, 0.11, 0.2, 2, 4, 20, 40 ppm) exposure began 1 day after mating until litter was 90 days old one litter of mice exposed to each dietary concentration	Low-level exposure (PbB 2–13 µg/dL) reduced red cell synthesis ($p<0.05$); high-level exposure (PbB 0.6–2 µg/dL) enhanced red cell synthesis ($p<0.05$).	PbB 0.6 to <2.0 µg/dL or >2.0-13 µg/dL
Iavicoli et al. (2004)	Mouse/Swiss, adult	Lead acetate in feed; exposure began 1 day after mating until litter was 90 days old	In females: accelerated time to puberty at PbB <3 µg/dL; delayed time to puberty at 3–13 µg/dL.	PbB 0.6 to <2.0 µg/dL or >2.0-13 µg/dL
Lögdberg et al. (1987)	Monkey/ Squirrel, adult	Lead acetate p.o. exposure of gravid squirrel monkeys from week 9 of gestation through PND 0	Increase in pre- and perinatal mortality among squirrel monkeys receiving lead acetate p.o. during the last two-thirds of pregnancy (45% vs. 7–8% among controls); mean maternal PbB was 54 µg/dL (39–82 µg/dL); statistically significant reductions in mean birth weight were observed in lead exposed monkeys as compared to controls; effects occurred without clinical manifestation of toxic effects in the mothers.	PbB 54 µgdL (39–82 µgdL)
Lögdberg et al. (1998)	Monkey/ Squirrel, adult	Lead acetate (varying concentrations ≤ 0.1% in diet); maternal dosing from 5–8.5 weeks pregnant to PND 1; 11 control monkeys, 3 low-lead exposure group (PbB 24 µg/dL), 7 medium lead group (PbB 40 µg/dL, 5 high-lead group (PbB 56 µg/dL)	Dose-dependent reduction in placental weight ($p<0.0007$); various pathological lesions were seen in the placentas (n=4), including hemorrhages, hyalinization of the parenchyma with destruction of the villi and massive vacuolization of chorion epithelium; effects occurred without clinical manifestation of toxic effects in the mothers.	Mean maternal PbB 37 µgdL (22–82 µgdL) 24 (22–26) µg/dL (low dose) 40 (35–46) µg/dL (mid dose) 56 (43–82) µg/dL (high dose)
McGivern et al. (1991†)	Rat/Sprague-Dawley, adult	0.1% lead acetate in drinking water from GD 14 to parturition	Male offspring of dams exhibited reduced sperm counts, altered male reproductive behavior, and enlarged prostates later in life; females exhibited delayed puberty, menstrual irregularities, and an absence of observable corpora lutea; males and females exhibited irregular release patterns of both FSH and LH later in life.	PbB 73 µg/dL
Nayak et al. (1989)	Mouse/Swiss Webster, adult	Lead nitrate dissolved in NaCl solution, administered intravenously, via caudal vein at dose levels of 100, 150, 200 mg/kg; one time exposure on GD 9	Chemical analysis showed lead was readily transferred across placenta; lead caused moderate, statistically significant, increase in frequency of SCEs in maternal bone marrow cells and significant reduction in NRs at the 2 highest dose levels (150 and 200 mg/kg); animals showed several specific chromosomal aberrations, mostly deletions, in maternal bone, marrow, and fetal cells; aneupoidy was found to be frequently associated with the lowest dose levels of lead nitrate (100 mg/kg); increased embryonic resorption and reduced placental weights.	PbB levels at birth in the exposure groups for these studies were >180 µg/dL
Piasek and Kostial (1991)	Rat/Wistar, 10 weeks old	7500 ppm lead acetate in drinking water for 9 weeks	Decrease in litter size, pup survival, and birth weight; food consumption, body weight, and fertility were not altered in 20 week exposure period.	Maternal PbB >300 µg/dL Offspring PbB >220 µg/dL

Table AX5-4.1 (cont'd). Effect of Lead on Reproduction and Development in Mammals* Effects on Offspring

Citation	Species/ Strain/Age	Dose/Route/ Form/Duration	Endpoint	Blood Lead Concentration (PbB)
Pinon-Lataillade et al. (1995)	Mouse/NMRI, adult	0–0.5% lead acetate in drinking water exposed to lead during gestation until post-GD 60	Lead exposure during gestation reduces litter size; reduced birth weight and growth rates.	PbB <4–132 µgdL
Pillai and Gupta (2005)	Rat/Charles Foster, 200–220 g	Subcutaneoous injection of 0.05 mg/kg-d lead acetate for 5–7 days prior to mating through PND 21	Long term exposure of rats (premating, gestational, and lactational) to moderate levels of lead acetate (s.c.) resulted in reduced activities of hepatic steroid (E2) metabolizing enzymes (17-ß-hydroxy steroid oxidoreductase and UDP glucuronyl transferase) and decreased hepatic CYP450 content.	PbB not reported
Ronis et al. (1996†)	Rat/Sprague-Dawley, 22, 55 days or plug-positive time-impregnated	0.6% lead acetate in drinking water for various durations: PND 24–74 (pubertal exposure), PND 60–74 (post pubertal exposure); 11 males and females in pubertal exposure group (10 each in control pubertal group); 6 males and females post-pubertal exposure and control groups	Reduction in serum testosterone levels in male, not female; in female suppression of circulating E2 ($p<0.05$) and LH ($p<0.05$); reduction in male secondary sex organ weight ($p<0.0005$); delayed vaginal opening and disrupted diestrous in females ($p<0.005$); increased incidence of stillbirth (2% control vs. 19% Pb) ($p<0.005$).	In utero PbB 250–300 µg/dL Pre-pubertal PbB 30–60 µg/dL Post-pubertal PbB 30–60 µg/dL PbBs in the dams and offspring in this experiment were >200 µg/dL.
Ronis et al. (1998a†)	Rat/Sprague-Dawley, various ages	0.6% lead acetate in drinking water ad libitum for various durations: GD 5 to PND 1, GD 5 to weaning, PND 1 to weaning 3 control litters, 2 gestation exposure litters, 2 lactation exposure litters, 2 gestation and lactation exposure litters, 2 postnatal litters, 2 chronic litters (4 male and 4 female pups per litter)	Dose-dependent delay in sexual maturation (delayed vaginal opening) ($p<0.0002$) following prenatal lead exposure that continued until adulthood (85 days old); reduced birth weight ($p<0.05$), more pronounced among male pups.	Group: pup PbB Naïve: ~6 µg/dL Control: <2 µg/dL Gest: ~10 µg/dL Lact: ~3 µg/dL Gest+Lact: ~13 µg/dL Postnatal: ~260 µg/dL Chronic: ~287 µg/dL
Ronis et al. (1998b†)	Rat/Sprague-Dawley, adult	Lead acetate in drinking water (0.05% to 0.45% w/v); dams exposed until weaning, exposure of pups which continued until PND 21, 35, 55, or 85; 5 control litters (0%), 10 low-dose litters (0.05%), 8 mid-dose litters (0.15%), 9 high-dose litters (0.45%); 4 male and 4 female pups per litter	Prenatal lead exposure that continues until adulthood (85 days old) delays sexual maturation in female pups in a dose-related manner ($p<0.05$); birth weight reduced ($p<0.05$), more pronounced among male pups; decreased growth rates ($p<0.05$) in both sexes accompanied by decrease in plasma concentrations of IGF1 through puberty ($p<0.05$) and a significant increase in pituitary and growth hormone during puberty ($p<0.05$).	PbBs in the pups between the ages of 21 and 85 days were >100 µg/dL and reached up to 388 µg/dL.

Table AX5-4.1 (cont'd). Effect of Lead on Reproduction and Development in Mammals* Effects on Offspring

Citation	Species/ Strain/Age	Dose/Route/ Form/Duration	Endpoint	Blood Lead Concentration (PbB)
Ronis et al. (1998c)	Rat/Sprague-Dawley, adult	Lead acetate 0.05, 0.15, or 0.45% in drinking water beginning GD 5 continuing until PND 21, 35, 55, or 85; 5 control litters (0%), 10 low-dose litters (0.05%), 8 mid-dose litters (0.15%), 9 high-dose litters (0.45%); |4 male and 4 female pups per litter	Dose-responsive decrease in birth weight ($p<0.05$), and crown-to-rump length ($p<0.05$); dose-responsive delay in sexual maturity in male ($p<0.05$) and female ($p<0.05$); neonatal decrease in sex steroids ($p<0.05$); pubertal decrease in testosterone (male) ($p<0.05$) and E2 (female) ($p<0.05$); decrease estrous cyclicity at high dose ($p<0.05$).	Dams: 0, 48, 88, or 181 µg/dL Pups PND 1: <1, ~40, ~70, or >120 µg/dL Pups PND 21: <1, >50, >160, or ~237 µg/dL Pups PND 35: <1, ~22, >70, or >278 µg/dL Pups PND 55: <1, >68, >137, or ~380 µg/dL Pups PND 85: <1, >43, >122, or >214 µg/dL
Ronis et al. (2001†)	Rat/Sprague-Dawley, neonate, male (100 days) and female pup	Lead acetate in drinking water to 825 or 2475 ppm ad libitum from G'D 4 to GD 55 postpartum; 1 male and female pup/litter (5 litters per group) control group, 1 male and female pup/litter (5 litters per group) 825 ppm lead acetate group, 1 male and female pup/litter (5 litters per group) 2475 ppm lead acetate group	Dose-dependent decrease of the load of failure in male ($p<0.05$); no difference in plasma levels of vitamin D metabolites; reduced somatic growth ($p<0.05$), longitudinal bone growth ($p<0.05$), and bone strength during the pubertal period ($p<0.05$); sex steroid replacement did not restore skeletal parameters in lead exposed rats; L-Dopa increased plasma IGF_1 concentrations, rates of bone growth, and bone strength measures in controls while having no effect in lead exposed groups; DO gap x-ray density and proximal new endostreal bone formation were decreased in the distration gaps of the lead-treated animals ($p<0.01$); distraction initiated at 0.2 mm 30 to 60 days of age.	PbB at 825 ppm was 67-192 µg/dL PbB at 2475 ppm was 120-388 µg/dL
Sant'Ana et al. (2001)	Rat/Wistar, 90 days old	0.1 and 1% lead in drinking water 7 days	1% Pb exposure reduced offspring body weight during treatment, no changes observed after 0.1% exposure; no altered offspring sexual maturation, higher Pb improved sexual behavior, while 0.1% reduced it; 0.1% Pb caused decrease in testis weight, an increase in seminal vesicle weight, and no changes in plasma testosterone levels, hypothalamic VMA levels were increased compared to control group; reduced birth weight and growth rates.	PbB 36.12±9.49 µg/dL or 13.08±9.42 µg/dL
Singh et al. (1993)b	Rat/ITRC, albino (NOS), 6 weeks old	250, 500, 1000, and 2000 ppm lead nitrate in drinking water from GD 6 to GD 14	Significantly reduced litter size, reduced fetal weight, and a reduced crown-to-rump length, increased resorption and a higher blood-lead uptake in those groups receiving 1000 and 2000 ppm Pb; these also had a higher placental uptake; however the level was the same in both groups; fetal lead uptake remained the same whether or not 2000 ppm lead was given to an iron-deficient or normal iron groups of mothers.	PbB not reported
Watson et al. (1997)	Rat/Sprague-Dawley, adult	Lead in drinking water at 34 ppm from weaning of mothers through gestation and weaning of offspring until birth; 6 pups control group, 6 pups experimental group	Reduced body weight ($p=0.04$); parotid function was decreased by nearly 30% ($p=0.30$); higher mean caries scores than the control pups ($p=0.005$); pre- and perinatal lead exposure had significantly increased susceptibility to dental caries ($p=0.015$).	PbB 48±13 µg/dL

Table AX5-4.1 (cont'd). Effect of Lead on Reproduction and Development in Mammals* Effects on Offspring

Citation	Species/ Strain/Age	Dose/Route/ Form/Duration	Endpoint	Blood Lead Concentration (PbB)
Wiebe et al. (1998)	Rats/Sprague-Dawley, adult	20 or 200 ppm lead chloride in drinking water; prior to pregnancy, during pregnancy, lactation	Exposure to lead did not affect tissue weights but did cause a significant decrease in gonadotropin-receptor binding in the prepubertal, pubertal, and adult females; conversion of progesterone to androstenedione and dihydrotestosterone was significantly decreased in 21-day old rats, in 150-day old females, the exposure to lead resulted in significantly increased conversion to the 5-alpha-reduced steroids, normally high during puberty.	PbB 4.0±1.4 to 6.6±2.3 µg/dL

*Not including effects on the nervous or immune systems.

†Candidate key study.

cGMP, cyclic guanosine--3',5'-monophosphate; DO, distraction osteogenesis; DOPAc, 3,4-dihydroxyphenylacetic acid; E_2, estradiol; ERG, electroretinographic; FSH, follicle stimulating hormone; GD, gestational day; IGF_1, insulin-like growth factor 1; i.v., intravenous; kDA, kilodalton; LH, luteinizing hormone; NOS, not otherwise specified; PbB, blood lead concentration; PDE, phosphodiesterase; PND, post-natal day; p.o., per os (oral administration); s.c., subcutaneous; SEM, standard error mean; UDP, uridine diphosphate; VMA, vanilmandelic acid

Table AX5-4.2. Effect of Lead on Reproduction and Development in Mammals* Effects on Males

Citation	Species/ Strain/Age	Dose/Route/ Form/Duration	Endpoint	Blood Lead Concentration (PbB)
Acharya et al. (2003)	Mouse/Swiss, 6–8 weeks old	200 mg/kg lead acetate through i.p. injection of lead; one time injection	Testicular weight loss with constant increase in the incidence of abnormal sperm population; decrease in sperm count; testicular ascorbic acid also declined significantly; significant rise in LPP of tissue; LPP is indicative of oxidative stress in treated mice testes.	Not reported
Adhikari et al. (2000)	Rat/Druckrey, 28 days old	0.0, 0.4, 4.0, 40.0 µM lead acetate in vitro for 24 and 48 hours	Germ cells progressively detached from Sertoli cell monolayer into medium in a concentration and duration dependent manner Viability of the detached cells showed a decrease with increase in time and concentration of Pb; leakage of LDH recorded at higher dose of 4.0 and 40.0 µM.	PbB not applicable–in vitro study
Adhikari et al. (2001)	Rat/Druckrey, 28 days old	5, 10, and 20 mg/kg lead in distilled water by gavage for 2 weeks	Induced significant numbers of germ cells to undergo apoptosis in the semiferous tubules of rats treated with highest dose; DNA fragmentation was not detected at any of the doses; level of lead accumulation in testes increased in a dose-dependent manner.	PbB not reported
Alexaki et al., 1990	Bulls/Holstein, 3–5 years old	In vitro fertilization 2.5 or 0.25 µg/mL	Sperm motility reduced significantly at 2.5 µg/mL; lower concentration had no effect on sperm motility.	PbB not applicable–in vitro study
al-Hakkak et al. (1998)	Mouse/ BALB/c, weaning	0, 25, 50 mg lead monoxide alloy/kg in chow for 35–70 days	Reduced number of spermatogenia and spermatocytes in the 50 mg group after 70 days; reduced number of implantations after mating (after 35 days exposure).	PbB not reported
Barratt et al. (1989)	Rat/Wistar, 70 days old	0, 0.3, 33, 330 mg lead acetate/kg-d in drinking water, by gavage for 63 days	Increased number of abnormal post-testicular sperm in the highest exposure group; reduced number of spermatozoa at PbB >4.5 µgdL.	PbB 2, 4.5, 7, 80 µgdL PbBs >40 µg/dL
Bataineh et al. (1998)	Rat/Sprague-Dawley, adult	1000 ppm lead acetate in drinking water for 12 weeks	Fertility was reduced in males.	PbB not reported
Batra et al. (2001)	Rat/Portan, 8 weeks old	10, 50, 200 mg/kg lead acetate orally for 3 months	Lead in testis and epididymis increased with dose; administration of zinc reduced lead levels; dose related changes in activities of enzyme alkaline phosphatase and Na+-K+-ATPase, which decreased with increased dose of lead; improvement in activities of enzymes was seen in groups given lead and zinc; disorganization and disruption of spermatogenesis with accumulation of immature cells in lumen of tubule; highest dose of lead resulted in arrest of spermatogenesis, and decrease in germ cell layer population; highest dose levels, damage of basement membrane, disorganization of epithelium and vacuolization cells; tubules were found almost empty, indicating arrest of spermatogenesis.	PbB not reported
Batra et al. (2004)	Rat/Portan, 8 weeks old	10, 50, 200 mg/kg lead acetate orally for 3 months	LH and FSH concentrations were decreased at 200 mg/kg; decrease in fertility status at 200 mg/kg; decline in various cell populations at 200 mg/kg; 50 mg/kg group hormone levels, cell numbers, and fertility status were found close to normal.	PbB not reported
Bizarro et al. (2003)	Mouse/CD-1, adult	0.01 M lead acetate twice a week for 4 weeks	Dose-time relationship was found; ROS role.	PbB not reported
Boscolo et al. (1998)	Rat/Sprague-Dawley, weanling	60 mg lead acetate/mL in drinking water for 18 months	Increased vacuolization in Sertoli cells; no other ultrastructural modifications; no impairment of spermatogenesis.	PbB 4–17 µgdL

Table AX5-4.2 (cont'd). Effect of Lead on Reproduction and Development in Mammals* Effects on Males

Citation	Species/ Strain/Age	Dose/Route/ Form/Duration	Endpoint	Blood Lead Concentration (PbB)
Chowdhury et al. (2001)	Mouse/ BALB/c, 3 months old	0.0, 0.2, 0.5, 1.0, 2.0 µg/mL lead acetate in culture medium for 2 hours (superovulated ova and sperm)	Significant dose dependent decrease in the number of sperm attaching to the ova in both exposed groups; decrease in the incorporation of radio-labeled thymdine, uridine, and methionine.	PbB not applicable–in vitro study
Chowdhury et al. (1984)	Rat/Albino, (NOS), adult	Dietary concentrations of 0.25, 0.50, or 1.0 g/L lead acetate for 60 days	Testicular atrophy along with cellular degeneration was conspicuous at 1 g/L; high cholesterol concentration and significantly low ascorbic acid concentration were found in the testes at 1 g/L; lowest dose (0.25 g/L) had no significant morphological and biochemical alterations, whereas as 0.5 g/L resulted in partial inhibition of spermatogenesis.	PbB 54–143 µgdL
Chowdhury et al. (1986)	Rat/NOS, adult	0, 1, 2, 4, 6 mg lead acetate/kg-d i.p. for 30 days	Dose-related decrease of testis weight; at 187 µgdL: degenerative changes in testicular tissues; at 325 µgdL: degenerative changes and inquiry of spermatogenetic cells; edematous dissociation in interstitial tissue.	PbB 20, 62, 87, 187, or 325 µgdL
Chowdhury et al. (1987)	Rat/Charles Foster, 150±5 g	0, 1, 2, 4, 6 mg lead acetate/kg-d/i.p. for 30 days	Dose related decrease of testis weight at 56 µg of spermatoids; at 91 µgdL: inhibition of post-meiotic spermatogenic cell; at 196 µgdL: decreased spermatogenic cell count (6), detachment of germinal call layers; at 332 µgdL: Decreased spermatogenic cell count, degenerative changes, Interstitial edema, and atrophy of Leydig cells.	PbB 56–3332 µgdL
Coffigny et al. (1994†)	Rat/Sprague-Dawley, adult	Inhalation exposure to 5 mg/m3 lead oxide daily for 13 days during gestation (GD 2, 3, 6–10, 13–17, 20)	Adult male offspring exhibit no change in sperm parameters or sex hormones T, FSH, and LH (because of duration or timing).	PbB 71.1 µg/dL (dam) PbB 83.2 µg/dL (fetal)
Corpas et al. (1995)	Rat/Wistar, adult	300 mg/L lead acetate via drinking water beginning GD 1 through 5 day postnatal or throughout gestation and early lactation	Testicular weight and gross testicular structure were not altered; seminiferous tubule diameter and the number of prospermatogonia were reduced; total DNA, RNA, and protein content of the testes in treated rats was significantly reduced, DNA:RNA ratio remained unaltered.	PbB 14 µgdL
Corpas et al. (2002a)	Rat/Wistar, adult	300 mg/L acetate lead in drinking water beginning at mating until PND 12 and 21	Neither abnormalities in the liver structure nor depositions of lead, toxicant produced biochemical alterations; pups exhibited decrease in hemoglobin, iron and alkaline, and acid phosphatase levels and an increase in Pb content; protein, DNA, and lipid total amounts were reduced, and hepatic glycogen content was diminished at 12 and 21 PN, with a higher dose of glucose in blood; decrease in alkaline phosphatase in liver of pups at day 21 PN, but acid phosphatase was unaltered.	PbB 22 µgdL
Corpas et al. (2002b)	Rat/Wistar, adult	300 mg/L acetate lead in drinking water beginning at mating until PND 12 and 21	Neither abnormalities in the liver structure nor depositions of lead, toxicant produced biochemical alterations; pups exhibited decrease in hemoglobin, iron and alkaline, and acid phosphatase levels and an increase in Pb content; protein, DNA, and lipid total amounts were reduced, and hepatic glycogen content was diminished at 12 and 21 PN, with a higher dose of glucose in blood; decrease in alkaline phosphatase in liver of pups at day 21 PN, but acid phosphatase was unaltered.	PbB 22 µgdL

Table AX5-4.2 (cont'd). Effect of Lead on Reproduction and Development in Mammals* Effects on Males

Citation	Species/ Strain/Age	Dose/Route/ Form/Duration	Endpoint	Blood Lead Concentration (PbB)
Cory-Slechta et al. (2004†)	Rat/Long-Evans, adult	Lead acetate in drinking water beginning 2 months before breeding until the end of lactation	Observed potential effects of lead and stress in female; Pb alone (in male) and Pb plus stress (in females) permanently elevated corticosterene levels in offspring.	PbB 30–40 µg/dL
Foote (1999)	Rabbit/Dutch-belted, adult	0, 0.005, 0.01, and 0.025 mM PbCl2 in vitro; one time dose	Six out of 22 males tested showed appreciable spontaneous hyperactivation, lead did not affect hyperactivation, or associated capacitation.	PbB not applicable–in vitro study
Foster et al. (1993)	Monkey/ Cynomolgus, adult	0–1500 µg lead acetate/kg-d in gelatin capsules p.o. for various durations: 9 control monkeys, 4 monkeys in lifetime group (birth to 9 years), 4 in infancy group (first 400 days of life), 4 in post-infancy exposure (from 300 days to 9 years)	Suppressed LH response to GnRH stimulation in the lifetime group (p=0.0370); Sertoli cell function (reduction in the inhibin to FSH ratio) (p=0.0286) in lifetime and post-infancy groups.	Lifetime group 3–26 µgdL at 4-5 years; infancy group 5–36 µgdL at 100–300 days, 3–3 µgdL at 4-5 years; post-infancy group 20-35 µg/dL
Foster et al. (1996a)	Monkey/ Cynomolgus, adult	0–1500 µg lead acetate/kg-d in gelatin capsules p.o. from birth until 9 years of age: 8 control monkeys, 4 monkeys in low group (6–20 µg/dL), 7 monkeys in high group (22–148 µg/dL)	Mean PbB of 56 µg/dL showed no significant alterations in parameters of semen quality (count, viability, motility, or morphology).	PbB 10±3 or 56±49 µg/dL
Foster et al. (1998)	Monkey/ Cynomolgus, adult	0–1500 µg lead acetate/kg-d in gelatin capsules p.o. for various durations: birth to 10 years (lifetime); PND 300 to 10 years (post-infancy); birth to 300 days (infancy); 3 control monkeys, 4 lifetime, 4 infancy, 5 post-infancy	Circulating concentrations of FSH, LH, and testosterone were not altered by treatment; semen characteristics (count, motility, morphology) were not affected by treatment possibly because not all Sertoli cells were injured; degeneration of seminiferous epithelium in infancy and lifetime groups (no difference in severity between these groups); ultrastructural alterations in seminal vesicles, most prominent in infancy and post-infancy groups.	PbB ~35 µg/dL
Gandley et al. (1999)	Rat/Sprague-Dawley, adult	Male rats received lead acetate 25 or 250 ppm in drinking water for 35 days prior to mating	High dose reduced fertility; low dose altered genomic expression in offspring.	PbB 15–23 µg/dL or 27-60 µg/dL
Gorbel et al. (2002)	Rat/(NOS), 90 days old	3 mg (P1) or 6 mg (P2) lead acetate in drinking water for 15, 30, 45, 60, or 90 days	Male rats, absolute and relative weights of testis, epididymis, prostate and seminal vesicles were found to significantly decrease at day 15 in P2 group and at day 45 in P1 group, at day 60 these absolute values and relative weights returned to control values; at day 15 arrest of cell germ maturation, changes in the Sertoli cells, and presence of apoptotic cells were observed; serum testosterone level was found to be lowered at day 15 in both P1 and P2, and peaked at day 60, then returned to normal values.	PbB not reported

Table AX5-4.2 (cont'd). Effect of Lead on Reproduction and Development in Mammals* Effects on Males

Citation	Species/ Strain/Age	Dose/Route/ Form/Duration	Endpoint	Blood Lead Concentration (PbB)
Graca et al. (2004)	Mouse/CD-1, 2 months old	Subcutaneous injection of 74 mg/kg-d of lead chloride for 1 to 3 days	Reversible changes in sperm (count) and ultrastructural changes in testes (reduced diameter of seminiferous tubules).	PbB not reported
Hsu et al. (1997)	Rat/Sprague-Dawley, 7 weeks old	10 mg/kg lead acetate through i.p. injection to males for 6 or 9 weeks	Six-week group had unchanged epididymal sperm counts, percent of motile sperms, and motile epididymal sperm counts compared with control group; 9-week group showed statistically lower epididymal sperm counts, and lower motile epididymal sperm counts; good correlation between blood lead and sperm lead; significantly higher counts of chemiluminescence, they were positively associated with sperm lead level; epididymal sperm counts, motility, and motile epididymal sperm counts were negatively associated with sperm chemiluminescence; SOPR were positively associated with epididymal sperm counts, motility and motile epididymal sperm counts, sperm chemiluminescence was negatively associated with SOPR.	PbB after 6 weeks 32 µg/dL, after 9 weeks 48±4.3 µg/dL
Hsu et al. (1998a)	Rat/Sprague-Dawley, 100-120 g	20 or 50 mg lead acetate via i.p. route weekly to males for 6 weeks	Serum testosterone levels were reduced; percentage of capacitation and the chemiluminescence were significantly increased in fresh cauda epididymal spermatozoa; serum testosterone levels were negatively associated with the percentage of acrosome-reacted spermatozoa; sperm chemiluminescence was positively correlated with the percentage of both capacitated and acrosome-reacted spermatozoa; SOPR was negatively associated with the percentage of both capacitated and acrosome-reacted spermatozoa.	PbBs >40 µg/dL
Hsu et al. (1998b)	Rat/Sprague-Dawley, 7 weeks old	10 mg/kg lead acetate weekly via i.p. injection to males for 6 weeks	Intake of VE and/or VC in lead exposed rats prevented the lead associated sperm ROS generation, increased the epididymal sperm motility, enhanced the capacity of sperm to penetrate eggs harvested from unexposed female rats in vitro; protective effect of VE and VC not associated with reduced blood or sperm lead levels.	PbB 30.1±3.4 to 36.1±4.6 PbBs >40 µg/dL
Huang et al. (2002)	Mouse MA-10 cells	10^{-8} to 10^{-5} M lead incubated for 3 hours	Higher decreases in human chorionic gonadotropin (hCG)-stimulated progesterone production, expressions of StAR protein, and the activity of 3ß-HSD compared to 2 hours; no affect on P450scc enzyme activity.	PbB not applicable–in vitro study
Johansson (1989)	Mouse, 9 weeks old	0–1 g lead chloride/L in drinking water for 112 days	No effects on frequency of motile spermatozoa, nor on swimming speed; decreased fertilizing capacity of the spermatozoa by in vitro fertilization; premature acrosome reaction .	PbB 0.5–40 µgdL
Johansson and Pellicciari (1998)	Mouse/NMRI, 9 weeks old	1 g/L lead chloride in drinking water for 16 weeks	Decreased uptake of PI was found in spermatozoa from the vas deferens of the lead-exposed mice; after thermal denaturation of the DNA, the spermatozoa showed a higher uptake of PI in comparison to those of the controls; after reductive cleavage of S-S bonds with DTT and staining with a thiol-specific reagent significantly fewer reactive disulfide bonds were also observed in the spermatozoa; significant delay in the capacity for NCD was noted.	PbB 42±1.6 µg/dL
Johansson and Wide (1986)	Mouse/NMRI, 9 weeks old	0–1 g/L lead chloride in drinking water for 84 days	No effects on sperm count; no effects on serum testosterone; reduced number of implantations after mating.	PbB <0.5–32 µg/dL Mean tissue lead content difference between lead treated and controls: testicular 11 µg/g (epididymal 67 µg/g) PbB <0.5 µg/100 mL

Table AX5-4.2 (cont'd). Effect of Lead on Reproduction and Development in Mammals* Effects on Males

Citation	Species/ Strain/Age	Dose/Route/ Form/Duration	Endpoint	Blood Lead Concentration (PbB)
Johansson et al. (1987)	Mouse/NMRI, 9–10 weeks old	1 g/L lead chloride in drinking water for 16 weeks	Spermatozoa had significantly lower ability to fertilize mouse eggs; morphologically abnormal embryos were found.	PbB not reported
Kempinas et al. (1998)	Rat/Wistar, adult	0.5 g/L and 1.0 g/L lead acetate in drinking water for 90 days	PbB exhibited a significant increase in both groups; decrease in hematocrit and hemoglobin, together with a rise in glucose levels; no signs of lesion were detected upon histological examination of testes, caput, and cauda epididymidis; an increase in ductal diameter, and a decrease in epithelial height were observed in the cauda epididymidis; concentration of spermatozoa stored in the caudal region of the epididymis exhibited a significant increase in lead-treated animals.	PbB 65–103 µg/dL
Kempinas et al., 1990	Rat/NOS, pubertal	(1.0 g/L) lead acetate in drinking water in addition to i.v. injections of lead acetate (0.1 mg/100 g bw) every 10 days, 20 days	Basal levels of testosterone were higher both in the plasma and in the testes of acutely intoxicated animals; levels of LH were not affected in either group, nor was the LHRH content of the median eminence; density of LH/hCG binding sites in testicular homogenates was reduced by saturnism in both groups, apparent affinity constant of the hormone-receptor, complex significantly increased.	PbB ~40 µg/dL
		(1.0 g/L) lead acetate in drinking water in addition to i.v. injections of lead acetate (0.1 mg/100 g bw) every 15 days, 9 months		
Kempinas et al. (1994)	Rat/Wistar, 50 days old	0–1 g/lead acetate/L in drinking water + 0.1 mg/kg i.v. every 10 days for 20 days	Increased plasma and testicular testosterone concentrations; no effects on testicular weight; reduced weight of prostate; increased weight of seminal vesicle and seminal secretions.	PbB 10–41 µg/dL
		0–1 g lead acetate/L in drinking water + 0.1 µgkg i.v. every 15 days for 270 days		PbB 8.5–40 µg/dL
Klein et al. (1994)	Rat/Sprague-Dawley, 100 days old	0.1, 0.3, or 0.6% lead acetate in distilled water for 21 days	2-3 fold enhancement of mRNA levels of GnRH and the tropic hormone LH; 3-fold enhancement of intracellular stores of LH; mRNA levels of LH and GnRH and pituitary levels of stored LH are proportional to blood levels of lead.	PbB 42–102 µg/dL
Liu et al. (2001)	Mouse, MA-10 cells	10^{-8} to 10^{-5} lead acetate in vitro for 2 hours	Significantly inhibited hCG- and dbcAMP-stimulated progesterone production in MA-10 cells; steroid production stimulated by hCG or dbcAMP were reduced by lead; expression of StAR protein and the activities of P450 side-chain cleavage (P450) and 3ß-HSD enzymes detected; expression of StAR protein stimulated by bdcAMP was suppressed by lead at about 50%; progesterone productions treated with 22R-hydroxycholesterol or pregnenolone were reduced 30–40% in lead treated MA-10 cells.	PbB not applicable–in vitro study
Liu et al. (2003)	Mouse, MA-10 cells	10^{-8} to 10^{-5} lead acetate in vitro for 6 hours incubated	Lead significantly inhibited hCG- and dbcAMP-stimulated progesterone production from 20 to 35% in MA-10 cells at 6 hours; lead suppressed the expression of steriodogenesis acute regulatory (StAR) protein from 30 to 55%; activities P450 side-chain cleavage (P450scc) enzyme and 3ß-HSD were reduced by lead from 15 to 25%.	PbB not applicable–in vitro study

Table AX5-4.2 (cont'd). Effect of Lead on Reproduction and Development in Mammals* Effects on Males

Citation	Species/ Strain/Age	Dose/Route/ Form/Duration	Endpoint	Blood Lead Concentration (PbB)
Marchlewicz et al. (1993)	Rat/Wistar, 90 days old	0–1% lead acetate in drinking water for 270 days	No histological or weight changes in testicle or epididymis; fever spermatozoa in all zones of the epididymis.	PbB not reported
McGivern et al. (1991†)	Rat/Sprague-Dawley, adult	0.1% lead acetate in drinking water from GD 14 to parturition: 8 control litters; 6 lead acetate litters (5 males per litter)	Decreased sperm count (21% at 70 days and 24% at 165 days; $p<0.05$); reduced male behavior ($p<0.05$); enlarged prostate (25% increase in weight; $p<0.07$); irregular release patterns of both FSH and LH ($p<0.05$).	Control PbB <5 µg/dL at birth Maternal PbB 73 µg/dL at birth Pup PbB 64 µg/dL at birth
McMurry et al. (1995)	Rat/Cotton, adult	0, 100, or 1000 ppm lead in drinking water for 7 or 13 weeks	Immune function was sensitive to lead exposure; spleen mass was reduced in cotton rats receiving 100 ppm lead; total leukocytes, lymphocytes, neutrophils, eosinophils, total splenocyte yield, packed cell volume, hemoglobin, and mean corpuscular hemoglobin were sensitive to lead exposure; reduced mass of liver, seminal vesicles, and epididymis in males after 7 week exposure.	PbB not reported
Mishra and Acharya(2004)	Mouse/Swiss, 9–10 weeks old	10 mg/kg lead acetate in drinking water for 5 to 8 weeks	Stimulates lipid peroxidation in the testicular tissue, associated with increased generation of noxious ROS; reduced sperm count, increased sperm abnormality	PbB not reported
Moorman et al. (1998)	Rabbit/NOS, adult	3.85 mg/kg lead acetate subcutaneous injection for 15 weeks	Increased blood levels associated with adverse changes in the sperm count, ejaculate volume, percent motile sperm, swimming velocities, and morphology; hormonal responses were minimal; dose-dependent inhibition of sperm formation; semen quality, threshold estimates ranged from 16 to 24 µg/dL.	PbB 0, 20, 40, 50, 70, 80, 90, and 110 µg/dL
Murthy et al. (1991)	Rat/ITRC, (NOS), weanling	0–250 ppm lead acetate in drinking water for 70 days	At 20 µg/dL no impairment of spermatogenesis; vacuolization of Sertoli cell cytoplasm and increase in number and size of lysosomes.	PbB 20.34±1.79 µg/dL
Murthy et al. (1995)	Rat/Druckrey, adult	Pb 5 mg/kg i.p. lead acetate in drinking water for 16 days	Swelling of nuclei and acrosomes round spermatids; in Sertoli cells, nuclei appeared fragmented, whereas the cytoplasm exhibited a vacuolated appearance and a few structures delimitated by a double membrane that contains microtubules arranged in parallel and cross-striated fin fibrils, cell tight junction remain intact; no significant change in epididymal sperm motility and counts, testicular blood levels were found to be elevated after lead exposure.	PbB 7.39 µgdL
Nathan et al. (1992)	Rat/Sprague-Dawley, adult	0, 0.05, 0.1, 0.5, or 1% lead acetate in drinking water for 70 days	No effects on spermatogenesis in all groups; at 124 µgdL: decreased seminal vesicle weight; decreased serum testosterone in the 0.5% group at 10 weeks; no effects in the other exposure categories; no effects on serum FSH, LH, nor pituitary LH content.	PbB 2.3, 40, 44, 80, or 124 µgdL
Pace et al. (2005)	Mouse/BALB/c, adult	0.1 ppm lead acetate in drinking water (lactational exposure as neonates and drinking water from PND 21 to PND 42)	Reduction in fertility when mated with unexposed females; no change in sperm count; increase in number of apoptotic cells in testes.	Neonatal PbB 59.5 µg/dL Post PND 21 PbB 20.3 µg/dL
Piasecka et al. (1995)	Rat/Wistar, adult	1% aqueous solution of lead acetate for 9 months	Lead-loaded (electron dense) inclusions were found in the cytoplasm of the epididymal principal cells, especially in the caput of epididymis, also present, but smaller, in smooth muscle cells; inclusions were located in the vacuoles, rarely without any surrounding membrane; similar lead-containing structures were found in the epididymal lumen.	PbB not reported

Table AX5-4.2 (cont'd). Effect of Lead on Reproduction and Development in Mammals* Effects on Males

Citation	Species/ Strain/Age	Dose/Route/ Form/Duration	Endpoint	Blood Lead Concentration (PbB)
Piasek and Kostial(1987)	Rat/Albino, (NOS), adult	1500, 3500, and 5500 ppm of lead acetate in drinking water for 18 weeks	No overt signs of general toxicity in adult female rats, only at the end of the exposure period the mean body weight of males exposed to two higher levels was slightly lower; no affect of lead exposure on male fertility either after first or after second mating; values in the pups did not differ from control group.	PbB not reported
Pinon-Lataillade et al. (1993)	Rat/Sprague-Dawley, 90 days old	0–0.3% lead acetate in drinking water for 70 days 5 mg/m2 lead oxide in aerosol for 6 hours/day, 5 days/week, 90 days	Decreased weight of seminal vesicles in inhalation study; no effects on spermatogenesis (epididymal sperm count, spermatozoal motility or morphology) or plasma testosterone, LH, and FSH; no effects on fertility; decrease in epididymal sperm count of progeny of sires of the inhalation group, however without effect on their fertility.	PbB 58±1.7 µgdL (oral) PbB 51.1±1.8 µgdL (inhalation)
Pinon-Lataillade et al. (1995)	Mouse/NMRI, adult	0–0.5% lead acetate in drinking water, day 1 of gestation until 60 days of age	No effects on testicular histology, nor on number and morphology of epididymal spermatozoa; no effects on plasma FSH, LH, and testosterone, nor on testicular testosterone; decreased weight of testes, epididymis, seminal vesicles, and ventral prostate; no effects on fertility.	PbB <4–132 µgdL
Rodamilans et al. (1998)	Mouse/BALB/c, 63 days old	0–366 mg lead acetate/L in drinking water for 30, 60, 90, 120, 150, 180 days	Reduction of intra-testicular testosterone concentrations after 30 days; reduction of andrenostenedione concentrations after 150 days; no changes in intratesticular progesterone and hydroxy-progesterone.	PbB 48–67 µgdL
Ronis et al. (1996†)	Rat/Sprague-Dawley, adult	0.6% lead acetate in drinking water for various durations: PND 24–74 (pubertal exposure); PND 60–74 (post pubertal exposure); 11 males and females in pubertal exposure group (10 each in control pubertal group); 6 males and females post-pubertal exposure and control groups	PbB>250 µg/dL reduced circulating testosterone levels in male rats 40–50% ($p<0.05$); reduction in male secondary sex organ weight ($p<0.005$); delayed vaginal opening ($p<0.0001$); disrupted estrous cycle in females (50% of rats); increased incidence of stillbirth (2% control vs. 19% Pb) ($p<0.005$).	Pubertal PbB 30–60 µg/dL Post pubertal PbB 30–60 µg/dL Mean PbBs in male rats 30–60 µg/dL, respectively
Ronis et al. (1998a)	Rat/Sprague-Dawley, adult	0.6% lead acetate in drinking water ad libitum for various durations as follows: GD 5 to PND 1; GD 5 to weaning; PND 1 to weaning; 3 control litters, 2 gestation exposure litters, 2 lactation exposure litters, 2 gestation and lactation exposure litters, 2 postnatal exposure litters, 2 chronic exposure litters; 4 male and 4 female pups per litter	Suppression of adult mean serum testosterone levels was only observed in male pups exposed to lead continuously from GD 5 throughout life ($p<0.05$).	Group: male PbB Naïve: 5.5±2.0 µg/dL Control: 1.9±0.2 µg/dL Gest: 9.1±0.7 µg/dL Lact: 3.3±0.4 µg/dL Gest+Lact: 16.1±2.3 µg/dL Postnatal: 226.0±29 µg/dL Chronic: 316.0±53 µg/dL

Table AX5-4.2 (cont'd). Effect of Lead on Reproduction and Development in Mammals* Effects on Males

Citation	Species/ Strain/Age	Dose/Route/ Form/Duration	Endpoint	Blood Lead Concentration (PbB)
Ronis et al. (1998b)	Rat/Sprague-Dawley, adult	Lead acetate in drinking water (0.05% to 0.45% w/v); dams exposed until weaning, exposure of pups which continued until PND 21, 35, 55, or 85; 5 control litters (0%), 10 low-dose litters (0.05%), 8 mid-dose litters (0.15%), 9 high-dose litters (0.45%); 4 male and 4 female pups per litter	Dose-response reduction in birth weight ($p<0.05$), more pronounced in male pups; decreased growth rates in both sexes ($p<0.05$) were accompanied by a statistically significant decrease in plasma concentrations of IGF1 through puberty PND 35 and 55 ($p<0.05$); increase in pituitary growth hormone during puberty ($p<0.05$).	Mean PbB in offspring at 0.05% (w/v) 49±6 µg/dL Mean PbB in offspring at 0.15% (w/v) 126±16 µg/dL Mean PbB in offspring at 0.45% (w/v) 263±28 µg/dL
Ronis et al. (1998c†)	Rat/Sprague-Dawley, adult	Lead acetate 0.05, 0.15, or 0.45% in drinking water beginning GD 5 continuing until PND 21, 35, 55, or 85; 5 control litters (0%), 10 low-dose litters (0.05%), 8 mid-dose litters (0.15%), 9 high-dose litters (0.45%); 4 male and 4 female pups per litter	Dose-responsive decrease in birth weight ($p<0.05$); dose-responsive decrease in crown-to-rump length ($p<0.05$); dose-dependent delay in sexual maturity ($p<0.05$); decrease in prostate weight ($p<0.05$); decrease in plasma concentration of testosterone during puberty ($p<0.05$); decrease in plasma LH ($p<0.05$); elevated pituitary LH content ($p<0.05$); decrease in plasma testosterone/LH ratio at high dose ($p<0.05$).	Dams: 0, 48, 88, or 181 µg/dL Pups PND 1: <1, 40, 83, or 120 µg/dL Pups PND 21: <1, 46, 196, or 236 µg/dL Pups PND 35: <1, 20, 70, or 278 µg/dL Pups PND 55: <1, 68, 137, or 379 µg/dL Pups PND 85: <1, 59, 129, or 214 µg/dL
Sant'Ana et al. (2001)	Rat/Wistar, 90 days old	0.1 and 1% lead acetate in drinking water for 7 days	0.1% Pb caused decrease in testis weight, an increase in seminal vesicle weight and no changes in plasma testosterone levels, hypothalamic VMA levels were increased compared to control group.	PbB 36.12±9.49 µg/dL and 13.08±9.42 µg/dL
Saxena et al. (1984)	Rat/ITRC, albino (NOS), 12 weeks old	8 mg/kg lead acetate i.p. for 15 days	Histoenzymic and histological alterations in the testes; degeneration of seminiferous tubules; patchy areas showing marked loss in the activity of succinic dehydrogenase and adenosine triphosphatase, whereas alkaline phosphatase activity showed only slight inhibition.	PbB not reported
Saxena et al. (1986)	Rat/ITRC, albino (NOS), 40–50 g	5, 8, or 12 mg Pb+2/kg lead acetate i.p. for 15 days	Increasing dose of lead resulted in significant loss of body weight, as well as testicular weight in groups 3 and 4; cholesterol in the testis of rats markedly decreased at all given doses of lead and was statistically significant in groups 3 and 4; in phospholipid contents, the significant decrease was observed only at two highest doses, while at the lowest dose the decrease was not significant; activity of ATPase remained unaffected at all three doses of lead; no significant increase in lead content in the testis was noticed at lower dose levels as compared to control; however, significant increase was found in groups 3 and 4 which was dose dependent.	PbB not reported
Saxena et al. (1987)	Rat/Wistar, 40-50 g	8 mg Pb2/kg-d lead acetate i.p. for 100 days (from PND 21 to PND 120)	Disturbed spermatogenesis; Leydig cell degeneration; altered enzyme activity (G6PDH).	PbB not reported

Table AX5-4.2 (cont'd). Effect of Lead on Reproduction and Development in Mammals* Effects on Males

Citation	Species/ Strain/Age	Dose/Route/ Form/Duration	Endpoint	Blood Lead Concentration (PbB)
Saxena et al., 1990	ITRC albino, (NOS), adult	8 mg/kg-day lead acetate for 45 days	Alterations in SDH, G6PDH activity, cholesterol, and ascorbic acid contents and reduced sperm counts associated with marked pathological changes in the testis, after combined treatment with lead and immobilization stress in comparison to either alone.	PbB >200 µg/dL
Singh et al. (1993a)	Monkey/ Cynomolgus, birth Birth: 300 days:	0–1500 µg lead acetate/kg-d in gelatin capsules for various durations: 3 control monkeys, 4 monkeys in infancy group (exposure first 400 days), 5 in post-infancy group (exposure 300 days to 9 years of age), 4 in lifetime group (exposure from birth until 9 years)	Degeneration of seminiferous epithelium in all exposed groups (frequency not specified); ultrastructural alterations in seminal vesicles, most prominent in infancy and post-infancy groups (frequency not specified).	Chronic PbB <40–50 µg/dL
Sokol(1987)	Rat/Wistar, 52 days old	0–0.3% lead acetate in drinking water for 30 days	Hyper-responsiveness to stimulation with both GnRH and LH (10); blunted response to naloxone stimulation (10).	PbB 30±5 µg/dL
Sokol (1989)	Rat/Wistar, 27 days old	0–0.6% lead acetate in drinking water for 30 days + 30 days recovery	Suppressed intratesticular sperm counts, sperm production rate, and serum testosterone in both lead treated groups (10-10); sperm parameters and serum testosterone normalized at the end of the recovery period in the pre-pubertal animals (27 days at start) (10) but not in the pubertal animals (52 days at start) (5).	<3–43 µgdL (<4–18 µgdL after recovery period)
	52 days old	0–0.6% lead acetate in drinking water for 30 days + 30 days recovery		B1 <3–43 µgdL (<4–18 µgdL after recovery period)
Sokol, 1990	Rat/Wistar, 52 days old	0–0.6% lead acetate in drinking water for 7, 14, 30, 60 days	Decreased sperm concentration, sperm production rate and suppressed serum testosterone concentrations after 14 days of exposure; not dose related (NS).	Controls: <8 µgdL at any time exposed: 42, 60, 58, 75 µgdL after 7, 14, 30, and 60 days, respectively
Sokol and Berman(1991)	Rat/Wistar, NOS	0, 0.1, or 0.3% lead acetate in drinking water for 30 days beginning at 42, 52, or 70 days old; 8–11 control rats for each age, 8–11 rats for each age in 0.1% group, 8–11 rats for each age in 0.3% group	Dose-related suppression of spermatogenesis (decreased sperm count and sperm production rate) in the exposed rats of the two highest age groups ($p<0.05$); dose-related suppression of serum testosterone in 52-day old rats ($p=0.04$) and in 70-day old rats ($p<0.003$).	0% All <7 µg/dL 42 d 25 µg/dL 0.1% 52 d 35 µg/dL 70 d 37 µg/dL 42 d 36 µg/dL 0.3% 52 d 60 µg/dL 70 d 42 µg/dL
Sokol et al., 1985†	Rat/Wistar, 52 days old	0.1 or 0.3% lead acetate in drinking water for 30 days	Negative correlations between PbB levels and serum and intratesticular testosterone values; dose-dependent reduction in intratesticular sperm count; FSH values were suppressed; no change in LH; decrease in ventral prostatic weight; no difference in testicular or seminal vesicle weights.	PbB 34±3 µg/dL or PbB 60±4 µg/dL
Sokol et al. (1994)	Rat/Sprague-Dawley, 100 days old	0.3% lead acetate in drinking water for 14, 30, or 60 days	Lead exposed fertilized fewer eggs; increased duration of exposure did not result in more significant percentage of eggs not fertilized; no ultrastructural changes were noted in the spermatozoa of animals; no difference in histogram patterns of testicular cells.	PbB ~40 µg/dL

Table AX5-4.2 (cont'd). Effect of Lead on Reproduction and Development in Mammals* Effects on Males

Citation	Species/ Strain/Age	Dose/Route/ Form/Duration	Endpoint	Blood Lead Concentration (PbB)
Sokol et al. (2002)	Rat/Sprague-Dawley, adult	lead acetate in water for 1 week	Dose-related increase in gonadotropin-releasing hormone (GnRH) mRNA; no effect on the serum concentrations of hypothalamic gonadotropin-releasing hormone (GnRH) or LH.	PbB 12–28 µg/dL
Thoreux-Manlay et al. (1995a)	Rat/Sprague-Dawley, 97 days old	0–8 mg lead acetate/kg i.p. for 5 days/week, 35 days	No effects on spermatogenesis; decreased plasma and testicular testosterone by 80%; decreased plasma LH by 32%, indications for impaired Leydig cell function, no effects on fertility.	PbB not reported
Thoreux-Manlay et al. (1995b)	Rat/Sprague-Dawley, adult	8 mg/kg-d lead for 5 days/week, 35 days	Germ cells and Sertoli cells were not major target of lead, accessory sex glands were target; epididymal function was unchanged; plasma and testicular testosterone dropped about 80%, plasma LH only dropped 32%.	PbB 1700 µgdL
Wadi and Ahmad (1999)	Mouse/CF-1, adult	0.25 and 0.5% lead acetate in drinking water for 6 weeks	Low dose significantly reduced number of sperm within epididymis; high dose reduced both the sperm count and percentage of motile sperm and increased the percentage of abnormal sperm within the epididymis; no significant effect on testis weight, high dose significantly decreased the epididymis and seminal vesicles weights as well as overall body weight gain; LH, FSH, and testosterone were not affected.	PbB not reported
Wenda-Rózewicka et al. (1996)	Rat/Wistar, adult	1% aqueous solution of lead acetate for 9 months	Electron microscopic studies did not reveal any ultrastructural changes in the semiferous epithelium or in Sertoli cells; macrophages of testicular interstitial tissue contained (electron dense) lead-loaded inclusions, usually located inside phagolisosome-like vacuoles; x-ray micro-analysis revealed that the inclusions contained lead.	PbB not reported
Yu et al. (1996)	Rat/Sprague-Dawley, neonates	Neonatal and lactational exposure to 0.3% lead acetate in drinking water beginning PND 1 to PND 21	Neonatal exposure to lead decreased cold-water swimming endurance (a standard test for stress endurance) and delayed onset of puberty in males and female offspring, which was exacerbated by swimming stress.	PbB 70 µg/dL

*Not including effects on the nervous or immune systems.

†Candidate key study.

3ß-HSD, 3ß-hydroxysteriod dehydrogenase; dbcAMP, dibutyryl cyclic adenosine-3',5'-monophosphate; DTT, dithiothreitol; FSH, follicle stimulating hormone; G6PDH, glucose-6-phosphate dehydrogenase; GD, gestational day; GnRH, gonadotropin releasing hormone; hCG, human chorionic gonadotropin; IGF_1, insulin-like growth factor 1; i.p., intraperitoneal; LDH, lactate dehydrogenase; LH, luteinizing hormone; LHRH, luteinizing hormone releasing hormone; LPP, lipid peroxidation potential; NCD, nuclear chromatin decondensation rate; NOS, not otherwise specified; PbB, blood lead concentration; PND, post-natal day; p.o., per os (oral administration); ROS, reactive oxygen species; SDH, succinic acid dehydrogenase; SOPR, sperm-oocyte penetration rate; StAR, steroidogenic acute regulatory protein; VC, vitamin C; VE, vitamin E; VMA, vanilmandelic acid

Table AX5-4.3. Effect of Lead on Reproduction and Development in Mammals* Effects on Females

Citation	Species/ Strain/Age	Dose/Route/ Form/Duration	Endpoint	Blood Lead Concentration (PbB)
Burright et al. (1989)	Mouse/HET, neonates	0.5% lead acetate solution via milk, or drinking water chronic beginning PND 1	Plasma prolactin levels implied that lead exposure alone decreased circulating prolactin in primiparous; low prolactin levels in non-behaviorally tested females suggests that dietary lead alone may alter plasma-hormone in these lactating HET dams; pattern of plasma prolactin appear to be inconsistent with the observation that lead exposure decreases dopamine; prolactin levels of lead exposed dams were very low.	PbB ~100 µg/dL
Coffigny et al. (1994†)	Rat/Sprague-Dawley, adult	Inhalation exposure to 5 mg/m3 lead oxide daily for 13 days during gestation (GD 2, 3, 6–10, 13–17, 20)	No effects on the incidence of pregnancy, prenatal death, or malformations when male and female rats from mothers who had been exposed.	PbB 71.1 µg/dL (dam) PbB 83.2 µg/dL (fetal)
Corpas et al. (2002a)	Rat/Wistar, adult	300 mg/L acetate lead in drinking water from mating until PND 12 or PND 21	Neither abnormalities in the liver structure nor depositions of lead, toxicant produced biochemical alterations; pups exhibited decrease in hemoglobin, iron and alkaline, and acid phosphatase levels and an increase in Pb content; protein, DNA, and lipid total amounts were reduced, and hepatic glycogen content was diminished at 12 and 21 PN, with a higher dose of glucose in blood; decrease in alkaline phosphatase in liver of pups at day 21 PN, but acid phosphatase was unaltered.	PbB 22 µgdL
Cory-Slechta et al. (2004†)	Rat/Long-Evans, adult	Lead acetate in drinking water beginning 2 months before breeding through weaning	Observed potential effects of lead and stress in female; Pb alone (in male) and Pb plus stress (in females) permanently elevated corticosterene levels in offspring.	PbB 30–40 µg/dL
Dearth et al. (2002†)	Rat/Fisher 344, 150–175 g	12 mg/mL lead acetate gavage from 30 days prior breeding until pups were weaned 21 day after birth; 10–32 litters per group, control group, gestation and lactation exposure, gestation only exposure, lactation only exposure	Delay in onset of puberty ($p<0.05$); reduced serum levels of IGF1 ($p<0.001$), LH ($p<0.001$), and E2 ($p<0.001$).	Maternal PbB ~40 µg/dL Pups PbB as follows: Gest+lact: ~38 µg/dL PND 10 Gest+lact: ~15 µg/dL PND 21 Gest+lact: ~3 µg/dL PND 30 Gest: ~14 µg/dL PND 10 Gest: ~3 µg/dL PND 21 Gest: ~1 µg/dL PND 30 Lact: ~28 µg/dL PND 10 Lact: ~15 µg/dL PND 21 Lact: ~3 µg/dL PND 30
Dearth et al. (2004)	Rat/Sprague-Dawley and Fisher-344, adult	12 mg/mL lead acetate by gavage 30 days prior to breeding through PND 21 (gestation and lactation exposure)	Lead delayed the timing of puberty in PbB 37.3 µg/dL lead group and suppressed serum levels of LH and E2, these effects did not occur in PbB 29.9 µg/dL lead group, when doubling dose to 29.9 µg/dL group the PbB levels rose to 62.6 µg/dL, yet no effect was noted; results indicate that offspring are more sensitive to maternal lead exposure with regard to puberty related insults than are 29.9 µg/dL rats.	PbB 29.9 µg/dL (Sprague-Dawley) PbB 37.3 µg/dL (Fisher)

Table AX5-4.3 (cont'd). Effect of Lead on Reproduction and Development in Mammals* Effects on Females

Citation	Species/ Strain/Age	Dose/Route/ Form/Duration	Endpoint	Blood Lead Concentration (PbB)
Foster (1992)	Monkey/ Cynomolgus, 0-10 years old	Daily dosing for up to 10 years with gelatin capsules containing lead acetate (1.5 mg/kg); 8 control group monkeys, 8 lifetime exposure (birth–10 years), 8 childhood exposure (birth–400 days), and 8 adolescent exposure (postnatal day 300–10 years of age)	Statistically significant reductions in circulating levels of LH ($p<0.042$), FSH ($p<0.041$), and E2 ($p<0.0001$) during menstrual cycle; progesterone concentrations were unchanged and menstrual cycle was not significantly affected.	PbB <40 µg/dL
Foster et al. (1992)	Monkey/ Cynomolgus, 10 years old	Daily dosing for up to 10 years with gelatin capsules containing lead acetate (1.5 mg/kg); 8 control group monkeys, 8 childhood (birth–400 days), 7 adolescent (postnatal day 300–10 years), 7 lifetime (birth–10 years)	No effect on endometrial response to gonadal steroids as determined by ultrasound.	PbB <40 µg/dL
Foster et al. (1996b)	Monkey/ Cynomolgus, 15–20 years old	Chronic exposure to lead acetate 50 to 2000 µg/kg-d p.o. beginning at birth for 15–20 years; 20 control monkeys, 4 monkeys in 50 µg/kg-d group, 3 monkeys in 100 µg/kg-d, 2 monkeys in 500 µg/kg-d group, and 3 monkeys in 2000 µg/kg-d group	Reduced corpora luteal production of progesterone ($p=0.04$), without alterations in E2, 20alpha-hydroxyprogesterone, or menstrual cyclicity.	PbB 10–15 µg/dL in low group (50 or 100 µg/kg-d) PbB 25–30 µg/dL in moderate group (500 or 2000 µg/kg-d)
Franks et al. (1989)	Monkey/Rhesus, adult	Lead acetate in drinking water (2–8 mg/kg-d) for 33 months; 7 control and 10 lead monkeys	Reduced circulating concentration of progesterone ($p<0.05$); treatment with lead did not prevent ovulation, but produced longer and more variable menstrual cycles and shorter menstrual flow.	PbB 68.9±6.54 µg/dL
Fuentes et al. (1996)	Mouse/Swiss, adult	14, 28, 56, and 112 mg/kg lead acetate via i.p; one time exposure on GD 9	Absolute placental weight at 112 mg/kg and relative placental weight at 14, 56, and 112 mg/kg were diminished significantly; most sections of placenta showed vascular congestion, and increase of intracellular spaces, and deposits of hyaline material of perivascular predominance; trophoblast hyperplasia was also observed, whereas there was a reinforcement of the fibrovascular network in the labyrinth	PbB not reported
Gorbel et al. (2002)	Rat/NOS, 3 months old	3 mg (P1) or 6 mg (P2) lead acetate in drinking water for 15, 30, 45, 60, or 90 days	Female rats absolute and relative weights of ovary and uterus were unchanged, vaginal smears practiced in females revealed the estrus phase; fertility was found to be reduced; lead level in blood was poorly correlated with the level of poisoning.	PbB not reported

Table AX5-4.3 (cont'd). Effect of Lead on Reproduction and Development in Mammals* Effects on Females

Citation	Species/ Strain/Age	Dose/Route/ Form/Duration	Endpoint	Blood Lead Concentration (PbB)
Iavicoli et al. (2004)	Mouse/Swiss, 33–37 days old	0.02, 0.06, 0.11, 0.20, 2.00, 4.00, 20.00, 40.00 ppm in food lead acetate concentration beginning GD 1 to 3 months after birth	Increase in food consumption; however, did low-dose group increase food consumption because of sweet nature of lead? body weight may contribute to delay in onset of puberty and confound results.	PbB 0.69, 1.32, 1.58, 1.94, 3.46, 3.80, 8.35, 13.20 µg/dL
Junaid et al. (1997)	Mouse/Swiss, adult	0, 2, 4, or 8 mg/kg-d lead acetate, subchronic exposure, 5 days/week, 60 days	Altered follicular development.	PbB 22.3–56.5 µg/dL
Laughlin et al. (1987)	Monkey/Rhesus, adult	Lead acetate in drinking water at 3.6, 5.9, or 8.1 mg/kg-d for 1–2 years; 7 control and 10 experimental monkeys per group	Reductions in cycle frequency ($p<0.01$); fewer days of flow ($p<0.01$); longer and more variable cycle intervals ($p<0.025$).	PbB 44–89 µg/dL 51.2 µg/dL (low dose) 80.7 µg/dL (mid dose) 88.4 µg/dL (high dose)
Lögdberg et al. (1987)	Monkey/ Squirrel, adult	Lead acetate in drinking water from 9th week of gestation to PND 1; per oral exposure similar to Laughlin et al. (1987)	Increase in pre- and perinatal mortality during the last two-thirds of pregnancy; statistically significant reduction in mean birth weight was observed in lead exposed monkeys as compared to controls.	Mean maternal PbB 54 µg/dL (39–82 µg/dL)
Lögdberg et al. (1998)	Monkey/ Squirrel, adult	Lead acetate maternal dosing from 5–8.5 weeks pregnant to PND 1 11 control monkeys, 3 low-lead exposure group (PbB 24 µg/dL), 7 medium lead group (PbB 40 µg/dL, 5 high-lead group (PbB 56 µg/dL)	Dose-dependent reduction in placental weight ($p<0.0007$); various pathological lesions were seen in the placentas, including hemorrhages, hyalinization of the parenchyma with destruction of the villi, and massive vacuolization of chorion epithelium.	PbB 37 µgdL (22–82 µgdL) 24 (22–26) µg/dL (low dose) 40 (35–46) µg/dL (mid dose) 56 (43–82) µg/dL (high dose)
McGivern et al. (1991†)	Rat/Sprague-Dawley, adult	0.1% lead acetate in drinking water from GD 14 to parturition	Female rats showed delay in vaginal opening; 50% exhibited prolonged and irregular periods of diestrous and lack observable corpora lutea; both sexes showed irregular release patterns of both FSH and LH.	PbB 73 µg/dL
Nilsson et al. (1991)	Mouse/NMRI, adult	75 µg/g bw lead chloride via i.v.; one time injection on gestation day 4	Electron microscopy showed that the uterine lumen, which was closed in control mice, was opened in lead-injected mice; suggested that lead caused increase in uterine secretion; study suggested lead could have a direct effect on the function of the uterine epithelium and that lead was secreted into the uterine lumen and affect the blastocysts.	PbB not reported
Piasek and Kostial(1991)	Rat/Wistar, 10 weeks old	7500 ppm lead acetate in drinking water for 9 weeks	Decrease in litter size, pup survival, and birth weight; food consumption, body weight, and fertility were not altered in 20 week exposure period.	Maternal PbB >300 µg/dL Offspring PbB >220 µg/dL
Pinon-Lataillade et al. (1995)	Mouse/NMRI, adult	0–0.5% lead acetate in drinking water exposed to lead during gestation until post-GD 60	Exhibited reduced fertility as evidenced by smaller litters and fewer implantation sites.	PbB 70 µg/dL

Table AX5-4.3 (cont'd). Effect of Lead on Reproduction and Development in Mammals* Effects on Females

Citation	Species/ Strain/Age	Dose/Route/ Form/Duration	Endpoint	Blood Lead Concentration (PbB)
Priya et al. (2004)	Rat/Charles Foster, 6–9 months old	0.03 µM lead in vitro for 1 hour	LH binding was dropped to 84% in Pb treated cells; lead exposed cells showed 31% reduction in the enzymes 17ß-HSDH and 17ß-HS; lead can cause a reduction in LH and FSH binding, which significantly alters steroid production in vitro and exerts a direct influence on granulose cell function.	PbB not applicable–in vitro study
Ronis et al. (1996)	Rat/Sprague-Dawley, various ages	lead acetate in the drinking water or male and female rats for the following durations: PND 24–74 (pubertal exposure); PND 60–74 (post pubertal exposure)	Data suggest that both the temporary and the long-lasting effects of lead on reproductive endpoints in male and female experimental animals are mediated by the effects of lead on multiple points along the hypothalamic-pituitary-gonad axis; exposure of male and female Sprague-Dawley rats pre-pubertally (age 24–74 days) to lead acetate in the drinking water resulted in significant reduction in testis weight and in the weight of secondary sex organs in males; these effects were not observed in rats exposed post-pubertally (day 60–74); there is convincing evidence that pre-pubertal female rats exposed in utero and during lactation have reduced levels of circulating E2 and LH.	Materal PbB 30–60 µg/dL Offspring PbB >200 µg/dL.
Ronis et al. (1998a†)	Rat/Sprague-Dawley, adult	0.6% lead acetate in drinking water; ad libitum for various durations as follows: GD 5 to PND 1, GD 5 to weaning, PND 1 to weaning	Female pups exposed to lead from birth through adulthood or from GD 5 through adulthood were observed to have significantly delayed vaginal opening and disrupted estrus cycling; these effects on female reproductive physiology were not observed in animals where lead exposure was confined only to pregnancy or lactation.	Pups continuously exposed to lead 225 to 325 µg/dL
Ronis et al. (1998b)	Rat/Sprague-Dawley, adult	Ad libitum intake of lead acetate (0.05 to 0.45% w/v); lead exposure of dams until weaning, exposure of pups until day 21, 35, 55, 85	Prenatal lead exposure that continues until adulthood (85 days old) delays sexual maturation in female pups in a dose-related manner; dose-dependent delay in sexual maturation (delayed vaginal opening) among female rats following prenatal lead exposure that continued until adulthood (85 days old); a growth hormone-mediated effect on growth that differs depends upon the developmental state of the animal. birth weight was significantly reduced and more pronounced among male pups; decreased growth rates in both sexes were accompanied by a statistically significant decrease in plasma concentrations of IGF1 through puberty and a significant increase in pituitary growth hormone during puberty; growth suppression of male and female rats involves disruption of growth hormone secretion during puberty.	Mean PbB in offspring at 0.05% (w/v) 49±6 µg/dL Mean PbB in offspring at 0.15% (w/v) 126±16 µg/dL Mean PbB in offspring at 0.45% (w/v) 263±28 µg/dL
Ronis et al. (1998c)	Rat/Sprague-Dawley, adult	0.05, 0.15, or 0.45% lead acetate in drinking water beginning GD 5 for 21, 35, 55, 85 days	Dose-responsive decrease in birth weight and crown-to-rump length was observed in litters; dose-dependent delay in sexual maturity (delay in vaginal opening); decrease in neonatal sex steroid levels and suppression of E2 during puberty; elevation in pituitary LH content was observed during early puberty; E2 cycle was significantly disrupted at the highest lead dose; data suggests that the reproductive axis is particularly sensitive to lead during specific developmental periods, resulting in delayed sexual maturation produced by sex steroid biosynthesis.	PbB in dams 181±14 µg/dL PbB in pups ranged from 197±82 to 263±38 µg/dL, increasing with age of pups
Sierra and Tiffany-Castiglioni (1992)	Guinea pig/NOS, adult	0, 5.5, or 11 mg/kg lead acetate, oral dose from GD 22 until GD 52 or 62	Hypothalamic levels of SRIF; lower serum concentrations of progesterone at higher dose only; hypothalamic levels of GnRH and SRIF were reduced in a dose-dependent manner by lead treatment in both dams and fetuses; reduction of SRIF levels in 52-day old fetus was particularly severe (92%) in the 11 mg group.	PbB not reported
Srivastava et al. (2004)	Rat/Fisher 344, adult	12 mg/mL lead acetate by gavage for 30 days prior to breeding until weaning	Lead decreased StAR protein expression and lowered E2 levels; suggested that the primary action of Pb to suppress E2 is through its known action to suppress the serum levels of LH and not due to decreased responsiveness of StAR synthesizing machinery.	PbB of dams 39±3.5 SEM µg/dL and offspring PbB 2.9±0.28 SEM µg/dL

Table AX5-4.3 (cont'd). Effect of Lead on Reproduction and Development in Mammals* Effects on Females

Citation	Species/ Strain/Age	Dose/Route/ Form/Duration	Endpoint	Blood Lead Concentration (PbB)
Taupeau et al. (2001)	Mouse/C57blxCBA, 8 weeks old	10 mg/kg-d lead nitrate via i.v for 15 days	Low lead concentration in the ovary caused dysfunction of folliculogenesis, with fewer primordial follicles and an increase in atretic antral follicles.	PbB not reported
Tchernitchin et al. (1998a)	Rat/Sprague-Dawley, 14 days old	172 µg/g bw lead from day 14 every 2nd day until day 20	Lead inhibits estrogen-induced uterine eosinophilia at 6 and 24 hours after treatment; lead also inhibits estrogen-induced edema in deep and superficial endometrial stoma at 24 hours but not 6 hours after treatment; myometrial hypertrophy is inhibited under the effect of exposure at 24 hours of treatment.	PbB 47 µg/dL
Tchernitchin et al. (1998b)	Rat/Sprague-Dawley, 20 or 21 days old	(75 mg/g bw) lead via i.v. one time exposure at 1 or 24 before hormone stimulation	Enhanced some parameters of estrogen stimulation and inhibited other estrogenic responses; interaction with responses to estrogen was different depending on whether lead pretreatment was 1 or 24 hours before hormone stimulation; estrogenic responses mostly affected were uterine eosinophilia, endometrial edema, uterine liminal 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.	PbB not reported
Wide, 1985	Mouse/NMRI, 10 weeks old	20 µg/dL/g bw lead chloride via i.v. single exposure on days 8, 12, or 16 after mating	Litter size and fetal survival varied significantly; small litters and increased numbers of fetal deaths were observed in mice exposed to lead on day 8 of intrauterine life; live fetuses were normal with respect to weight and morphological appearance; ovarian follicle counts revealed a significantly smaller number of primordial follicles in the latter group, it suggested that the exposure to lead at a time of early organogenesis caused the observed fertility decrease by interfering with the development of the female germ cells.	PbB not reported
Wide and D'Argy (1986)	Mouse/NMRI, adult	20 µg/g bw by i.v. single injection on GD 8	Primordial germ cells showed a normal body distribution but were significantly fewer at all four stages compared with those of control embryos of corresponding age; lead had interfered with the production or activity of alkaline phosphatase.	PbB not reported
Wiebe and Barr (1998)	Rat/Sprague-Dawley, adult	20 or 200 ppm lead chloride in drinking water; 3 exposure durations; prior to mating through weaning, GD 7 to weaning, PND 21 to PND 35	Treatment with lead prior to mating resulted in significant increase in E2-receptor affinity in 21-day old offspring without a change in E2 receptor number; treatment from day 7 of pregnancy until weaning of the pups resulted in approximately 35% decrease in E2 receptors per mg uterine protein when these offspring reached 150 days of age; lead treatment from 21–35 days old or until 150 days resulted in a significant decrease in uterine E2 receptor number at 35 and 150 day, respectively.	PbB likely 4.0±1.4 to 6.6±2.3 µg/dL (similar design as Wiebe et al. (1988))
Wiebe et al. (1998)	Rat/Sprague-Dawley, adult	20 or 200 ppm lead chloride in drinking water; 4 exposure durations; prior to mating through weaning, GD 7 to weaning, PND 21 to PND 35, prior to mating only	Exposure to lead did not affect tissue weights but did cause a significant decrease in gonadotropin-receptor binding in the pre-pubertal, pubertal, and adult females; conversion of progesterone to androstenedione and dihydrotestosterone was significantly decreased in 21-day old rats and in 150-day old females; significantly increased conversion to the 5-alpha-reduced steroids, normally high during puberty.	PbB range 4.0±1.4 to 6.6±2.3 µg/dL

Table AX5-4.3 (cont'd). Effect of Lead on Reproduction and Development in Mammals* Effects on Females

Citation	Species/ Strain/Age	Dose/Route/ Form/Duration	Endpoint	Blood Lead Concentration (PbB)
Yu et al. (1996)	Rat/Sprague-Dawley, adult	Neonatal and lactational exposure to 0.3% lead acetate in drinking water (PND 30)	Neonatal exposure to lead decreased cold-water swimming endurance (a standard test for stress endurance); delayed onset of puberty in males and female offspring, which was exacerbated by swimming stress.	PbB 70 µg/dL

*Not including effects on the nervous or immune systems.

†Candidate key study.

E_2, estradiol; FSH, follicle stimulating hormone; GD, gestational day; GnRH, gonadotropin releasing hormone; HET, Binghamton Heterogeneous Stock; IGF_1, insulin-like growth factor 1; i.p., intraperitoneal; LH, luteinizing hormone; NOS, not otherwise specified; PbB, blood lead concentration; PND, post-natal day; p.o., per os (oral administration); SRIF, somatostatin; StAR, steroidogenic acute regulatory protein

CHAPTER 5 ANNEX

ANNEX TABLES AX5-5

Table AX5-5.1. In Vivo and In Vitro Studies of the Effects of Lead Exposure on Production and Metabolism of Reactive Oxygen Species (ROS), Nitric Oxide (NO), and Soluble Guanylate Cyclease (sCG).

				Pb Exposure			Measured Parameters			
Reference	**Species/ Tissue**	**Age/ Weight**	**n**	**Dosage**	**Duration**	**Pb Level**	**CVS**	**Other**	**Interventions**	**Results**
Khalil-Manesh et al. (1994)	Male SD rats	200 g	N/A	Pb-acetate, 100 ppm in water	6 months	7 ± 3.6 µg/d	BP, tail art. ring response to NE	ET-3, cGMP	DMSA R_X	Pb caused HTN, ↑ET_3, ↓U cGMP (NS) (no effect on NE reactivity). DMSA R_x lowered BP and Vasc response to NE & raised cGMP
Gonick et al. (1997)	Male SD rats	2 months 200 g	6	Pb-acetate, 100 ppm in water	3 months	12.4 ± 1.8 µg/dL	BP	cGMP, NO_2 + NO_3, ET-1, ET-3, MDA, eNOS, iNOS	—	HTN, ↑MDA, ↑eNOS, ↑iNOS (protein and activity in kidney)
Ding et al. (1998)	Male SD rats	2 months	N/A	Pb-acetate, 100 ppm in water	3 months	3.2 ± 0.2 µg/dL	BP	urine NO_2 + NO_3, plasma MDA	DMSA (0.5% H_2O) x 2 wks, IV infusions of L. Arg., SOD & SNP	Pb caused HTN, ↓urine NO_2+NO_3, ↑plasma MDA. DMSA lowered BP, blood lead & MDA + raised urine NO_2 + NO_3. L-Arg lowered BP and MDA, raised NO_2+NO_3, SNP lowered BP
Vaziri (1997)	Male SD rats	190 g	12	Pb-acetate, 100 ppm in water	3 months	17 ± 9 µg/dL	BP	plasma MDA urine NO_2 + NO_3	Antioxidant R_x (Lazaroid)	Pb caused HTN, ↑MDA, ↓urine NO_2+NO_3 in untreated animals. Antioxidant R_x improved HTN, urine NO_2 + NO_3 and lowered MDA without changes in blood Pb level
Dursun (2005)	Male SD rats		24	Pb acetate 8 mg/kg IP	2 weeks		BP, RBF	Ur Na, Ur NO_2 + NO_3, 24 hr UrNa (Na^+ intake Not given)		↑BP, ↓RBF, ↓UrNO_2 + NO_3, unchanged UrNa^+

Table AX5-5.1 (cont'd). In Vivo and In Vitro Studies of the Effects of Lead Exposure on Production and Metabolism of Reactive Oxygen Species (ROS), Nitric Oxide (NO), and Soluble Guanylate Cyclase (sCG).

				Pb Exposure			Measured Parameters			
Reference	**Species/ Tissue**	**Age/ Weight**	**n**	**Dosage**	**Duration**	**Pb Level**	**CVS**	**Other**	**Interventions**	**Results**
Vaziri et al. (1999)	Male SD rats	200 g	6 per group per time point	Pb-acetate, 100 ppm in water	3 months	8.2 ± .8 & 10.8 ± 1 µg per g. Kidney tissue in untreated & antiox-treated groups	BP	Aorta & kidney eNOS protein abundance, Ur NO_2 + NO	Subgroups treated with high-dose vitamin E	Pb exposure resulted in a time-dependent rise in BP, aorta & kidney eNOS & iNOS. This was associated w/ a paradoxical fall in NO availability (Ur NO_2 ± NO_3). Antioxidant R_x attenuated upregulation of iNOS & eNOS & raised NO availability.
Vaziri et al. (2001)	Male SD rats	200 g	6 per group	Pb acetate	3 months	N/A	BP	Aorta, heart, kidney & brain NOS isoforms, urine NO_2 + NO_3	Subgroups studied after 2 wks of R_x w/tempol and those studied 2 wks after cessation of tempol R_x	Pb exposure resulted in rises in BP, eNOS, iNOS & nNOS in the tested tissues + ↓urine NO_x. Tempol administration attenuated HTN, reduced NOS expressions & increased urine NO_x. The effects of tempol disappeared within 2 weeks of its discontinuation.
Vaziri and Ding (2001)	Human coronary endothelial cells	N/A	≥4 per experiment	0 and 1 ppm lead acetate	24 hrs w/ Pb or Na acetate followed by 24 hrs w/ tempol or vehicle	1 ppm medium	N/A	eNOS expression	Co-treatment w/$O_2^{\bullet}$ scavenger, tempol	Pb exposure for 48 hours upregulated eNOS expression. Co-treatment w/ tempol resulted in dose-dependent reversal of Pb-induced upregulation of eNOS but had no effect on control cells.

Table AX5-5.1 (cont'd). In Vivo and In Vitro Studies of the Effects of Lead Exposure on Production and Metabolism of Reactive Oxygen Species (ROS), Nitric Oxide (NO), and Soluble Guanylate Cyclase (sCG).

				Pb Exposure			Measured Parameters			
Reference	**Species/ Tissue**	**Age/ Weight**	**n**	**Dosage**	**Duration**	**Pb Level**	**CVS**	**Other**	**Interventions**	**Results**
Vaziri et al. (1999)	Male SD rats	200 g	6 per group	100 ppm in water	3 months	8.3 - 10.8 µg/g kidney tissue	BP	Urine NO_2 + NO_3, tissue and plasma nitrotyrosine (marker of NO-ROS interaction).	Antioxidant R_x (Vit E)	Pb exposure raised BP, reduced Ur NO_2 + NO_3 & increased nitrotyrosine abundance in plasma, heart, kidney, brain & liver. Anti ox R_x ameliorated HTN, lowered nitrotyrosine & raised Ur NO_2 + NO_3.
Vaziri et al. (2003)	Male SD rats	200 g	6 per group	100 ppm in water	3 months	N/A	BP	Urine NO_2 + NO_3, kidney, heart, brain SOD, catalase, GPX, NAD(P)H oxidase abundance	Tempol ($O_2^{\bullet}$ scavenger infusion)	Pb caused HTN, ↑NAD(P)H oxidase ($gp91^{phox}$), ↑SOD, un-changed catalase and GPX, ↓UrNO_2 + NO_3. Tempol resulted in ↓BP + ↑urine NO_2 + NO_3 in lead-exposed but not control rats.
Ni et al. (2004)	Cultured human coronary endothelial & VSM cells.	N/A	≥4 per experiment	0,1 & 10 ppm Pb acetate	short exposure (5-30 min) & long exposure (60 hours)	0, 1, 10 ppm	N/A	$O_2^{\bullet}$ and H_2O_2 productions SOD, catalase, GPX & NAD(P)H oxidase ($gp91^{phox}$)	None	Short-term incubation with Pb at 1 & 10 ppm raised $O_2^{\bullet}$ & H_2O_2 productions by both endothelial & VSM cells, long-term incubation resulted in further rise in H_2O_2 generation & normalization of detectable $O_{2y}^{\bullet}$. This was associated with increases in NAD(P)H oxidase & SOD & reduced or unchanged catalase & GPX.

Table AX5-5.1 (cont'd). In Vivo and In Vitro Studies of the Effects of Lead Exposure on Production and Metabolism of Reactive Oxygen Species (ROS), Nitric Oxide (NO), and Soluble Guanylate Cyclase (sCG).

				Pb Exposure			Measured Parameters			
Reference	**Species/ Tissue**	**Age/ Weight**	**n**	**Dosage**	**Duration**	**Pb Level**	**CVS**	**Other**	**Interventions**	**Results**
Ding et al. (2001)	Male SD rats	2 months 200 g	N/A	100 ppm	3 months	Blood lead 12.4 ± 1.8 µg/dL vs. 1 mg/dL in controls	BP	Response to DMTU administration, tissue nitrotyrosine, hydroxyl radical	IV infusion of DMTU	Pb caused HTN, ↑plasma nitrotyrosine, ↑plasma.OH concentration all reversed with .OH-scavenger, DMTU infusion
Ding et al. (2000)	Cultured rat aorta endothelial cells	N/A	≥ 4 experiment	0-1 ppm	1, 2, 24, 84 hours	0-1 ppm culture media	N/A	Hydroxyl radical production using the following reaction (Na Salicylate + . OH → 2,3dihydroxy benzoic acid), MDA	None	Pb exposure resulted in conc-dependent rise in MDA and . OH production by cultured endothelial cells.
Attri (2003)	Male Wistar Kyoto rats	150-200 g	10 per group	Pb acetate, 100 ppm in water ± Vit C 20 mg/day/rat	1-3 months	Blood Pb 1.5 mg/dL at 1 mo 2.4 mg/dL at 2 mos 4.1 mg/dL at 3 mos	BP	Total antioxidant capacity, ferric-reducing antioxidant power, NO metabolites, MDA, 8-hydroxyguanosine	Response to vitamin C.	Pb caused ↑BP, ↑MDA, ↑DNA damage/ oxidation, ↓NO_x, ↓antioxidant. and ferric-reducing antioxidant. Concomitant R_x with Vit-C ameliorated all abnormalities.
Malvezzi (2001)	Male Wistar rats	5-6 wks (170 g)	4-10 per group	Pb acetate 750 ppm in water	100 days	Blood, bone, kidney, aorta, liver	BP	—	Response to L. arg, DMSA, L. arg .+ DMSA (given together w/Pb in last 30 days	↑BP w/lead, partial ↓BP w/L. Arg or DMSA, greater reduction w/both, blood and aorta PB remained ↑ in all but DMSA + L. Arg group. Significant Pb mobilization shown in other organs.

Table AX5-5.1 (cont'd). In Vivo and In Vitro Studies of the Effects of Lead Exposure on Production and Metabolism of Reactive Oxygen Species (ROS), Nitric Oxide (NO), and Soluble Guanylate Cyclase (sCG).

				Pb Exposure			Measured Parameters			
Reference	**Species/ Tissue**	**Age/ Weight**	**n**	**Dosage**	**Duration**	**Pb Level**	**CVS**	**Other**	**Interventions**	**Results**
Khalil-Manesh et al. (1993)	Male SD rats	8 wks	N/A	Pb acetate 100 or 5000 ppm in water	1-12 months	29 ± 4 μg/dL	BP, vascular contractility to NE in vitro	cGMP, ET-3, ANP	—	Pb caused HTN, ↓serum and urine cGMP, ↑serum ET-3 without changing ANP or response to NE
Marques et al. (2001)	Male Wistar rats	3 months	20	Pb acetate 5 ppm ± Vit C (3 mmol/L) in water	30 day	N/A	BP, arch-, SNP-vasorelaxation response in aorta rings	sGC protein mRNA & activity. cGMP production, eNOS protein	CoR_x with Vit C	Pb caused HTN, ↓relaxation to Ach & SNP, ↑eNOS, ↓sGC protein mRNA and activity. These abnormalities were prevented by antioxidant R_x.
Farmland et al. (2005)	Male SD rats	200 g	8	Pb acetate 100 ppm in water	3 months	N/A	BP	Aorta sGC, SOD, catalase, glutathione peroxidase	—	↓sGC, ↑CuZn SOD activity, unchanged catalase & GPX activities.
Courtois et al. (2003)	Rat thoracic aorta	N/A	6/experiment	0-1 ppm	24 hr	0-1 ppm	cGMP production	sGC expression, superoxide production, COX-2	Vit C, COS-2 inhibitor	Pb caused ↓sGC, ↓cGMP, ↑O_2, ↑COX-2. All abnormalities improved by Vit C. COX-2 inhibitor improved sGC expression but not O_2 production.

Table AX5-5.2. Studies of the Effects of Lead Exposure on PKC Activity, NF_kB Activation, and Apoptosis

				Pb Exposure			Measured Parameters			
Reference	Species/ Tissue	Age/ Weight	n	Dosage	Duration	Pb Level	CVS	Other	Interventions	Results
Watts et al. (1995)	Isolated rabbit mesenteric artery	N/A	5-6 sets per experiment	Pb acetate $10^{-10}-10^{-3}$M	immediate (contraction)	$5^{-10}-10^{-3}$M medium	vascular contraction		Preincubation w/PKC activators, PKC inhibitor or verapamil for 30-60 minutes + endothelium denudation	Pb acetate induced contraction which was potentiated by PKC activators & attenuated by PKC inhibitor (role of PKC). CCB attenuated Pb-induced contraction (contribution of Ca^{2+} entry). Removal of endothelium did not affect lead-induced vasoconstriction.
Rodriquez-Iturbe et al. (2005)	Male SD rats	200 g	8 Pb group 9 controls	Pb acetate 100 ppm in drinking water	3 months	N/A		NF_kB activation, apoptosis, Ang II positive cells, macrophage/T cell infiltration & nitrotyrosine staining in renal tissue		Pb-exposed animals showed tubuloniterstitial accumulation of activated T-cells, macrophages & Ang II positive cells, NF_kB activation increased apoptosis and nitrotyrosine staining in the kidney.

Table AX5-5.3. Studies of the Effects of Lead Exposure on Blood Pressure and Adrenergic System

Reference	Species/ Tissue	Age/ Weight	n	Pb Exposure		Pb Level	Measured Parameters		Reference	Species/Tissue
				Dosage	Duration		CVS	Other		
Chang et al. (1997)	Wistar rats	190-200 g	20	Pb acetate 0.5% in drinking water	2 months	blood 29.1 ± 1.9 µg/dL aorta; 1.9 ± 0.2 μg/g	BP	Plasma catecholamines + aorta; β receptor binding assay & cAMP generation	—	Pb exposure caused HTN, elevated plasma NE (unchanged plasma Epi). ↓ isoproterenol-stimulated plasma cAMP, ↓ β receptor density in aorta.
Tsao et al. (2000)	Wistar rats	190-200 g	70	Pb acetate 0-2% in drinking water	2 months	blood, heart, aorta, kidney	BP, β agonist-stimulated cAMP production (10 µM isoproterenol in vitro)	pl NEpi, cAMP β receptro densities	—	Pb exposure raised BP and pl NE + lowered aorta and heart β receptor density, basal and stimulated cAMP productions + increased kidney β receptor density and basal and stimulated cAMP productions.
Carmignani et al. (2000)	Male SD rats	3 mo	24	60 ppm	10 months	Blood 22.8 ± 1.2 µg/dL	BP, HR, cardiac contractility (dP/dt), blood flow	Plasma NE, Epi, dopamine, monoamine oxidase (MAO) activity, histology	—	Pb exposure raised BP and dp/dt, lowered carotid blood flow, (no change in HR) raised plasma NE and Epi and MAO (all tissues) lowered plasma NOx + ↓aorta media thickness, ↑lymphocyte infiltration in periaortic fat, nonspecific change in kidney (congestion, edema, rare prox. tubular cell necrosis).

Table AX5-5.3 (cont'd). Studies of the Effects of Lead Exposure on Blood Pressure and Adrenergic System

Reference	Species/ Tissue	Age/ Weight	n	Pb Exposure		Pb Level	Measured Parameters		Interventions	Results
				Dosage	Duration		CVS	Other		
Lai et al. (2002)	Male SD rats	300 g	Acute response	In vivo: Intrathecal injection of PbCl2, 10-100 µM. In vitro: Thoracic cord slices exposed to 5-50 µM PbCl2	—	—	BP, HR, (In vivo) w/without ganglionic blockade (Hexomethonium)	Electophysiologic measures (In vitro) before/after saline washout	—	In vivo: IT injection of PbCl2 raised BP and HR. This was reversed by ganglionic blockade. In vitro: Pb raised excitatory & lowered inhibitory postsynaptic potentials which were reversed by removal of lead (saline washout)
Chang et al. (2005)	Male Wistar rats	10 wks	70	2% Pb acetate (drinking water)	2 mo, observed for 7 mo after cessation	blood: 85 µg/dL aorta: 8 µ/g/g heart: 1 µg/g kidney: 60 µg/g	BP	Plasma NE, β recaptor density (aorta, heart, kidney)	Cessation of Pb exposure	Pb exposure raised BP, plasma NE, & renal tissue β receptor & lowered aorta/heart β receptor density. Plasma and tissue lead fell to near-control values within 7 mo. after Pb cessation. This was associated with significant reductions (not normalization) of BP, plasma NE and partial correction of tissue β receptor densities (Bone lead was not measured).

Table AX5-5.4. Studies of the Effects of Lead Exposure on Renin-angiotensin System, Kallikrein-Kinin System, Prostaglandins, Endothelin, and Atrial Natriuretic Peptide (ANP)

Reference	Species/ Tissue	Age/ Weight	n	Pb Exposure		Pb Level	Measured Parameters		Interventions	Results
				Dosage	Duration		CVS	Other		
Carmignani et al. (1999)	Male SD rates	Weaning	16	Pb acetate 60 ppm drinking water	10 months	Blood 24.2 ± 1.8 µg/dL	BP, HR, carotid blood flow	Plasma ACE, Kininase Kallikrein activities dp/dt	—	Lead exposure raised BP & dp/dt, lowered carotid blood flow without changing HR. This was associated with marked increase in plasma ACE, Kininase II and Kininase I activities.
Sharifi et al. (2004)	Male SD rates	200 g	32	Pb acetate 100 ppm drinking water	2-8 wks	—	BP	ACE activity in plasma, aorta, heart, kidney	—	Lead exposure raised BP, ACE activity in plasma & tested tissues markedly increased peaking within 2-4 wks followed by a decline to subnormal values.
Gonick at al. (1998)	Male SD rates	2 mo	21	Pb acetate 100 ppm drinking water	12 wks	—	BP	Urinary Tx B2, 6-keto PGF1	—	Lead exposure raised BP but did not affect urinary PG metabolite excretion rates.
Dorman and Freeman (2002)	VSMC (rat aorta)	—	—	0.0, 0.02, 0.2, 2.0 mg/dL	up to 48 hrs	0.0 to 2 mg/dL	—	Arachidonic acid (AA), DNA synthesis, cell proliferation + cell viability	Ang II, FCS	Pb augmented Ang II stimulated AA release in a concentration-dependent fashion. At low concentrations, Pb augmented Ang II-stimulated DNA synthesis & lowered cell count in unstimulated cells.
Giridhar and Isom (1990)	Male SD rats	150-175 g	20	Pb acetate 0.01, 0.01, 0.5, 1.0 mg/Kg, BiW, IP	30 days	—	—	ANF	—	Pb exposure resulted in fluid retention (↓urine flow + unchanged fluid intake + weight gain). This was associated with decreased plasma & hypothalamic ANF levels.

Table AX5-5.5. Studies of Effect of Lead on Vascular Contractility

Reference	Species/ Tissue	Age/ Weight	n	Pb Exposure		Pb Level	Measured Parameters		Interventions	Results
				Dosage	Duration		CVS	Other		
Shelkovnikov and Gonick (2001)	Rat aorta rings			Pb acetate 10^{-8} to 10^{-4}	short incubations	—		Vasoconstriction/ vasodilation		Lead acetated did not cause vasoconstriction & did not modify the response to NE, isoproterenol, phorbol ester or acetylcholine but raised contractile response to submaximal Ca^{2+} concentration
Purdy et al. (1997)	Male SD rats	8 weeks		Pb acetate 100 ppm in water	3 months	—	BP	Aorta ring response to NE, phenylephrine, acetylcholine, and nitroprusside		Pb exposure raised BP. Aorta ring vasoconstrictive response to NE & phenylephrine & vasodilatory response to acetylcholine & nitroprusside were unchanged.
Oishi (1996)	Male Wistar rats			Pb acetate	1-3 months			Mesenteric art & aorta response to acetylcholine in presence or absence of NOS inhibitor (L-NAME)		Vasorelaxation response to acetylcholine in presence of L-NAME was significantly reduced in mesenteric art, but not aorta of lead-exposed animals (Inhibition of hyperpolarizing factor)
Valencia et al. (2001)	Wistar rat thoracic aorta rings	7 weeks	6 sets/ experiment	Pb acetate 0.1-3.1mM	rapid response in vitro	—		In vitro contractile response		Lead induced a concentration-dependent vasoconstriction in intact & endothelial-denuded rings in presence or absence of α-1 blocker, PKC inhibitor, L. type Ca^{2+} channel blocker or intra- & extracellular Ca^{2+} depletion. However, the response was abrogated by lanthanum (a general Ca channel blocker)

Table AX5-5.6. Effects of Lead on Cultured Endothelial Cell Proliferation, Angiogenesis, and Production of Heparan Sulfate Proteoglycans and tPA

Reference	Species/ Tissue	Age/ Weight	n	Pb Exposure		Pb Level	Measured Parameters		Interventions	Results
				Dosage	Duration		CVS	Other		
Kaji et al. (1995)	Bovine aorta endothelial cells	—	4 sets per experiment	Pb nitrate 5-50 μM	24 hrs		—	endothelial damage	Co-incubation with cadmium	Addition of Pb alone resulted in mild deendothelialization of the monolayers & markedly increased cadmium-associated endothelial damage.
Kaji et al. (1995)		—	4-5 sets per experiment	Pb nitrate 0.5-5 μM	24 hrs		—	$_3$H-thymidine incorporation, cell count, morphology, LDH release	stimulation w/βFGF & αFGF	Incubation w/Pb resulted in a concentration-dependent reduction of DNA synthesis & cell count, caused some shape change (polygonal →spindle) & reduced βFGF- and αFGF-mediated proliferation.
Fujiwara et al. (1998)	Bovine aorta endothelial cells	—	6 set per experiment	Pb nitrate 5 and 10 μM	48 hrs		—	appearance of cells in denuded areas of monolayer, DNA synth	stimulation w/Zn	Pb inhibited appearance of endothelial cells in the denuded section of monolayer & attenuated the healing response to Zinc
Kishimoto et al. (1995)	Human-umbilical vein endothelia cells	—	3 sets per experiment	Pb acetate 1-100 μM	24 hrs		—	formation of tube-like structures (angio-genesis assay, on Matrigel (BM)	—	Lead inhibited tube formation concentration-dependently & tube lengthening time dependently.
Ueda D., et al. (1997)	Human umbilical vein endothelial cells	—	3 sets per experiment	Pb acetate 1-100 μM	24 hrs		—	tube formation on Matrigel matrix	PKC activator and inhibitor	Lead inhibited tube formation concentration-dependently & tube lengthening time dependently. These effects were independent of PKC.

Table AX5-5.6 (cont'd). Effects of Lead on Cultured Endothelial Cell Proliferation, Angiogenesis, and Production of Heparan Sulfate Proteoglycans and tPA

Reference	Species/ Tissue	Age/ Weight	n	Pb Exposure		Pb Level	Measured Parameters		Interventions	Results
				Dosage	Duration		CVS	Other		
Fujiwara & Kaji (1995)	Bovine aorta endothelial cells	—	4 sets per experiment	Pb nitrate 0.5, 1, 2 μM	12-48 hrs			-βFGF production/ distribution -Heparan sulfate production (sulfate incorporation) -DNA synthesis (cell proliferation) _βFGF binding assay	Heparin, Anti-βFGF antibody	Pb & anti-βFGF alone or together equally reduced DNA synthesis. PB did not change endogenous βFGF production but reduced its HSPG-bound component. This was due to diminished heparan sulfate synthesis as opposed to interference with βFGF binding property.
Kaji et al. (1991)	Bovine aorta endothelial cells	—	4-5 sets per experiment	Pb nitrate 0, 1-20 μM	24-48 hrs			Glycosaminoglycan (GAG) synthesis (sulfate incorporation)		At 10 μM, Pb significantly reduced production of total GAGs. Heparan sulfate was reduced more severely than other GAGs. Cell surface GAG was reduced more severely than found in the medium.
Kaji et al. (1997)	Bovine aorta endothelial cells (confluent)	—	N/A	Pb chloride 10 μM	24 hrs	N/A		Synthesis of heparan sulfate proteoglycans (HSPGs) & their core proteins		In confluent cells, lead suppressed incorporation of precursors into HSPG in the cell layer to a geater extent than chondroitin/dermatan sulfate proteoglycans. Lead suppressed low-molecular weight HSPGs more than the high-molecular weight subclass. The core proteins were slightly increased by Pb exposure.

Table AX5-5.6 (cont'd). Effects of Lead on Cultured Endothelial Cell Proliferation, Angiogenesis, and Production of Heparan Sulfate Proteoglycans and tPA

Reference	Species/ Tissue	Age/ Weight	n	Pb Exposure		Pb Level	Measured Parameters		Interventions	Results
				Dosage	Duration		CVS	Other		
Fujiwara & Kaji (1999)	Bovine aorta endothelial cells (growing 10% BCS)	—	4 sets per experiment	Pb nitrate 0.1-2 μM	48 hrs			Sulfate & glucosamine incorporation in GAGs, quantification of high & low MW-HSPG, identification of perlecan core protein		In growing cells, Pb depressed high-MW HSPGs production but had little effect on low-MW HSPGx (~50 KD). The core protein of perlecan (400 KD) was significantly reduced by Pb exposure.
Kaji et al. (1992)	Human umbilical vein endothelial cells (confluent)	—	5 sets per experiment	0.01-1 μM				t-PA release, DNA synth, protein synth (leucine incorporation)	Thrombin and ET-1 stimulations	Lead exposure reduced basal & thrombin-stimulation t-PA release & worsened ET-1 induced inhibition of t-PA release.

Table AX5-5.7. Studies of the Effect of Lead on Cultured Vascular Smooth Muscle Cells

Reference	Species/ Tissue	Age/ Weight	n	Pb Exposure		Pb Level	Measured Parameters		Reference	Species/Tissue
				Dosage	Duration		CVS	Other		
Fugiwara et al. (1995)	Bovine aorta vascular smooth muscle cell		4 sets per experiment	lead nitrate 0.5-10 μM	24 hrs		—	DNA synthesis	Coincubation w/βFGF, αFGF. pDGF	Pb caused a concentration-dependent increase in DNA synthesis. Co-incubation w/βFGF & Pb resulted in an additive stimulation of VSMC DNA synth. However, Pb inhibited PDGF & αFGF-induced DNA synthesis.
Corsia RV (1995)	rat aorta VSMC cells (80-90% confluent)		≥3 sets per experiment	lead citrate 100 & 500 μg/L	time to confluence (~90% for control experiments)			cell density (cell #/Cm^2), cell morphology, membrance lipid analysis, receptor densities (Ang-II, α, β, ANP		At low concentration, Pb caused VSMC hyperplasia, phenotypic transformation from spindle-to-cobblestone (neointima-like) shape, reduced Ang II receptor density without changing α, β, ANP receptors, increased arachidonic acid content of cell membrane.
Yamamoto C (1997)	Human aorta VSMC & fetal lung fibroblasts (confluent)		5 sets per experiment	lead chloride 0.5-10 μM	24 hrs			t-PA & PAI-1 release		At 2 μM or higher concentrations, lead resulted in a concentration-dependent decline in t-PA release in both cell types. Lead increased PAI-1 release in fibroblasts but lowered PAI-1 in VSMC.

CHAPTER 5 ANNEX

ANNEX TABLES AX5-6

Table AX5-6.1. Genotoxic/Carcinogenic Effects of Lead – Laboratory Animal Studies

Compound	Dose and Duration	Cell Type	Co-exposure	Effects	Reference
Lead Acetate	0.5, 5, or 25 ppm given in drinking water – duration not given. Number of animals per group was not given.	Female C3HSt mice infected with MMTV (Murine mammary tumor virus) – age not given	Selenium, 0.15-1 ppm (duration not given) in diet (Se prevents spontaneous tumors in these mice)	Lead acetate exposed mice exhibited greater mortality unrelated to the tumor formation. 25 ppm suppressed tumor formation, but increased the aggressiveness of the tumors. 5 ppm increased tumor formation, but had no effect on growth rates. 0.5 ppm with low selenium exhibited 80% tumor formation and reduced weight gains that recovered. 0.5 ppm with high selenium exhibited normal weight gain, but tumor incidence still reached 80%. Control data described as "significantly lower" but not given. Methods poorly described and data not shown.	Schrauzer (1987)
Lead as lead acetate	0-4000 ppm given in drinking water for 104 weeks.	Wild type (WT) and metallothionine null (MT null) mice	None	Renal proliferative lesions were much more common and severe in MT null mice than WT mice. MT null mice could not form renal inclusion bodies even with prolonged lead exposure and this could have contributed to increase in the carcinogenic potential of lead.	Waakes et. al. (2004)
Lead Acetate	50, 250, or 1000 ppm given in drinking water for 15 weeks. Number of mice per group in initial exposures not given. Number of mice at analysis ranged from 19-25.	Female albino Swiss Mice – 3 weeks old	Urethane 1.5 mg/g given i.p.	No signs of lead poisoning. No lead effects on growth or weight gain. Urethane added to induce lung tumors. Lead did not affect urethane metabolism. Lead did not affect number of tumors or affect tumor size. Lead alone was not evaluated. Lead levels did increase in tissues.	Blakley (1987)

Table AX5-6.1 (cont'd). Genotoxic/Carcinogenic Effects of Lead – Laboratory Animal Studies

Compound	Dose and Duration	Cell Type	Co-exposure	Effects	Reference
Lead Acetate	50 or 1000 ppm 50 or 1000 ppm given in drinking water for 280 days. Number of mice pre group in initial exposures not given. Number of mice at end were 50 per dose.	Female albino Swiss Mice – 8 weeks old	None	Mice have high rate of spontaneous leukemia from endemic viral infection. No signs of lead poisoning. No lead effects on growth or weight gain. Lead did increase leukemia-related mortality possibly due to immunosuppression. Lead levels did increase in tissues. Data indicate that lead may be immunosuppressive, though the exact mechanism is not understood.	Blakley (1987)
Lead Acetate	60 mg/kg injected s.c. weekly for 5 weeks followed by observation for 80 weeks. 13 treated and 14 control rats.	Fisher F344/NSle rats – 3 weeks old	None	Lead induced tumors at the site of injection in 42% of rats though data was not shown. Control data not indicated or shown. Lead accumulated in tumor tissue, tooth, and bone. This data was shown.	Teraki and Uchiumi (1990)
Lead Acetate	1 or 100 µg/L given in the drinking water for 31 weeks 8 animals per group	Male Wistar Rats - weanlings	0.2-4 % calcium carbonate given in the diet for 31 weeks.	No differences in drinking water or food consumption. High lead and high calcium reduced growth. No deaths in low calcium groups. 10/24 rats from high calcium diet died (4 from controls and 3 each from lead groups). All 10 had kidney or bladder stones. 0/8 rats in low calcium no lead had kidney pathology 2/8 rats in low calcium low lead had nephrocalcinosis. 7/8 rats in low calcium high lead had nephrocalcinosis. 3/4 rats in high calcium no lead had nephrocalcinosis. 1/5 rats in high calcium low lead had a renal pelvic carcinoma. 3/5 rats had nephrocalcinosis. 3/5 rats in high calcium high lead had transitional cell hyperplasia. 2/5 rats had invasive renal pelvic carcinoma. Lead tissue levels were same regardless of dietary calcium levels.	Bogden et al. (1990)

Table AX5-6.2. Genotoxic/Carcinogenic Effects of Lead – Human Cell Cultures

Compound	Dose and Duration	Cell Type	Co-exposure	Effects	Reference
Lead Chromate	Anchorage Independence (0.1-1 µM for 48 h)	Human Foreskin Fibroblasts In H-MEM +15% FCS	None	Lead chromate-induced concentration-dependent increase in anchorage independence.	Biedermann and Landolph (1987)
Lead Chromate	Anchorage Independence (0.1-1 µM for 48h)	Human Foreskin Fibroblasts In H-MEM +15% FCS	None	Lead chromate-induced concentration-dependent increase in anchorage independence.	Biedermann and Landolph (1990)
Lead Chromate	Morphological Transformation (2 µg/mL for 24 h, performed 3 times immediately after passage) Anchorage Independence (0.2-2 µg/mL or cells isolated during morphological transformation) Neoplastic Transformation (cells isolated during morphological transformation)	HOS TE 85 in DMEM + 10% FBS	None	Lead chromate induced foci of morphological transformation after repeated exposure and passaging. Lead chromate did not induce anchorage independence, but cells from the foci obtained during morphological transformation. Lead chromate did not induce neoplastic transformation in the cells from the foci obtained during morphological transformation. Studied as a chromate compound. Role of lead not mentioned or considered.	Sidhu et al. (1991)
Lead Acetate	Anchorage Independence (500-2000 µM for 24 h)	Human Foreskin Fibroblasts (Chinese) In DMEM +10% FCS	3-aminotriazole (3-AT) (80 mM to inhibit catalase)	Lead acetate-induced concentration-dependent increase in anchorage independence. Anchorage independence not affected by 3-AT.	Hwua and Yang (1998)

Table AX5-6.3. Genotoxic/Carcinogenic Effects of Lead – Carcinogenesis Animal Cell Cultures

Compound	Dose and Duration	Cell Type	Co-exposure	Effects	Reference
Lead Acetate	Morphological Transformation (10-50 μM for 48 h)	Primary SHE cells in AMEM + 10% FBS	None	Lead acetate was weakly positive inducing a 0.19-1.6% increase in transformation. There was a weak dose response. There were no statistical analyses of these data.	Zelikoff et al. (1988)
Lead Chloride	Morphological Transformation (doses not given)	C3H10T1/2 cells in EMEM +10% FBS	None	Lead chloride did not induce morphological transformation.	Patierno et al. (1988), and Patierno and Landolph (1989) (both papers present the same data)
Lead Chromate	Enhancement of Simian Adenovirus (SA7) induced morphological transformation. (80-1,240 μM for 20 h)	Primary SHE cells in DMEM + 10% FBS	None	Lead chromate enhanced SA7-induced morphological transformation. Studied as a chromate compound. Role of lead not mentioned or considered.	Schechtman et al. (1986)
Lead Chromate	Morphological Transformation (10-50 μM for 24 h) Anchorage Independence for cells isolated during morphological transformation Neoplastic Transformation for cells isolated during morphological transformation.	C3H10T1/2 cells in EMEM + 10% FBS	None	Lead chromate induced morphological and neoplastic transformation. Cells exhibiting morphological transformation grew in soft agar and grew in nude mice. Studied as a chromate compound.	Patierno et al. (1988) and Patierno and Landolph (1989) (both papers present the same data)
Lead Chromate (and pigments containing lead chromate)	Morphological Transformation (0.04 – 8 μg/mL as Cr for 7 days) Anchorage Independence for cells isolated during morphological transformation Neoplastic Transformation for cells isolated during morphological transformation	Primary SHE cells in DMEM + 10% FCS	None	Lead chromate induced morphological and neoplastic transformation. Cells exhibiting morphological transformation grew in soft agar and grew in nude mice. Studied as a chromate compound.	Elias et al. (1989)
Lead Chromate	Morphological Transformation (0.02 – 0.88 μg/mL as Cr for 7 days)	Primary SHE cells in DMEM + 10% FCS	None	Lead chromate induced morphological transformation more potently (9-fold) than other chromate compounds.	Elias et al. (1991)

Table AX5-6.3 (cont'd). Genotoxic/Carcinogenic Effects of Lead – Carcinogenesis Animal Cell Cultures

Compound	Dose and Duration	Cell Type	Co-exposure	Effects	Reference
Lead Nitrate	Morphological Transformation (0.04 – 8 µg/mL as Cr for 7 days)	Primary SHE cells in DMEM + 10% FCS	Calcium chromate	Lead nitrate alone did not induce significant levels of transformation. Lead nitrate plus calcium chromate increased the potency of calcium chromate to that of lead chromate. Data suggest lead ions are synergistic with chromate ions in inducing neoplastic transformation.	Elias et al. (1991)

Abbreviations:

Cells
SHE = Syrian hamster embryo;
C3H10T/12 cells are a mouse embryo cell line

Medium and Components
AMEM = Alpha Minimal Essential Medium;
DMEM = Dulbecco's Minimal Essential Medium;
EMEM = Eagle's Minimal Essential Medium;
FBS = Fetal Bovine Serum
FCS = Fetal Calf Serum
H-MEM = Minimum essential medium/nutrient mixture–F12-Ham
HOS TE = Human osteosarcoma cell line TE

Differences between the serum are unclear as insufficient details are provided by authors to distinguish.

Table AX5-6.4. Genotoxic/Carcinogenic Effects of Lead – Genotoxicity Laboratory Animal Studies.

Compound	Dose and Duration	Species	Co-exposure	Effects	Reference
Lead Acetate	2.5 mg/100 g given i.p. as daily injection for 5-15 days or 10-20 mg/100 g given i.p. a single injection and animals studied after 15 days 5 animals per group. Chromosome damage in bone marrow	Female Norway Rat	Selenium (0.012-0.047 mg/100g or 0.094-0.188 mg/100 g given i.p. with lead)	Lead induced chromosome damage after chronic treatment. It was not dose dependent as only 1 dose was studied. The effects of selenium on lead effects are unclear as selenium alone induced substantial chromosome damage. The single dose exposure also induced chromosome damage, but untreated controls were not done in this regimen. There is some mention that this dose regimen is toxic to the animals as selenium modulated the lethal effects, but no explanation of how many animals died.	Chakraborty et al. (1987)
Lead Acetate	25-400 mg/kg given ip as single injection and animals studied after 24 h For some chromosome damage studies animals were treated with 25-200 mg/kg given ip as a series of 3, 5, or 7 daily injections and animals studied after 24 h after the last injection. 5 animals per group. Chromosome damage, sister chromatid exchange, in bone marrow and spermatocytes.	Male Swiss Mice – 9-12 weeks old	None	Lead induced chromosome damage in a dose dependent manner at 100-400 mg/kg after a single dose or repeated does exposure in bone marrow cells. In spermatocytes, lead also induced chromosome damage in a dose dependent manner at 50-400 mg/kg after a single dose or repeated dose exposure in bone marrow cells. Lead induced SCE at 50 and 100 mg/kg. A lower dose was negative and higher doses were not done.	Fahmy (1999)
Lead Acetate	200 or 400 mg/kg given by gavage daily for 5 days 5 animals per group Chromosome aberrations in bone marrow and spermatocytes	Male Swiss Mice – 9-12 weeks old	Calcium chloride (40 or 80 mg/kg by gavage daily for 3 days given 2 weeks after lead exposure)	Lead induced chromosome damage at both 200 and 400 mg/kg in bone marrow cells and spermatocytes. Calcium appeared to block this effect.	Aboul-Ela (2002)

Table AX5-6.4 (cont'd). Genotoxic/Carcinogenic Effects of Lead – Genotoxicity Laboratory Animal Studies.

Compound	Dose and Duration	Species	Co-exposure	Effects	Reference
Lead Nitrate	100-200 mg/kg given iv on 9th day of gestation onwards for 9 days. Mothers and fetuses analyzed on G18. Group size not given. Resorptions, fetal viability and chromosome damage, SCE and NOR in the mother and fetus were examined. Mother – bone marrow; fetus liver or lung 3 mothers and fetuses per dose were analyzed.	ICR Swiss Webster Mice – 6-8 week old	None	Lead levels were found in both mother and fetus indicating no problems crossing the placenta. All doses indicated increased resorption and decreased placental weights. No effects on fetal weight. Significant increase in SCE in mothers at 150 and 200 mg/kg. No increase in SCE in fetuses. Significant decrease in NOR in both mother and fetuses. No gaps or breaks in mothers or fetuses. Some weak aneuploidy at lowest dose. Some karyotypic chromosome damage was seen. No explanation of how many cells analyzed per animal (3 animals per dose were analyzed) as only 20- 40 cells were analyzed. There was no dose response and no statistical analyses for chromosome damage. No details on how many animals analyzed for metaphase damage or how many cells per animal. Data interpretation is also complicated as too few metaphases were analyzed 10-25 for SCE. Not given for CA. No detail on potential maternal toxicity.	Nayak et al. (1989b)
Lead Nitrate	10, 20, or 40 mg/kg given ip 24 h 6 animals per group. Chromosome damage and Mitotic Index in bone marrow	Swiss Albino Mice – 8 weeks old	Phyllanthus fruit extract (685 mg/kg) or ascorbic acid (16.66 mg/kg) given by gavage for 7 days	Lead nitrate increased the amount of chromosome damage at each dose. But there was no dose response and a similar level of damage was seen for each dose. Phyllanthus fruit extract reduced the amount of damage at each dose. Acrorbic acid reduced the damage at the lowest dose but increased it at the higher doses. Higher concentrations of lead nitrate reduced the mitotic index. This effect was reversed by ascorbate and Phyllanthus only at the moderate dose.	Dhir et al. (1990)
Lead Nitrate	5, 10, or 20 mg/kg given ip 24 h 6 animals per group. Chromosome damage in bone marrow. 50 metaphases per animal for a total of 300 (X6).	Swiss Albino Mice – 8 weeks old	Ferric chloride (18 mg/kg) given ip for 24 h administered 1 h before-, 1 h after- or together with- lead nitrate	Lead nitrate increased the amount of chromosome damage in a dose-dependent manner. Iron exhibited some modifications of lead induced damage: If administered 1 h before lead plus simultaneously it reduced the damage. If administered with lead only at same time it reduced damage in the lower doses. If lead was started 1 h before iron there was no effect. Thus iron may antagonize lead perhaps by blocking uptake.	Dhir et al. (1992)a

Table AX5-6.4 (cont'd). Genotoxic/Carcinogenic Effects of Lead – Genotoxicity Laboratory Animal Studies.

Compound	Dose and Duration	Species	Co-exposure	Effects	Reference
Lead Nitrate	5 or 10 mg/kg given by gavage for 24 h 6 animals per group. Chromosome aberrations in bone marrow	Swiss Albino Mice – 7-8 weeks old	Zirconium oxychloride (110 or 220 mg/kg) given by gavage for 24 h administered 2 h before-, 2 h after- or together with- lead nitrate	Lead nitrate increased the amount of chromosome damage in a dose-associated manner. Zirconium induced a dose-associated increase in chromosome damage. Zirconium exhibited minimal modification of lead nitrate-induced damage when administered 2 h before or after lead nitrate. Administering the two together increased the damage.	Dhir et al. (1992)b
Lead Nitrate	10, or 20 mg/kg given ip 48 h 12 animals per group. Micronucleus formation in bone marrow	Swiss Albino Mice – 6 weeks old	Phyllanthus fruit extract (685 mg/kg) or ascorbic acid (16.66 mg/kg) given by gavage for 7 days	Lead nitrate increased the amount of micronuclei at both doses in a dose-associated manner. The 48 h recovery time was lower than 24 h but still elevated. Phyllanthus fruit extract reduced the amount of damage at both doses. Ascorbic acid reduced the damage at the lowest dose but increased it at the higher dose.	Kumar et al. (1990)
Lead Nitrate	10, 20, or 40 mg/kg given ip 24 h 5 animals per group. SCE in bone marrow	Swiss Albino Mice – 6-8 weeks old	Phyllanthus fruit extract (685 mg/kg) or ascorbic acid (16.66 mg/kg) given by gavage for 7 days	Lead nitrate increased the amount of SCE in a dose-dependent manner. Lead nitrate had no effect of the proliferative rate index (consideration of metaphases in different divison numbers) Phyllanthus fruit extract and ascorbic acid reduced the amount of damage at each dose.	Dhir et al. (1993)
Lead Nitrate	0.625-80 mg/kg given ip for 12, 24 or 36 h. 12 animals per group Micronucleus formation in bone marrow. 4000 erythorocytes scored per animal	Swiss Albino Mice – 6-8 weeks old	None	Lead nitrate induced micronuclei but they did not increase with dose. Lead induced more micronuclei in males than in females. The ratio of polychromatic to normochromatic erythrocytes was elevated in lead nitrate treated cells, but again did not increase with dose.	Jagetia and Aruna (1998)
Lead Nitrate	0.7-89.6 mg/kg given by gavage for 24, 48, or 72 h, or 1 or 2 weeks. 5 animals per group. Cell viability by trypan blue Single strand breaks in white blood cells	Swiss Albino Mice – 4 weeks old	None	Viability was high (92-96%) at all doses. Lead nitrate induced single strand breaks but they did not increase with dose. In fact the 3 highest doses were all similar in magnitude and less than the 5 lowest doses. The 5 lowest doses were also similar in magnitude.	Devi et al. (2000)

Table AX5-6.4 (cont'd). Genotoxic/Carcinogenic Effects of Lead – Genotoxicity Laboratory Animal Studies.

Compound	Dose and Duration	Species	Co-exposure	Effects	Reference
Lead Acetate	10 mg/kg given by gavage 5 times a week for 4 weeks. 10 animals per group Chromosome Aberrations with 20 metaphases scored per animal	Male Wistar rats – 30 days old	Cypermethrin	No effects on weight gain. Lead Acetate induced an increase in aneuploidy, and the percent of cells with damage, but did not increase structural damage or alterations in organ weight. Cypermethrin and lead together increased structural aberrations that were predominately acentric fragments. However, this was compared to untreated controls and not the individual treatments. Considering the individual treatments, the two together are less than additive.	Nehez et al. (2000)

Table AX5-6.5. Genotoxic/Carcinogenic Effects of Lead – Genotoxicity Human Cell Cultures Mutagenesis

Compound	Dose and Duration	Cell Type	Co-exposure	Effects	Reference
Lead Acetate, In vitro	Cytotoxicity – tritium incorporation (0.1-100 µM/mL for 2-24 h) Mutagenesis – HPRT modified to labeling of 6-thioguanine resistant cells (0.1-100 µM/mL for 2-24 h)	Human Keratinocytes-pooled in MEM and low calcium MEM+2% FBS	None	Decrease in tritium incorporation at 10-100 µM/mL. 6 µM/L was selected as the concentration to study as tritium-incorporation was highest and greater than control. Tritium incorporation in the presence of 6-thioguanine (TG) was optimal after 4 h lead acetate exposure and 5 days of expression time. It was concluded that the significant increase relative to control indicated mutations. The argument made was that because these cells are TG resistant they must be mutated. However, this argument was not proven by sequencing or colony formation in TG.	Ye (1993)
Lead Acetate	Cytotoxicity (500-2,000 µM for 24 h) Mutagenicity – HPRT assay (500-2,000 µM for 24 h)	Human Foreskin Fibroblasts (Chinese) in DMEM +10% FCS	3-aminotriazole (3-AT) (80 mM to inhibit catalase)	LC50 = 500 µM. Cytotoxicity not affected by 3-AT. Lead acetate was not mutagenic. Mutagenicity not affected by 3-AT.	Hwua and Yang (1998)
Lead Chromate	Mutagenicity as 6-thioguanine resistance (0.25-1 µM for 24 h)	Human Foreskin Fibroblasts In EMEM +15% FCS	None	Lead chromate was not mutagenic.	Biedermann and Landolph (1990)

Abbreviations:

Medium and Components

MEM = Minimal Essential Medium;
DMEM = Dulbecco's Minimal Essential Medium;
EMEM = Eagle's Minimal Essential Medium;
FBS = Fetal Bovine Serum
FCS = Fetal Calf Serum

Differences between the serum types are unclear as insufficient details are provided by authors to distinguish.

Table AX5-6.6. Genotoxic/Carcinogenic Effects of Lead – Genotoxicity Human Cell Cultures Clastogenicity

Compound	Assay (Concentration and Exposure Time)	Cell Type and Culture Medium	Co-exposure	Effects	Reference
Lead Chromate	Chromosome Aberrations (0.08-2 μg/cm^2 for 24 h)	Human Foreskin Fibroblasts (Caucasian) in EMEM + 15%FBS	None	Lead Chromate induced chromosome damage in a concentration dependent manner. This study was focused on chromate.	Wise et al. (1992)
Lead Chromate	Chromosome Aberrations (0.1-5 μg/cm^2 for 24 h)	Primary Human Lung Cells in DMEM/F12 + 15%FBS	None	Lead Chromate induced chromosome damage in a concentration dependent manner. This study was focused on chromate.	Wise et al. (2002)
Lead Chromate	Chromosome Aberrations (0.1-5 μg/cm^2 for 24 h)	Primary Human Lung Cells and WTHBF-6 –human lung cells with hTERT in DMEM/F12 + 15%FBS	None	Lead Chromate induced chromosome damage in a concentration dependent manner. Effects were similar in both cell types establishing the WTHBF-6 cells as a useful model. This study was focused on chromate.	Wise et al., (2004a)
Lead Chromate	Chromosome Aberrations (0.1-5 μg/cm^2 for 24 h)	WTHBF-6 –human lung cells with hTERT in DMEM/F12 + 15%CCS	Vitamin C (2 mM co-exposure for 24 h)	Lead Chromate induced chromosome damage in a concentration dependent manner. Vitamin C blocked Cr ion uptake and the chromosome damage after lead chromate exposure. This study was focused on chromate.	Xie et al. (2004)
Lead Chromate	Chromosome Aberrations (0.05-5 μg/cm^2 for 24 h)	WTHBF-6 –human lung cells with hTERT in DMEM/F12 + 15%CCS	Vitamin C (2 mM co-exposure for 24 h)	Lead Chromate induced chromosome damage in a concentration dependent manner. This study was focused on showing chromate and not lead ions were the clastogenic species.	Wise et al. (2004b)
Lead Chromate	Chromosome Aberrations (0.05-5 μg/cm^2 for 24 h)	WTHBF-6 –human lung cells with hTERT in DMEM/F12 + 15%CCS	None	Lead Chromate induced chromosome damage in a concentration dependent manner. This study was focused on comparing particulate chromate compounds.	Wise et al. (2004c)

Table AX5-6.6 (cont'd). Genotoxic/Carcinogenic Effects of Lead – Genotoxicity Human Cell Cultures Clastogenicity

Compound	Assay (Concentration and Exposure Time)	Cell Type and Culture Medium	Co-exposure	Effects	Reference
Lead Glutamate	Lead ion uptake – ICPMS (250-2,000 µM for 24 h) Chromosome Aberrations (250-2,000 µM for 24 h)	WTHBF-6 –human lung cells with hTERT in DMEM/F12 + 15%CCS	None	Lead glutamate induced a concentration-dependent increase in intracellular lead ions. Lead glutamate did not induce chromosome damage.	Wise et al. (2005)
Lead Glutamate	Lead ion uptake – ICPMS (250-2,000 µM for 24 h) Mitotic Index (250-2,000 µM for 24 h) Growth Curve (250-2,000 µM for 24 h) Cell cycle Analysis (250-2,000 µM for 24 h)	WTHBF-6 –human lung cells with hTERT in DMEM/F12 + 15%CCS	None	Lead glutamate induced a concentration-dependent increase in intracellular lead ions. Lead glutamate increased the mitotic index, but inhibited growth and did not induce chromosome damage.	
Radioactive Lead Ions no further specification	LET = 13,600keV/µM Fluence of 2 $X10^6$ particles/ cm^2 Chromosome Aberrations	Human Foreskin Fibroblasts in DF-12 + 10% FCS	None	Lead induced chromosome damage that recurred with time and cell passaging. Analysis limited to approximately 25 metaphases. Focused on radioactive effects of lead	Martins et al. (1993)

Abbreviations:
hTERT = hTERT is the catalytic subunit of human telomerase

Medium and Components
EMEM = Eagle's Minimal Essential Medium;
DMEM/F12 = Dulbecco's Minimal Essential Medium/Ham's F12;

CCS = Cosmic Calf Serum
FBS = Fetal Bovine Serum
FCS = Fetal Calf Serum

Differences between the serum types are unclear as insufficient details are provided by authors to distinguish.

Table AX5-6.7. Genotoxic/Carcinogenic Effects of Lead – Genotoxicity Human Cell Cultures DNA Damage

Compound	Assay (Concentration and Exposure Time)	Cell Type and Culture Medium	Co-exposure	Effects	Reference
Lead Acetate	DNA strand breaks as nucleiod sedimentation (500 µM for 20-25 h)	HeLa Cells in AMEM + 5%FBS	None See also Table AX5-6-16	Lead acetate alone did not induce single strand breaks.	Hartwig et al (1990)
Lead Acetate	DNA strand breaks as nucleoid sedimentation assay (100 µM for 30 min - 4 h)	HeLa Cells in HEPES/ glucose buffer	Buthionine sulfoximine (BSO) to deplete cells of thiols	Lead acetate did not induce DNA strand breaks.	Snyder and Lachmann (1989)
Lead Chromate	DNA adducts (0.4-0.8 µg/cm^2 for 24 h)	Primary Human Small Airway Cells in Clonetics growth medium	None	Lead chromate induced lead inclusion bodies and Cr-DNA adducts and Pb-DNA adducts in a concentration-dependent manner.	Singh et al. (1999)
Lead Chromate	DNA double strand breaks (0.1-5 µg/cm^2 for 24 h) by Comet assay and H2A.X foci formation	WTHBF-6 –human lung cells with hTERT in DMEM/F12 + 15%CCS	None	Lead Chromate induced DNA double strand breaks in a concentration dependent manner. This study showed the damage was due to chromate and not lead.	Xie et al. (2005)
Lead Acetate	DNA strand breaks and DNA protein crosslinks and oxidative lesions by comet assay (1-100 µM for 1 h)	Primary lymphocytes in RPMI 1640 without serum	Vitamins A (10 µM), C (10 µM), E (25 µM), calcium chloride (100 µM) magnesium chloride (100 µM) or zinc chloride (100 µM)	Lead acetate induced an increase in DNA single strand breaks at 1 µM that went down with increasing dose. The highest concentration was significantly less than the damage in untreated controls. For double strand breaks, all concentrations had more damage than the controls, but there was less damage in the highest concentrations than the two lower ones. Lead only induced a slight increase in the amount of DNA-protein crosslinks at the highest concentration. Co exposure to magnesium had no effect. Co-exposure to Vitamins A, C, and E or zinc exacerbated the DNA single strand break effects at the highest concentration. Co-exposure to calcium exacerbated the single strand break effect at all concentrations.	Wozniak and Blasiak (2003)

Table AX5-6.7 (cont'd). Genotoxic/Carcinogenic Effects of Lead – Genotoxicity Human Cell Cultures DNA Damage

Compound	Assay (Concentration and Exposure Time)	Cell Type and Culture Medium	Co-exposure	Effects	Reference
Lead Nitrate	DNA-protein crosslinks by SDS precipitation (1-10 mM for 6 h)	Human Burkitt's lymphoma cells – EBV transformed in RPMI 1640 + 10%FCS	None	Lead nitrate did not induced DNA protein crosslinks. Independent samples were analyzed by 5 different laboratories.	Costa et al. (1996)

Abbreviations:
hTERT = hTERT is the catalytic subunit of human telomerase.

Medium and Components
AMEM = Alpha Minimal Essential Medium;
EMEM = Eagle's Minimal Essential Medium;
DMEM/F12 = Dulbecco's Minimal Essential Medium/Ham's F12;
FBS = Fetal Bovine Serum
FCS = Fetal Calf Serum

Differences between the serum types are unclear as insufficient details are provided by authors to distinguish

Table AX5-6.8. Genotoxic/Carcinogenic Effects of Lead – Genotoxicity Animal Cell Cultures Mutagenicity

Compound	Assay and Duration	Cell Type	Co-exposure	Effects	Reference
Lead Acetate	Cytotoxicity (1-25 μM for 24 h) Mutagenesis- HPRT (0.5-5 μM for 44 h)	V79 in AMEM + 10%FBS	None See also Table AX5-6-16	LC50 = 3 μM Lead acetate alone was not mutagenic.	Hartwig et al (1990)
Lead Acetate -insoluble precipitate at high dose.	Cytotoxicity (0.5-2000 μM for 5 days) Mutagenesis - gpt (0.5-1700 μM for 5 days)	G12 – CHV79 cells with 1 copy gpt gene in Ham's F12 + 5%FBS	See also Table AX5-6-17	LC50 = 1700 μM Lead acetate was mutagenic, but only at toxic concentration (1700 μM) where precipitate formed not at lower concentrations (500 or 1000 μM). There were no statistical analyses of these data.	Roy and Rossman (1992)
Lead Chloride	Cytotoxicity (0.1-1 μM for 1 h) Mutagenicity – gpt assay (0.1-1 μM for 1 h)	AS52-CHO-gpt, lack hprt in HBSS followed by Ham's F12 + 5% FBS	None	LC74 = 1 μM (maximum concentration tested) Lead chloride induced a dose-dependent increase in the number of 6 thioguanine resistant mutants. Did not adjust and compare as previous studies.	Ariza et al. (1996)
Lead chloride	Cytotoxicity (0.1-1 μM for 1 h) Mutagenicity – gpt assay (0.1-1 μM for 1 h)	AS52-CHO-gpt, lack hprt in HBSS followed by Ham's F12 + 5% FBS	Allopurinol (50 μM) to inhibit xanthine oxidase	LC74 = 1 μM. Allopurinol had no effect on cytotoxicity. Lead chloride was mutagenic (0.8 and 1 μM). Allopurinol reduced mutagenesis.	Ariza et al. (1998)a
Lead chloride	Mutagenicity – gpt assay (0.1-1 μM for 1 h) PCR/Southern to analyze mutants for sequence	AS52-CHO-gpt, lack hprt in HBSS followed by Ham's F12 + 5% FBS	None	Lead chloride (0.1-0.4 μM) caused mostly point mutations. Higher concentrations (0.5-1 μM) caused more deletions. There were no statistical analyses of these data. Usually examined fewer mutations than control.	Ariza et al. (1998)b
Lead Chromate	Cytotoxicity (10 -100 μM for 24 h) HGPRT assay (10 -100 μM for 24 h)	V79 CHL – HPRT low clone in MEM + 10% FCS	NTA	Mutagenesis was assessed with HGPRT assay. Lead chromate was not mutagenic. Co-exposure to NTA caused Lead chromate to become mutagenic through increased solubilization. This mutagenic effect was completely attributed to the Cr(VI) ions.	Celotti et al. (1987)
Lead Chromate	Mutagenicity as Sodium/potassium ATPase (ouabain resistance) or 6-thioguanine resistance (25-100 μM for 5 h)	C3H10T1/2 cells in EMEM + 10% FBS	None	Lead chromate was not mutagenic.	Patierno et al. (1988) and Patierno and Landolph (1989) (both papers present the same data)

Table AX5-6.8 (cont'd). Genotoxic/Carcinogenic Effects of Lead – Genotoxicity Animal Cell Cultures Mutagenicity

Compound	Assay and Duration	Cell Type	Co-exposure	Effects	Reference
Lead Nitrate Precipitate at 1000 µM and higher.	Cytotoxicity (50-5,000 µM for 5 days) Mutagenesis at HPRT locus (50-2,000 µM for 5 days)	V79 CHL – HPRT low clone in Ham's F12 +10% FBS	None	LC50 = 2950 µM Lead nitrate was mutagenic at 500 µM, but there was no dose response as higher doses less mutagenic though still 2-4 fold higher. There were no statistical analyses of these data.	Zelikoff et al. (1988)
Lead nitrate -no insoluble precipitate	Cytotoxicity (0.5-2000 µM for 5 days) Mutagenesis - gpt (0.5-1700 µM for 5 days)	G12 – CHV79 cells with 1 copy gpt gene in Ham's F12 + 5%FBS	See also Table AX5-6-17	LC 50 = 1500 µM Lead nitrate was not mutagenic. There were no statistical analyses of these data.	Roy and Rossman (1992)
Lead Sulfide	Cytotoxicity (100-1,000 µM for 24 h) Mutagenicity at HPRT locus (100-1,000 µM for 24 h)	V79 CHL – HPRT low clone in Ham's F12 +10% FBS	None	LC50 = 580 µM; did not increase with longer exposures. Mutagenic at 376 and 563 µM. Not mutagenic lower or higher. Suggested cytotoxicity prevented mutagenesis at higher concentrations. There were no statistical analyses of these data.	Zelikoff et al. (1988)

Abbreviations:

V79 are a Chinese Hamster Lung Cell Line;
G12 – CHV79 are derived from V79;
CHO are a Chinese Hamster Ovary Cell Line ;
AS52 are derived from CHO;
C3H10T/12 cells are a mouse embryo cell line

Medium and Components
AMEM = Alpha Minimal Essential Medium;
EMEM = Eagle's Minimal Essential Medium;
HBSS = Hank's Balanced Salt Solution
FBS = Fetal Bovine Serum
FCS = Fetal Calf Serum

Differences between the serum types are unclear as insufficient details are provided by authors to distinguish.

Table AX5-6.9. Genotoxic/Carcinogenic Effects of Lead – Genotoxicity Animal Cell Cultures Clastogenicity

Compound	Assay (Concentration and Exposure Time)	Cell Type and Culture Medium	Co-exposure	Effects	Reference
Lead Chromate	Chromosome Aberrations (0.4-8 μg/cm^2 for 24 h)	Chinese Hamster Ovary AA8 cells in AMEM + 10%FBS	None	Lead chromate induced chromosome damage in a concentration dependent manner. This study was focused on chromate.	Wise et al. (1992)
Lead Chromate	Chromosome Aberrations (0.8-8 μg/cm^2 for 24 h)	Chinese Hamster Ovary AA8 cells in AMEM + 10%FBS	Vitamin C (1 mM for 24 h as co-exposure to block Cr uptake)	Lead chromate induced chromosome damage in a concentration dependent manner. This effect and uptake of Cr ions were blocked by vitamin C. This study was focused on chromate.	Wise et al. (1993)
Lead Chromate	Chromosome Aberrations (0.8 or 8 μg/cm^2 for 24 h)	Chinese Hamster Ovary AA8 cells in AMEM + 10%FBS	Vitamin E (25 μM as pretreatment for 24 h)	Lead chromate induced chromosome damage in a concentration dependent manner. Vitamin E blocked clastogenic activity of lead chromate, but had no effect on other lead compounds. This study found that the chromosome damage was mediated by chromate ions and not lead ions	Wise et al. (1994)
Lead Chromate	Chromosome Aberrations (0.8-8 μg/cm^2 for 24 h)	Chinese Hamster Ovary AA8 cells in AMEM + 10%FBS	Vitamin C (1 mM as pretreatment for 24 h) Vitamin E (25 μM as pretreatment for 24 h)	Lead chromate induced chromosome damage in a concentration dependent manner. Vitamins C and E blocked clastogenic activity of lead chromate. This study was focused on chromate.	Blankenship et al. (1997)
Lead Glutamate	Chromosome Aberrations (500-2,000 μM for 24 h)	Chinese Hamster Ovary AA8 cells in AMEM + 10%FBS	Vitamin E (25 μM as pretreatment for 24 h)	Lead glutamate induced chromosome damage at 1 mM but not at higher or lower concentrations. Vitamin E did not modify this effect.	Wise et al. (1994)
Lead Nitrate	Chromosome Aberrations (500-2,000 μM for 24 h) Insoluble precipitate at all concentrations	Chinese Hamster Ovary AA8 cells in AMEM + 10%FBS	Vitamin E (25 μM as pretreatment for 24 h)	Lead nitrate did not induce chromosome damage.	Wise et al. (1994)
Lead Nitrate	Chromosome Aberrations (3-30 μM for 2h +16 h recovery)	Chinese Hamster Ovary cells in EMEM + 10%FBS	None	Lead nitrate did not induce chromosome damage.	Lin et al. (1994)
Lead Nitrate	Chromosome aberrations (0.05- 1 μM for 3-12 h)	Chinese Hamster Ovary AA8 cells in DMEM +10% NCS	Crown ethers to modify effect through chelation and uptake	Lead nitrate did not induce chromosome damage.	Cai and Arenaz (1998)

Table AX5-6.9 (cont'd). Genotoxic/Carcinogenic Effects of Lead – Genotoxicity Animal Cell Cultures Clastogenicity

Compound	Assay (Concentration and Exposure Time)	Cell Type and Culture Medium	Co-exposure	Effects	Reference
Lead Acetate	SCE (1-10 μM for 26 h+)	V79 in AMEM + 10%FBS	None See also Table AX5-6-17	Lead acetate alone did not induce SCE. Only 25 cells per treatment analyzed.	Hartwig et al (1990)
Lead Acetate	Micronucleus assay (0.01-10 μM for 18 h)	Chinese Hamster V79 cells in DMEM + 10% FCS	None	Lead acetate induced an increase in micronuclei that increased with concentration and reached a plateau. Two experiments were done and presented separately as a Figure and a Table. The magnitude of the effects was small to modest and statistics were not done.	Bonacker et al. (2005)
Lead Nitrate	SCE Formation (500-3,000 μM for 24 h) Precipitate at 1000 μM and higher.	V79 CHL – HPRT low clone in Ham's F12 +10% FBS	None	No SCE. Only 30 cells analyzed per treatment.	Zelikoff et al. (1988)
Lead Nitrate	Micronucleus Formation (3-30 μM for 2h +16 h recovery) SCE (3-30 μM for 2h +16 h recovery)	CHO cells in EMEM + 10%FBS	None	Lead nitrate did not induce micronuclei formation Lead nitrate induces a concentration-dependent increase in SCE (3, 10, 30 μM).	Lin et al. (1994)
Lead Nitrate	SCE (0.05- 1 μM for 3-12 h)	CHO AA8 in DMEM +10% NCS	Crown ethers to modify effect through chelation and uptake	Lead nitrate caused a weak concentration-dependent increase in SCE. These data were not statistically analyzed. The effect was reduced by a crown ether probably because a similar reduction was seen in spontaneous SCE.	Cai and Arenaz (1998)

Table AX5-6.9 (cont'd). Genotoxic/Carcinogenic Effects of Lead – Genotoxicity Animal Cell Cultures Clastogenicity

Compound	Assay (Concentration and Exposure Time)	Cell Type and Culture Medium	Co-exposure	Effects	Reference
Lead Sulfide	SCE Formation (100-1,000 µM for 24 h)	V79 CHL – HPRT low clone in Ham's F12 +10% FBS	None	No SCE. Only 30 cells analyzed per treatment.	Zelikoff et al. (1988)

Abbreviations:
V79 are a Chinese Hamster Lung Cell Line;
CHO are a Chinese Hamster Ovary Cell Line ;
Medium and Components
AMEM = Alpha Minimal Essential Medium;
DMEM = Dulbecco's Minimal Essential Medium;
EMEM = Eagle's Minimal Essential Medium;
HBSS = Hank's Balanced Salt Solution
FBS = Fetal Bovine Serum
FCS = Fetal Calf Serum
NCS = Newborn Calf Serum

Differences between the serum types are unclear as insufficient details are provided by authors to distinguish.

Table AX5-6.10. Genotoxic/Carcinogenic Effects of Lead – Genotoxicity Animal Cell Cultures DNA Damage

Compound	Assay (Concentration and Exposure Time)	Cell Type and Culture Medium	Co-exposure	Effects	Reference
Lead Acetate	DNA damage as alkaline elution (exposure time and dose not given) Precipitate at 1000 μM and higher.	V79 CHL – HPRT low clone in Ham's F12 +10% FBS	None	No DNA damage (Single strand breaks, DNA-protein crosslinks or DNA-DNA crosslinks). However, the data was not shown	Zelikoff et al. (1988)
Lead Acetate	DNA strand breaks as nick translation (1700 μM for 5 days) Or Supercoiled relaxation (1000 μM for 5 days) Insoluble precipitate at high dose.	G12 – CHV79 cells with 1 copy gpt gene in Ham's F12 + 5%FBS	See also Table AX5-6-17	Lead acetate did not induce SSB. Lead acetate (1700 μM) did increase nick translation when an exogenous polymerase was added. There were no statistical analyses of these data.	Roy and Rossman (1992)
Lead Chromate	DNA damage as alkaline elution (0.4 -8 $\mu g/cm^2$ for 24 h plus 24 recovery)	Chinese Hamster Ovary AA8 cells in AMEM + 10%FBS	None	Lead chromate induced DNA single strand breaks in a concentration dependent manner, which were all repaired by 24 h post-treatment. Lead chromate induced DNA protein crosslinks in a concentration dependent manner, which persisted at 24 h post-treatment. Lead chromate did not induce DNA-DNA crosslinks. This study was focused on chromate.	Xu et al. (1992)
Lead Chromate	DNA adducts (0.8 or 8 $\mu g/cm^2$ for 24 h)	Chinese Hamster Ovary AA8 cells in AMEM + 10%FBS	Vitamin C (1 mM as pretreatment for 24 h) Vitamin E (25 μM as pretreatment for 24 h)	Lead chromate induced DNA adducts in a concentration dependent manner. Vitamins C and E blocked DNA adducts induced by lead chromate. This study was focused on chromate.	Blankenship et al. (1997)
Lead Nitrate	DNA Protein Crosslinks as SDS precipitation (50-5,000 μM for 4 h)	Novikoff ascites hepatoma cells	None	Lead Nitrate induced DNA protein crosslinks in a concentration dependent manner.	Wedrychowski et al. (1986)

Table AX5-6.10 (cont'd). Genotoxic/Carcinogenic Effects of Lead – Genotoxicity Animal Cell Cultures DNA Damage

Compound	Assay (Concentration and Exposure Time)	Cell Type and Culture Medium	Co-exposure	Effects	Reference
Lead Nitrate	DNA strand breaks as nick translation (1700 μM for 5 days)	G12 – CHV79 cells with 1 copy gpt gene in Ham's F12 + 5% FBS	See also Table AX5-6-17	Lead nitrate (1700 μM) did increase nick translation when an exogenous polymerase was added. There were no statistical analyses of these data.	Roy and Rossman (1992)

Abbreviations:

G12 – CHV79 are derived from V79;
V79 are a Chinese Hamster Lung Cell Line;
CHO are a Chinese Hamster Ovary Cell Line ;

Medium and Components
AMEM = Alpha Minimal Essential Medium;
FBS = Fetal Bovine Serum
FCS = Fetal Calf Serum

Differences between the serum types are unclear as insufficient details are provided by authors to distinguish.

Table AX5-6.11. Genotoxic/Carcinogenic Effects of Lead – Genotoxicity Non-Mammalian Cultures

Compound	Assay and Concentration	Cell Type	Co-exposure	Effects	Reference
Lead Chromate (and 13 pigments containing lead chromate)	Mutation Frequency (50-500 µg/plate) Anchorage Independence for cells isolated during morphological transformation Neoplastic Transformation for cells isolated during morphological transformation	Salmonella typhimurium +/- S9 fraction	Nitrilotriacetic acid (NTA to dissolve insoluble compounds) and Silica Encapsulation	Lead chromate and its related pigments did not induce mutagenicity. A few did when dissolved in NTA. Encapsulation prevented mutagenesis in those that were positive when dissolved in NTA. S9 had no effect. Studied as a chromate compound.	Connor and Pier (1990)

Table AX5-6.12. Genotoxic/Carcinogenic Effects of Lead – Genotoxicity as it Pertains to Potential Developmental Effects

Compound	Assay and Concentration	Species	Co-exposure	Effects	Reference
Lead Acetate	25-400 mg/kg given i.p as single injection and animals studied after 24 h Sperm morphology	Male Swiss Mice – 9-12 weeks old	None	Lead induced sperm head abnormalities at 50-100 mg/kg. A lower dose was negative and higher doses were not done.	Fahmy (1999)
Lead Acetate	200 or 400 mg/kg given by gavage daily for 5 days 5 animals per group Sperm Morphology	Male Swiss Mice – 9-12 weeks old	Calcium chloride (40 or 80 mg/kg by gavage daily for 3 days given 2 weeks after lead exposure)	Lead induced sperm abnormalities at 200 and 400 mg/kg. A lower dose was negative and higher doses were not done. Calcium appeared to block this effect.	Aboul-Ela (2002)

Table AX5-6.13. Genotoxic/Carcinogenic Effects of Lead – Genotoxicity as it Pertains to Potential Developmental Effects – Children

Compound	Exposure Regimen	Species	Co-exposure	Effects	Reference
Lead chloride	Administered in drinking water 1.33 g/L for 6 weeks	Male NMRI Mice – 9 weeks old	Cyclophosphamide – 120 mg/kg b.w. given i.p 7 days prior to start of breeding	Pb did not increase resorptions indicating no dominant lethal mutagenic effect. Pb appeared to have a small, but statistically insignificant reduction in the number of resorptions. Cyclophosphamide reduced live implants in female mice.	Kristensen et al. (1993).
Lead Nitrate	12.5-75 mg/kg given iv on 9th day of gestation for 9 days. Mothers and fetuses analyzed on G18. 5 animals per group Resorptions, fetal viability, and chromosome damage in the mother and fetus were examined.	ICR Swiss Webster Mice – 6-8 week old	None	12.5 – 50 mg/kg had no effect on resorption or fetal viability. 75 mg/kg demonstrated some increased resorption though statistics were not done. No chromosome damage was seen in untreated controls. A low level 1-3 and 2-5 aberrations were seen in mothers and fetuses respectively. There was no dose response and no statistical analyses. Data interpretation is also complicated as too few metaphases were analyzed 20-40 total. No descriptions of potential effects on maternal health parameters or fetal weights. No indication of how many animals included in the chromosomal analysis.	Nayak et al. (1989)a
Lead Nitrate	100-200 mg/kg given iv on 9th day of gestation for 9 days. Mothers and fetuses analyzed on G18. Group size not given. Resorptions, fetal viability and chromosome damage, SCE and NOR in the mother and fetus were examined. Mother – bone marrow; fetus liver or lung 3 mothers and fetuses per dose were analyzed.	ICR Swiss Webster Mice – 6-8 week old	None	Lead levels were found in both mother and fetus indicating no problems crossing the placenta. All doses indicated increased resorption and decreased placental weights. No effects on fetal weight. Significant increase in SCE in mothers at 150 and 200 mg/kg. No increase in SCE in fetuses. Significant decrease in NOR in both mother and fetuses. No gaps or breaks in mothers or fetuses. Some weak aneuploidy at lowest dose. Some karyotypic chromosome damage was seen. No explanation of how many cells analyzed per animal (3 animals per dose were analyzed) as only 20- 40 cells were analyzed. There was no dose response and no statistical analyses for chromosome damage. No details on how many animals analyzed for metaphase damage or how many cells per animal. Data interpretation is also complicated as too few metaphases were analyzed 10-25 for SCE. Not given for CA. No detail on potential maternal toxicity.	Nayak et al. (1989)b

Table AX5-6.14. Genotoxic/Carcinogenic Effects of Lead – Epigenetic Effects and Mixture Interactions – Animal

Compound	Exposure Regimen	Species	Co-exposure	Effects	Reference
Lead Acetate	Administered as an i.p. injection of 100 µl/kg. Animals were studied either 24 h after a daily dose for 3 days or for various times (5 min-48 h) after a single dose.	Male Wistar Rats – 10 weeks old	Actinomycin D (0.8 mg/kg) administered i.p. for 4 h before a single dose of lead acetate.	Lead acetate induced GST-P, which required the cis element, GPEI (GST-P enhancer I). Actinomycin D blocked the effects indicating that regulation was at the mRNA level. Lead acetate induced c-jun, which exhibited three peaks of exposure over 48 h. Lead acetate was more potent than lead nitrate.	Suzuki et al. (1996)
Lead Nitrate	Administered as an i.p. injection of 100 µmol/kg. Animals were studied either 24 h after a daily dose for 3 days or for various times (5 min-48 h) after a single dose.	Male Wistar Rats – 10 weeks old	Actinomycin D (0.8 mg/kg) administered i.p. for 4 h before a single dose of lead acetate.	Lead nitrate induced GST-P, which required the cis element, GPEI (GST-P enhancer I). Actinomycin D blocked the effects indicating that regulation was the mRNA level. Lead nitrate induced c-jun, which exhibited three peaks of exposure over 48 h. Lead nitrate was less potent than lead acetate.	Suzuki et al. (1996)
Lead Nitrate	Administered as an i.p. injection of 100 µmol/kg. Some rats were partially hepatectomized. Animals were studied 48 h after injection.	Male Sprague Dawley rats	Partial Hepatectomy	Lead nitrate induced GSH and GST 7-7 activity. Partial hepatectomy did not induce GSH or GST 7-7.	Dock (1989)
Lead Nitrate	Administered as an i.v. injection of 20, 50, or 100 µmol/kg. Animals were studied 24 h after injection.	Male Fisher 344 rats – 7 weeks old	2-methoxy-4-aminobenzene to induce P4501A2 or 3-methylcholanthrene to induce 4501A1	Lead nitrate selectively inhibited P4501A2 and its induction by 2-methoxy-4-aminobenzene at the mRNA and protein level in a dose-dependent manner. Lead nitrate had minimal effect on P4501A1 and its induction by 3- methyl cholanthrene. Lead nitrate did not affect microsomal activity. Lead nitrate induced GST-P in a dose-dependent manner.	Degawa et al (1993)

Table AX5-6.15. Genotoxic/Carcinogenic Effects of Lead – Epigenetic Effects and Mixture Interactions – Human

Compound	Assay (Concentration and Exposure Time)	Cell Type and Culture Medium	Co-exposure	Effects	Reference
Lead Acetate	Tyrosine aminotransferase expression and activity (0.3-10 µM for 24- 48 h) PKC activity: 10 µM for 48h	H4-IIE-C3 - human hepatoma cells in DMEM + 2.5% FCS	Dexamethasone (0.1 µM for 16 h), or calcium chloride (10 µM) or genistein (100 µM to block PKC activity)	Lead acetate inhibited glucocorticoid –induction of tyrosine aminotransferase in a time- and dose-dependent manner. Co-treatment with calcium reduced the effects of lead. Co-treatment with genistein increased the effects of lead. Lead acetate decreases PKC activity and its translocation from the cytosol to the particulate cellular fraction.	Tonner and Heiman (1997)
Lead Nitrate	EROD/MROD activity (10-100 µM for 24 h) NAD(P)H: quinone oxidoreductase activity (10-100 µM for 24 h) Glutathione-S-transferase Ya activity (10-100 µM for 24 h)	Hepa 1c1c7 wild type cells in DMEM + 10% FBS	TCDD (0.1 nM), 3-methyl cholanthrene (0.25 µM), beta-naptflavone (10 µM), benzo(a)pyrene (1 µM)	Lead did not affect EROD/MROD activity. Lead reduced CYP1A1 induction by TCDD, 3-methyl cholanthrene, beta-naptflavone, benzo(a)pyrene. Lead increased NAD(P)H: quinone oxidoreductase activity Lead increased NAD(P)H: quinone oxidoreductase activity induction by TCDD, 3-methyl cholanthrene, beta-naptflavone, benzo(a)pyrene. 10 µM increased Glutathione-S-transferase Ya activity. 25 and 100 µM increased Glutathione-S-transferase Ya activity. Lead nitrate did not affect Glutathione-S-transferase Ya induction by TCDD, 3-methyl cholanthrene, beta-naptflavone, benzo(a)pyrene.	Korashy and El-Kadi (2004)
Lead Nitrate	NAD(P)H: quinone oxidoreductase activity (25 µM for 24 h) Glutathione-S-transferase Ya activity (25 µM for 24 h)	C12- AHR-deficient Hepa 1c1c7 cells in DMEM + 10% FBS	TCDD (0.1 nM), 3-methyl cholanthrene (0.25 µM), beta-naptflavone (10 µM), benzo(a)pyrene (1 µM)	Lead nitrate did not increase NAD(P)H: quinone oxidoreductase and Glutathione-S-transferase Ya activity Lead increased NAD(P)H: quinone oxidoreductase activity induction by TCDD, 3-methyl cholanthrene, beta-naptflavone, benzo(a)pyrene. Lead did not affect Glutathione-S-transferase Ya induction by TCDD, 3-methyl cholanthrene, beta-naptflavone, benzo(a)pyrene.	Korashy and El-Kadi (2004)

Abbreviations:

Medium and Components

DMEM = Dulbecco's Minimal Essential Medium;

FBS = Fetal Bovine Serum
FCS = Fetal Calf Serum

Differences between the serum types are unclear as insufficient details are provided by authors to distinguish.

Table AX5-6.16. Genotoxic/Carcinogenic Effects of Lead – Epigenetic Effects and Mixture Interactions – DNA Repair – Human

Compound	Assay (Concentration and Exposure Time)	Cell Type and Culture Medium	Co-exposure	Effects	Reference
Lead Acetate	DNA strand breaks as nucleoid sedimentation (500 µM for 20-25 h)	HeLa Cells in AMEM + 5%FBS	UV (5 J/m^2)	Lead acetate alone did not induce single strand breaks. UV did induce strand breaks. Co-exposure of lead and UV cause DNA strand breaks to persist longer suggesting an inhibition of repair.	Hartwig et al (1990)

Abbreviations:
Medium and Components
AMEM = Alpha Minimal Essential Medium;
FBS = Fetal Bovine Serum

Table AX5-6.17. Genotoxic/Carcinogenic Effects of Lead – Epigenetic Effects and Mixture Interactions – DNA Repair – Animal

Compound	Assay (Concentration and Exposure Time)	Cell Type and Culture Medium	Co-exposure	Effects	Reference
Lead Acetate	Cytotoxicity (0.5-5 μM for 24 h) Mutagenesis- HPRT (0.5-5 μM for 44h) SCE (1-10 μM for 26 h+)	V79 in AMEM + 10%FBS	UV (5 J/m^2)	Lead acetate (3 and 5 μM) increased UV-induced increased cytotoxicity with no dose response (plateau). There were no statistical analyses of these data. Lead acetate (0.5-5) increased UV mutagenicity though with no dose response (plateau). There were no statistical analyses of these data Lead acetate (1-10 μM) increased UV-induced SCE. Significant at $p<0.01$. Only 25 cells per treatment analyzed.	Hartwig et al (1990)
Lead Acetate	Mutagenesis - gpt (0.5-1700 mM for 24 h) DNA strand breaks as supercoiled relaxation (1000 mM for 24 h)	G12 – CHV79 cells with 1 copy gpt gene in Ham's F12 + 5%FBS	UV (2 J/m^2), or MNNG (0.5 μg/L)	Lead acetate was co-mutagenic with UV and MNNG increasing frequency 2-fold. Lead acetate does not increase strand breaks induced by UV.	Roy and Rossman (1992)

Abbreviations:

G12 – CHV79 are derived from V79;
V79 are a Chinese Hamster Lung Cell Line;

Medium and Components
AMEM = Alpha Minimal Essential Medium;
FBS = Fetal Bovine Serum

Table AX5-6.18. Genotoxic/Carcinogenic Effects of Lead – Mitogenesis – Animal

Compound	Exposure Regimen	Species	Co-exposure	Effects	Reference
Lead Acetate	Administered lead acetate (12.5 mg/kg) i.p. Animals studied 24 h after injection.	Male B6 Mice	None	Lead acetate induced TNF-alpha in glial and neuronal cells in the cerebral cortex and subcortical white matter and on Purkinje cells in the cerebellum, but did not induced apoptosis in these areas	Cheng et al. (2002)
Lead Nitrate	Liver initiation induced by the resistant hepatocyte model Initiation followed by iv injection of lead nitrate (100 µM/kg) or partial hepatectomy Studied DNA synthesis (30 h after injection) and preneoplastic nodule formation (5 weeks after injection)	Male Wistar Rats-4 per group	Partial Hepatectomy	Lead nitrate stimulated DNA synthesis and liver cell proliferation Lead nitrate did not induce preneoplastic nodule. Partial hepatectomy did.	Columbano et al. (1987)
Lead Nitrate	Liver initiation induced by the resistant hepatocyte model (diethylnitrosamine followed by 2-acetylaminofluorene plus carbon tetrachloride) Initiation followed by iv injection of 4 doses of lead nitrate (100 µM/kg) given once every 20 days or partial hepatectomy, ethylene dibromide, or nafenopine Animal were evaluated for preneoplastic foci at 75 or 155 days after inititiation.	Male Wistar Rats-4 per group	Diethylnitrosamine	Lead nitrate, partial hepatectomy, ethylene dibromide, or nafenopine all stimulated DNA synthesis and liver cell proliferation Lead nitrate, ethylene dibromide, or nafenopine did not induce preneoplastic nodule. Partial hepatectomy did.	Columbano et al. (1990)
Lead Nitrate	Liver initiation induced by the orotic acid model (diethylnitrosamine plus orotic acid) Initiation followed by iv injection of lead nitrate (100 µM/kg) or partial hepatectomy, or by gavage: ethylene dibromide, or cyproterone DNA synthesis was examined at various time intervals (24 h -5 days) after injection.	Male Wistar Rats-4 per group	Diethylnitrosamine	Lead nitrate, partial hepatectomy, ethylene dibromide, or cyproterone all stimulated DNA synthesis within 30 minutes. Lead nitrate induced DNA synthesis for 5 days.	Coni et al. (1991)

Table AX5-6.18 (cont'd). Genotoxic/Carcinogenic Effects of Lead – Mitogenesis -Animal

Compound	Exposure Regimen	Species	Co-exposure	Effects	Reference
Lead Nitrate	Liver initiation induced by the resistant hepatocyte model (diethylnitrosamine followed by 2-acetylaminofluorene plus carbon tetrachloride) or the phenobarbital model (diethylnitrosamine plus orotic acid), or the orotic acid model (diethylnitrosamine plus orotic acid) Initiation followed by iv injection of lead nitrate (100 μM/kg) or partial hepatectomy, or carbon tetrachloride by gavage Animals were studied 6 weeks after initiation.	Male Wistar Rats-4 per group	Partial Hepatectomy, carbon tetrachloride	Lead nitrate, partial hepatectomy, carbon tetrachloride all stimulated DNA synthesis and liver cell proliferation Lead nitrate, did not induce preneoplastic nodules. Partial hepatectomy and carbon tetrachloride did.	Ledda-Columbano et al. (1992)
Lead Nitrate	Liver initiation induced by the resistant hepatocyte model (diethylnitrosamine followed by 2-acetylaminofluorene plus carbon tetrachloride) Initiation followed by iv injection of lead nitrate (100 μM/kg) or partial hepatectomy, or by gavage: ethylene dibromide, or cyproterone, or nafenopine Also tried either 1 or 2 additional iv injections of lead over 3 day intervals. Animals were studied at various intervals (1-6 days) after injection	Male Wistar Rats-4 per group	Diethylnitrosamine, 2-AAF	This study aimed to determine if mitogens induce nodules at different time points. Lead nitrate, ethylene dibromide, cyproterone, or nafenopine did not induce preneoplastic nodules at all. Partial hepatectomy did within 3 days. Multiple injections of lead nitrate did not induce preneoplastic lesions.	Coni et al. (1993)
Lead Nitrate	Administered as i.v. injection of lead nitrate (100 μM/kg) or partial hepatectomy, or by gavage: carbon tetrachloride, or ethylene dibromide, or cyproterone, or nafenopine Animals were studied at various time intervals (0.25-24 h) after injection.	Male Wistar Rats-4 per group	Partial Hepatectomy, carbon tetrachloride	Lead nitrate, ethylene dibromide, cyproterone, or nafenopine induced c-jun and c-myc but did not induce c-fos. Partial hepatectomy and carbon tetrachloride induced c-jun, c-fos, and c-myc.	Coni et al. (1993)
Lead Nitrate	Administered as i.v. injection of lead nitrate (100 μM/kg) or partial hepatectomy, or nafenopine by gavage. Animals were studied at various time intervals (24-96 h) after injection.	Male Wistar Rats-4 per group – 8 weeks old	None	Lead nitrate induced a high incidence of polyploidy and binucleated cells. These changes were irreversible after 2 weeks. Many of these cells were the newly synthesized cells. Partial hepatectomy and carbon tetrachloride induced tetraploid and octaploid mononucleated cells.	Melchiorri et al. (1993)
Lead Nitrate	Administered as i.v. injection of lead nitrate (10 μM/100 g) Studies for apoptosis at 12, 24, 36, 48, 72, 96, 120, 168, 336 h after injection	Male Wistar rats – 4 rats per group	None	Liver weight increased until day 5 then returned to control levels. DNA synthesis peaked at 36 h Apoptosis peaked at day 4 and then decreased gradually.	Nakajima et al. (1995)

Table AX5-6.18 (cont'd). Genotoxic/Carcinogenic Effects of Lead – Mitogenesis -Animal

Compound	Exposure Regimen	Species	Co-exposure	Effects	Reference
Lead Nitrate	Administered as i.v. injection of lead nitrate (100 µM/kg) or TNF-alpha. Animals were studied at various time intervals (12-48 h) after injection.	Male Wistar Rats- 4 per group – 5-6 weeks old	None	Lead nitrate and TNF-alpha induced similar proliferative responses.	Shinozuka, 1996
Lead Nitrate	Administered after diethylnitrosamine (200 mg/kg given i.p) as i.v. injection of lead nitrate (100 µM/kg) or instead carbon tetrachloride by gavage Animals were studied at various time intervals (3 -21 days) after injection.	Male Wistar rats – 4 per group	Carbon tetrachloride	Lead nitrate induced apoptosis affects both newly synthesized cells and non-replicative cells. Lead nitrate decreased the number and had no effect on the size of placental glutathione-S-transferase lesions. Carbon tetrachloride substantially increased these lesions both in number and in size.	Columbano et al. (1996)
Lead Nitrate	Administered as i.v. injection of lead nitrate (100 µM/kg) or partial hepatectomy, or by gavage: carbon tetrachloride, or cyproterone, or nafenopine Animals were studied at various time intervals (0.5-24h) after injection.	Male Wistar Rats – 8 weeks old	Partial Hepatectomy, ethylene dibromide, nafenopine, or cyproterone	Lead nitrate induced NF-kB, TNF-alpha and iNOS, but not AP-1. Carbon tetrachloride induced and activated NF-kB, TNF-alpha iNOS, and AP-1. Nafenopine and cyproteone did not induce or activate NF-kB,TNF-alpha iNOS, or AP-1.	Menegazzi et al. (1997)

Table AX5-6.19. Genotoxic/Carcinogenic Effects of Lead – Mitogenesis Human and Animal Cell Culture Studies

Compound	Assay (Concentration and Exposure Time)	Cell Type and Culture Medium	Co-exposure	Effects	Reference
Lead Acetate	Cell Proliferation (0.1-100 μM for 2-6 days) DNA synthesis (1-100 μM for 72 h) Tyrosine aminotransferase expression and activity (0.3-10 μM for 24- 48 h)	H4-II-C3 - human hepatoma cells in DMEM + 2.5% FCS	Dexamethasone (0.1 μM for 16 h)	Lead acetate inhibited cell growth in a time- and dose-dependent manner. Lead acetate inhibited DNA synthesis in a dose-dependent manner. Lead acetate alone did not inhibit tyrosine aminotransferase. Lead acetate inhibited glucocorticoid –induction of tyrosine aminotransferase in a time- and dose-dependent manner.	Heiman and Tonner (1995)
Lead Acetate	Cell proliferation (10 μM-1mM for 24 h -7 days)	REL cells- Rat Epithelial cells in Ham's F10 medium + 10% FBS	None	Lead acetate inhibited cell growth at all concentrations for 24 h – 7 days. Lead acetate did not affect gap junction capacity, which is often inhibited by tumor promoters.	Apostoli et al. (2000)
Lead Acetate	Cell growth (0.01-10 μM for 12-72 h) Expression of genes in cytokine pathways (0.01-10 μM for 24 h)	U-373MG – human glioma cell line in DMEM + 10 or 20% FBS	None	Lead acetate did not inhibit or enhance cell growth. Lead acetate enhanced the expression of TNF-alpha, but decreased interleukin- 1 beta, interleukin-6, gamma-aminobutyric acid transaminase, and glutamine synthetase under 10% FBS. Lead acetate further enhanced the expression of TNF-alpha under 20% serum, but had no effect at all on expression of the other genes.	Liu et al. (2000)
Lead Acetate	Cell proliferation (0.078-320 μM for 48 h) Apoptosis (1.25-80 μM) Cell cycle analysis	Rat-1 fibroblasts in EMEM +10% FBS	None	Lead acetate inhibited cell growth at 0.635-320 μM. Lead acetate induced apoptosis from 2.5-10 μM. Lead acetate caused GS/M and S-phase arrest.	Iavicoli et al., 2001
Lead Acetate	DNA synthesis (1-50 μM for 24 h) Expression of genes in mitogen activated pathways (1-50 μM for 5 min-4h)	1321N1 – human astrocytoma cells in DMEM + 0.1% BSA	None	Lead acetate induced DNA synthesis. Lead acetate induced activation of MAPK, ERK1. ERK2, MEK1 , MEK2, PKC, and $p90^{RSK}$. Lead acetate did not activate PI3K or $p70^{s6k}$	Lu et al. (2002)
Lead Acetate	Cell proliferation (1 μM for 24 h) Cell differentiation (1 μM for 48 h) PKC activation (1 μM for 24 h)	Primary oligodendrocyte progenitor cells – in DMEM + 1% FBS	None	Lead acetate inhibited basal and growth factor stimulated growth. Lead acetate inhibited cell differentiation in a PHC dependent-manner. Lead acetate redistributes PKC from the cytosol to the membrane, but did not increase PKC activity.	Deng and Portez (2002)

Table AX5-6.19 (cont'd). Genotoxic/Carcinogenic Effects of Lead – Mitogenesis Human and Animal Cell Culture Studies

Compound	Assay (Concentration and Exposure Time)	Cell Type and Culture Medium	Co-exposure	Effects	Reference
Lead Acetate	Expression of TNF-alpha (0.1-10 μM for 24 h)	U-373MG – human glioma cell line in DMEM + 20% FBS	None	Lead acetate did not induce apoptosis. Lead acetate increased the expression of TNF-alpha in a dose-dependent manner. TNF-alpha was not involved in lead-induced apoptosis.	Cheng et al. (2002)
Lead Chloride	Cell proliferation (10 μM-1mM for 24 -48 h)	REL cells- Rat Epithelial cells in Ham's F10 medium + 10% FBS	None	Lead chloride inhibited cell growth at all concentrations for 24-48 h. Lead chloride did not affect gap junction capacity, which is often inhibited by tumor promoters.	Apostoli et al. (2000)
Lead Oxide	Cell proliferation (10 μM-1mM for 24 h -7 days)	REL cells- Rat Epithelial cells in Ham's F10 medium + 10% FBS	None	Lead oxide inhibited cell growth at all concentrations for 24 h – 7 days. Lead oxide did not affect gap junction capacity, which is often inhibited by tumor promoters.	Apostoli et al. (2000)
Lead Sulfate	Cell proliferation (10 μM-1mM for 24 -48 h)	REL cells- Rat Epithelial cells in Ham's F10 medium + 10% FBS	None	Lead sulfate inhibited cell growth at all concentrations for 24-48 h. Lead sulfate did not affect gap junction capacity, which is often inhibited by tumor promoters.	Apostoli et al. (2000)
Lead Chromate	Apoptosis (350 μM for 24 h)	Chinese Hamster Ovary AA8 cells in AMEM + 10%FBS	None	Lead chromate induced apoptosis. This study was focused on chromate.	Blankenship et al. (1997)
Lead Chromate	Apoptosis (0.4-2 μg/cm^2 for 24 h)	Primary Human Small Airway Cells in Clonetics growth medium	None	Lead chromate induced apoptosis in a concentration-dependent manner.	Singh et al. (1999)
Lead Chromate	Growth Curve (0.5-5 μg/cm^2 24 h)	WTHBF-6 –human lung cells with hTERT in DMEM/F12 + 10%CCS	None	Lead chromate inhibited cell growth.	Holmes et al. (2005)
Lead Glutamate	Growth Curve (250-1,000 μM for 24 h)	WTHBF-6 –human lung cells with hTERT in DMEM/F12 + 10%CCS	None	Lead glutamate had no effect on growth.	Wise et al. (2005)
Lead Glutamate	Mitotic Index (250-2,000 μM for 24 h) Growth Curve (250-2,000 μM for 24 h) Cell cycle Analysis (250-2,000 μM for 24 h)	WTHBF-6 –human lung cells with hTERT in DMEM/F12 + 10%CCS	None	Lead glutamate induced a concentration-dependent increase in intracellular lead ions. Lead glutamate increased the mitotic index, but either had no effect or inhibited growth and induced mitotic arrest.	Wise et al. (2005)

Table AX5-6.19 (cont'd). Genotoxic/Carcinogenic Effects of Lead – Mitogenesis Human and Animal Cell Culture Studies

Compound	Assay (Concentration and Exposure Time)	Cell Type and Culture Medium	Co-exposure	Effects	Reference
Lead Nitrate	Mitotic Index (3-30 µM for 2h +16 h recovery)	CHO cells in EMEM + 10%FBS	None	Lower concentrations (1 and 3 µM) of lead nitrate significantly increased the mitotic index. Higher concentrations (10 and 30 µM) had no effect.	Lin et al. (1994)
Lead Nitrate	Mitotic Index (0.05- 1 µM for 3-12 h)	CHO AA8 in DMEM +10% NCS	Crown ethers to modify effect through chelation and uptake	Lead nitrate dramatically reduced the mitotic index at 1 µM though this was not statistically analyzed. There was no effect on mitotic index at lower concentrations. Crown ethers had no modifying effect.	Cai and Arenaz (1998)
Lead Nitrate	Apoptosis (15-240 µM for 3 h)	Rat Alveolar Macrophages in DMEM + 10% FBS	None	Lead nitrate induced apoptosis in a dose-dependent manner.	Shabani and Rabbani (2000)

Abbreviations:

G12 – CHV79 are derived from V79;
V79 are a Chinese Hamster Lung Cell Line;
hTERT = hTERT is the catalytic subunit of human telomerase

Medium and Components

AMEM = Alpha Minimal Essential Medium;
DMEM = Dulbecco's Minimal Essential Medium;
DMEM/F12 = Dulbecco's Minimal Essential Medium/Ham's F12;
EMEM = Eagle's Minimal Essential Medium;

BSA = Bovine Serum Albumin
CCS = Cosmic Calf Serum
FBS = Fetal Bovine Serum
FCS = Fetal Calf Serum
NCS = Newborn Calf Serum

Differences between the serum types are unclear as insufficient details are provided by authors to distinguish.

Table AX5-6.20. Genotoxic/Carcinogenic Effects of Lead – Mitogenesis Other

Compound	Assay (Concentration and Exposure Time)	Cell Type and Culture Medium	Co-exposure	Effects	Reference
Lead Acetate	Production of reactive oxygen species (1 mM for 180 min) Glutathione levels (1 mM for 0-180 min)	SH-SY5Y- Human neuroblastoma cells in DMEM + 7% FCS	Glutamate (1 mM) or PKC inhibitor (1 µM)	Lead acetate alone did not produce reactive oxygen species. Glutamate alone did. Lead acetate plus glutamate increase glutamate induced increases in reactive oxygen species. Lead acetate alone did not deplete glutathione. Glutamate alone did. Lead acetate plus glutamate decreased glumate-induced decrease in glutathione.	Naarala et al. (1995)
Lead Acetate	Catalase Activity (500-2,000 µM for 24 h)	Human Foreskin Fibroblasts (Chinese) in DMEM +10% FCS	3-aminotriazole (3-AT) (80 mM to inhibit catalase)	Lead acetate had no effect on catalase activity.	Hwua and Yang (1998)
Lead Acetate	Thiol Levels (100 µM for 30 min - 4 h)	HeLa in HEPES/glucose buffer	Buthionine sulfoximine (BSO) to deplete cells of thiols	Lead acetate only lowered thiols marginally	Snyder and Lachmann (1989)
Lead Chloride	Oxidative Metabolism (0.1-100 µM for 20 h) Phagocytosis (0.1-100 µM for 20h)	Macrophages from NMRI mice in EMEM (serum not given)	Zymosan and latex particles as substrates for phagocytosis	Lead inhibited oxidative metabolism. Lead inhibited phagocytosis, but only significantly at the highest dose.	Hilbertz et al. (1986)
Lead Chloride	Oxidative Enzyme Levels (0.1-1 µM for 1 h)	AS52-CHO-gpt, lack hprt in HBSS followed by Ham's F12 + 5% FBS	Allopurinol (50 µM) to inhibit xanthine oxidase	Lead chloride at low concentrations produced H_2O_2 at 1 h and not at 24 h. Lead chloride at high concentrations produced no change at 1 h and increased H_2O_2 at 24 h. Allopurinol inhibited H_2O_2 formation at high lead concentrations. Lead chloride had no effect on catalase, glutathione peroxidase, glutathione reductase. Lead chloride inhibited glutathione-S-transferase, CuZn-superoxide dismutase, and xanthine oxidase.	Ariza et al. (1998)

Abbreviations:
AS52 are derived from CHO;
CHO are a Chinese Hamster Ovary Cell Line;

Medium and Components
DMEM = Dulbecco's Minimal Essential Medium;
EMEM = Eagle's Minimal Essential Medium;
HBSS = Hank's Balanced Salt Solution
FBS = Fetal Bovine Serum
FCS = Fetal Calf Serum

Differences between the serum types are unclear as insufficient details are provided by authors to distinguish.

CHAPTER 5 ANNEX

ANNEX TABLES AX5-7

Table AX5-7.1. Light Microscopic, Ultrastructural, and Functional Changes

Author	Animal Species	Lead Dosage	Blood Lead	Findings
Khalil-Manesh et al. (1992a)	Sprague-Dawley rat	0.5% Pb acetate in drinking water for 12 mo.	Max 125.4 µg/dL Mean 55 µg/dL	Hyperfiltration at 3 mo. Decreased filtration at 12 mo. NAG and GST elevated. Nuclear inclusion bodies at all times. Tubulointerstitial scarring from 6 mo. No arterial or arteriolar pathology.
Khalil-Manesh et al. (1992b)	Sprague-Dawley rat	0.5% Pb discontinued after 6 mo 0.01% Pb discontinued after 6 mo DMSA 0.5% used in 1/2	Hi Pb @12 mo Disc 30.4 µg/dL Disc + DMSA 19.1 µg/dL Ctrl 3.1 µg/dL Lo Pb@12mo Disc 6.9 µg/dL DMSA5.5 µg/dL	High Pb: Nuclear inclusion bodies prominent. Tubulointerstitial disease severe but less than 12 mo continuous DMSA caused reduction in nuclear inclusion bodies and tubuloint decrease, and an increase in GFR. Low Pb: Neg pathology and increase in GFR with DMSA
Khalil-Manesh et al. (1993a)	Sprague-Dawley rat	0.01% Pb acetate for 12 mo.	Max 29.4 µg/dL Range 9-34 µg/dL	GFR increased at 1 and 3 mo. NAG increased but GST normal. Pathology neg except at 12 mo-mild tubular atrophy and interstitial fibrosis seen.
Sanchez-Fructuoso et al. (2002a)	Wistar rat	500 ppm (0.05%) Pb acetate for 2 mo, then EDTA	Max 52.9 µg/dL Day 90: 33.2 Day 137: 22.8 Ctrl: 5.90 µg/dL	Rats given Pb to day 90, then treated with EDTA or untreated to day 137. Marked decrease in kidney, liver, and brain Pb with EDTA but no change in femur Pb
Sanchez-Fructuoso et al. (2002b)	Wistar rat	500 ppm (0.05%) Pb acetate for 2 mo, then EDTA	Max 52.9 µg/dL Day 90: 33.2 Day 137: 22.8 Ctrl: 5.90 µg/dL	Hypertrophy and vacuolization of medium and small arteries, mucoid edema and muscular hypertrophy of arterioles, include bodies and fibrosis. EDTA slowed progression.
Papaioannou et al. (1998)	Dogs	12 mg Pb acetate i.p. x 10	—	Lead includes bodies intracytoplasmically in mesothelial and giant cells of peritoneum and in interstitial connective tissue cells of kidney. None in prox tubules of kidney.

Table AX5-7.1 (cont'd). Light Microscopic, Ultrastructural, and Functional Changes

Author	Animal Species	Lead Dosage	Blood Lead	Findings
Vyskocil et al. (1989)	Wistar rat	0.5%,1%, and 2% Pb acetate for 2-3 mo.	0.5%-105 µg/dL 1%-196 µg/dL 2%-320 µg/dL	0.5%-no morphologic or functional changes 1%-Incr in β-2 microglobulin excretion. 2%-Incr in β2micr, glucose, protein, lysozyme, and LDH. Hyperplasia and include bodies of prox tubules seen in both 1% and 2%
Vyskocil et al. (1995)	Wistar rat	1% or 0.1% Pb acetate for 2-4 mo.	1%- 173 µg/dL 0.1%-37.6 µg/dL	1% caused increase in β-2 microglobulin excretion and injury to proximal tubule. 0.1% caused no changes.
Vyskocil and Cizkova (1996)	Wistar rats	Unleaded petrol vapor (4mg/m^3) 8 hrs/day for 60 days	—	B-2 microglobulin excretion increased at 60 days
Sanchez et al. (2001)	Sprague-Dawley rat	0.06% Pb acetate for 4 mo.	13.9 µg/dL vs. <0.5 µg/dL in ctrl	Decrease in expression of laminin-1 and increase in expression of fibronectin in kidneys.
Herak-Kramberger et al. (2001)	Rat brush border membranes	500 µM Pb	—	58% loss of sealed brush border membrane vesicles. Lower loss of sealed basolateral membrane vesicles.
Fujiwara et al. (1995) and Kaji et al. (1995)	Bovine cultured vascular smooth muscle and endothelial cells	0.5 – 10 µM Pb nitrate	—	Stimulated proliferation in smooth muscle cells. Reduced proliferation in endothelial cells No leakage of LDH.

Table AX5-7.2. Lead and Free Radicals

Author	Animal Species	Lead Dosage	Blood Lead	Findings
Pereira et al. (1992)	Rats	ALA-treated (40 mg/kg every 2 days for 15 days)	—	Fatigued earlier than controls. Increase of CuZn SOD in brain, muscle and liver
Somashekaraiah et al. (1992)	Chick embryos	1.25 and 2.5 μmol/kg of Pb acetate	—	Lipoperoxides maximal at 9 hrs and returned to normal at 72 hrs. GSH depleted. GST, SOD and catalase increased in liver, brain and heart at 72 hrs
Bondy and Guo (1996)	Sprague-Dawley rat cerebral synaptosomes	0.5 mM Pb acetate	—	Generation of ROS not increased by Pb alone but increased when 50 μM iron sulfate added.
Blazka et al. (1994)	Mouse brain microvascular endothelial cell culture	10, 100, and 1,000 nM Pb acetate	—	Constitutive production of nitrite, but not inducible, decreased by Pb. Extracellular calcium abolishes this effect.
Quinn and Harris (1995)	Rat cerebellum homogenates	17-80 nM Pb nitrate	—	Constitutive NOS activity inhibited 50% by 17 nM Pb and 100% by 80 nM Pb. Reversed by increasing Ca concentration.
Ercal et al. (1996)	C57BL/6 mice	1300 ppm Pb acetate for 5 weeks. Nac, 5.5 mmol/kg, or DMSA, 1 mmol/kg, given in 6th week.	36.5 μg/dL in Pb-treated; 13.7 μg/dL in Pb + DMSA-treated.	Liver and brain GSH depleted by Pb and MDA increased. Both were restored by either DMSA or NAC. However, DMSA reduced blood, liver, and brain Pb levels while NAC did not.
Vaziri and co-workers (1997-2004)	Sprague-Dawley rats	See Section 5.5 for details	Variable.	See section 5.5 for details.
Farmand et al. (2005)	Sprague-Dawley rats	100 ppm Pb acetate for 3 months	—	CuZnSOD activity increased in kidney. CuZnSOD activity increased in aorta whereas protein abundance unchanged. Guanylate cyclase protein abundance in aorta decreased.
Gurer et al. (1999)	Fischer 344 rats	1100 ppm Pb acetate for 5 wks. Captopril for 6th wk	24.6μg/dL in Pb-treated. 23.8 μg/dL in Pb + Captopril-treated	MDA in liver, brain, and kidney increased by Pb. GSH decreased. Captopril reversed these findings.

Table AX5-7.2 (cont'd). Lead and Free Radicals

Author	Animal Species	Lead Dosage	Blood Lead	Findings
Acharya and Acharya (1997)	Swiss mice	200 mg/kg Pb acetate i.p. x 1	—	MDA-TBA increased x 4 in liver, brain, kidney, and testis by end of 1st wk and persisted for 4 wks.
Upasani et al. (2001)	Rats	100 ppm Pb acetate for 30 days. Groups given vit C, vit E, or algae	—	MDA, conj dienes, and H_2O_2 increased in liver, lung, and kidney by Pb. Treatment with vit C, vit E, or Blue Green algae reversed these findings.
Pande et al. (2001)	Wistar rats	Lead nitrate 50 mg/kg i.p. x 5 Lead + DMSA,MiADMSA,NAC, DMSA + NAC,DMSA + MiADMSA	—	DMSA most effective in blocking inhib of ALAD, elev of ZPP, and inhib of GSH. Combined DMSA+NAC most effective when given during or post-exposure.
Pande and Flora (2002)	Wistar rats	2000 ppm Pb acetate x 4 wks. DMSA,MiADMSA, DMSA + LA,MiADMSA + LA x 5 days	—	Lead caused decrease in ALAD, GSH, and increase ZPP. Lipoic acid (LA) did not chelate Pb in contrast to DMSA, but both agents increased ALAD and GSH
Flora et al. (2002)	Wistar rats	1000 ppm Pb acetate x 3 mo. DMSA or MiADMSA + vit C or vit E x 5 days	13.3 µg/dL lead Rx 3 µg/dL DMSA Rx <1µg/dL DMSA+ vit E	Both thiol chelators and 2 vitamins increased ALAD. GSH increase only after thiol chelators. Vitamin E or C with thiol chelators reduced blood Pb further.
Saxena and Flora (2004)	Wistar rats	2000 ppm Pb acetate x 6 wks. $CaNa_2EDTA$ + DMPS or MiADMSA x 5 day	15.1µg/dL lead Rx 9.8 µg/dL EDTA Rx 6 µg/dL EDTA + MiADMS	Lead caused inhib of ALAD and GSH and depl of ALAD in kidney, ALAS in liver, GSH in brain, increase in brain TBARS and GST. Combined Rx with CaNa2EDTA and MiADMSA most effective in reducing oxidative stress and tissue Pb burden.
Tandon et al. (2002)	Rats	2000 ppm Pb acetate x 9 wks. DMSA, MiADMSA, or NAC or combo x 6 days	—	Lead raised MDA, inhibited ALAD, increased catalase, and depleted GSH. DMSA plus NAC was most effective in reversing these changes.

Table AX5-7.2 (cont'd). Lead and Free Radicals

Author	Animal Species	Lead Dosage	Blood Lead	Findings
Sivaprasad et al. (2002)	Wistar rats	2000 ppm Pb acetate x 5 wks. LA and DMSA during 6th week.	—	Lead caused red in kidney GGT & NAG, decline in GSH, catalase, SOD, GPx and Glut reductase, and increased MDA. Lipoic acid +DMSA restored these changes
Senapati et al. (2000)	Rats	1% sol of 5mg/kg Pb acetate x43 d. Thiamine 25 mg/kg	—	Thiamine reduced Pb content and MDA levels of both liver and kidney and improved pathology.
Patra et al. (2001)	IVRI 2CQ rats	1 mg/kg Pb acetate for 4 wks. Vit E, vit C or methionine in 5th wk. Vit E + EDTA.	6.8 µg/dL Pb-Rx 6.3 µg/dL lead, vit E +EDTA	Lead in liver, kidney and brain reduced by vit E + EDTA treatment. MDA increased by Pb in all 3 organs but decreased by vit E + EDTA.
McGowan and Donaldson (1987)	Chicks	2000 ppm Pb acetate x 3 wks.	—	GSH, non-protein SH, lysine and methionine increased in liver and non-prot SH, glycine, cysteine and cystathionine in kidney. Cysteine reduced in plasma.

Table AX5-7.3. Chelation with DMSA

Author	Animal Species	Lead Dosage	Blood Lead	Findings
Cory-Slechta (1988)	Rats	50 ppm Pb acetate for 3-4 mo.	20 µg/dL-lead 1µg/dL-lead+25 mg/kg DMSA	DMSA 25-50 mg/kg i.p. for 1-5 d mobilized Pb from blood, brain, kidney and liver, but not femur.
Pappas et al. (1995)	Sprague-Dawley rats	550-1100 ppm Pb acetate for 35 days	52 µg/dL @ 550 ppm Pb 65 µg/dL @ 1100ppmlead	DMSA @16-240 mg/kg/day p.o. for 21 days given with and without concurrent Pb exposure. Rats showed dose-related reduction in Pb content of blood, brain, femur, kidney, and liver with or without concur Pb.
Smith and Flegal (1992)	Wistar rats	^{206}Pb 210 ng/mL for 1.5days.DMSA 20 mg/kg i.p.	5.1 ng/g-ctrl 3.0 ng/g-DMSA	Rats on low Pb diet given DMSA decreased soft tissue but not skeletal Pb. Lead redistributed to skeleton.
Varnai et al. (2001)	Wistar rats (suckling)	2 mg/kg/d for 8 d DMSA 0.5 mmol/kg 6x/d on d1-3 & 6-8	—	DMSA reduced Pb concentration in carcass, liver, kidneys, and brain by ~ 50%.

Table AX5-7.4. Effect of Chelator Combinations

Author	Animal Species	Lead Dosage	Blood Lead	Findings
Flora et al. (2004)	Wistar rats	1000 ppm Pb acetate for 4 mo	46 µg/dL-lead 12.8 µg/dL-combined Rx	5 days Rx with DMSA. $CaNa_2EDTA$, or DMSA + $CaNa_2EDTA$. Comb Rx resulted in increased ALAD & decreased Pb in blood, liver, brain, and femur.
Jones et al. (1994)	Mice	10 i.p. injections of Pb acetate, 5.0 mg/kg	—	Mice Rx'ed with DMSA, $CaNa_2EDTA$, $ZnNa_2EDTA$, or $ZnNa_3DTPA$ 1.0 mmol/kg/d 4-8 d. $CaNa_2EDTA$ most effective in removing brain Pb; DMSA in removing kidney and bone Pb.
Kostial et al. (1999)	Wistar rats (suckling)	5 mg Pb/kg i.p. x 1 Chel agents d 2 & 3	—	EDTA, DMSA, racemic DMSA, EDTA + DMSA, EDTA + rac DMSA given. EDTA reduced Zn in carcass & liver; rac DMSA reduced Zn in kidneys. DMSA reduced Pb w/o affecting Zn
Flora et al. (2004)	Wistar rats	1000 ppm Pb acetate x 2 mo	—	DMSA, taurine or DMSA + taurine given for 5 d. Both taurine & DMSA restored GSH. Comb of DMSA + taurine increased RBC SOD & decreased TBARS, while most effectively depleting blood, liver, & brain Pb.
Sivaprasad et al. (2004)	Wistar rats	2000 ppm Pb acetate x 5 wks	—	DMSA, lipoic acid or combination given during 6th week. Renal enzymes, kidney Pb and renal ALAD restored by combined Rx
Malvezzi et al. (2001)	Wistar rats	750 ppm Pb acetate x 70 days DMSA, arginine, DMSA + arg or H_2O x 30 d	67.8 µg/dL to 11.2 µg/dL in H_2O Rx'ed to 6.1 µg/dL in DMSA + arg	Lead increased BP and Pb levels in blood, liver, femur, kidney, and aorta. DMSA + L-arginine most effective in lowering BP and mobilizing Pb from tissues.
Tandon et al. 1997)	Rats	1000 ppm Pb acetate for 7 wks. Dithiocarbamate x 4 days	105.3 µg/dL in Pb. 86µg/dL in dithiocarbamate.	Two dithiocarbamates were compared: N-benzyl-D-glucamine and N-(4-methoxybenzyl)-D-glucamine. They were only partially effective in restoring ALAD, reducing liver & kidney, but not brain Pb. They depleted Zn, Cu, and Ca.

Table AX5-7.5. Effect of Other Metals on Lead

Author	Animal Species	Lead Dosage	Blood Lead	Findings
Maldonado-Vega et al. (1996)	Wistar rats (pregnant & non-pregnant)	100 ppm Pb acetate for 144-158 days	5.2 (ctrl) to 27.3 µg/dL in Pb-exposed 8 (non-preg) to 17 µg/dL in rats exposed only during lactation	Lead administered to period before lactation (144 d) or to mid-lactation (158 d).Lead in blood, kidney, liver, and bone increased. ALAD decreased and FEP incr. Lactation increased Blood Pb from 24.7 to 31.2 µg/dL and decreased bone Pb from 83.4 to 65.2 nmol/g.
Olivi et al. (2002)	MDCK canine kidney cells	1 µM	—	In response to agonists ADP or bradykinin levels of intracellular Ca increased 3-fold and 2-fold. Lead inhibited the response.
Bogden et al. (1991)	Wistar rats	0,1,100 ppm Pb for 31 wks 0.2% or 4.0% Ca diet	1.9 to 39.1 µg/dL on low Ca diet and 2.0 to 53.3 µg/dL on high Ca diet	At 100 ppm Pb high Ca diet produced higher BP and more renal cancers than low Ca diet and higher levels of Pb in brain, liver, bone, heart, and testis but lower levels in kidney. Serum Ca on high Ca diet was 13.2 mg/dL.
Skoczynska et al. (1994)	Buffalo rats	Pb 70 mg/kg 2x/wk for 7 wks Cd 20 mg/kg 1x/wk for 7 wks. All intragastric.	5.1 to 29.6 µg/dL in Pb-exposed. 37.4 µg/dL in Pb + Cd	Simultaneous Pb and Cd administration increased blood Pb but decreased Pb in liver and kidneys as compared to Pb administration alone.
Othman & Missiry (1998)	Albino rats	Pb acetate 100 µmol/kg I.M. x 1 Se 10 µmol/kg I.M. 2 hrs before Pb	—	Sodium selenite (Se is a well-known anti-oxidant) prevented lipid peroxidation (TBARS) and reduction in GSH caused by Pb. SOD & glut reductase also normalized.
Tandon et al. (1992)	Albino rats	Pb acetate 10 mg/kg/d p.o. x 6 wks. EDTA or DTPA given for 5 d w or w/o Se	17 to 138 µg/dL after Pb 58 µg/dL after EDTA. 50 µg/dL after EDTA + Se	Selenium had no additional benefit over chelators except for higher ALAD and lower ZPP in blood, lower Pb in liver and kidney.
Flora et al. (1989)	Albino rats	Pb acetate 10 mg/kg/d p.o. x 6 wks Thiamine, Zn or thiamine + Zn x 6 wks	6.2 to 120.9 µg/dL after Pb 44.1 µg/dL after thiamine + Zn	Thiamine given as 25 mg/kg/d and Zn sulfate as 25 mg/kg/d. ALAD restored by combined Rx. Liver and kidney Pb affected to a minor degree but brain Pb not affected.
Flora et al. (1994)	Wistar albino rats	Pb acetate 10 mg/kg/d x 56 d p.o. EDTA or EDTA + Zn x 5 days p.o.	4.6 to 43.0 µg/dL in Pb. 22.5 µg/dL in EDTA 16.5 µg/dL in EDTA + Zn.	CaNa2EDTA given as 0.3 mmol/kg/d i.p. and Zn sulfate as 10 or 50mg/kg/d. ALAD partially restored after EDTA + Zn but not after EDTA. EDTA reduced Pb in bone, kidney, and liver but not in brain. Zn conc increased in blood, kidney, & brain by 50 mg Zn dosage.

Table AX5-7.5 (cont'd). Effect of Other Metals on Lead

Author	Animal Species	Lead Dosage	Blood Lead	Findings
Satija and Vij (1995)	Albino rats	Pb acetate 20 mg/kg/d i.p. x 3 d Zn acetate 5 mg/kg/d i.p. x 3 d	—	Lead caused a decrease in Hgb, ALAD, & uroporphyrinogen I synthetase, partially restored by Zn. Total SH and non-protein SH reduced by Pb, partially restored by Zn.
Munoz et al. (1993)	Wistar rats	Pb acetate 60 ppm x 90 d, Zn or methionine given simultaneously	<60 µg/dL-Pb SAM reduces to average of 7.3 µg/dL	S-adenosyl-l-methionine (SAM) reduces blood Pb & uroporphyrinogen I synthetase. RBC ALAD reduced by Pb & 2 Rx. Liver ALAD decreased by Pb, increased by SAM.
Tandon et al. (1997)	Albino rats	Pb acetate 10 mg/kg/d x 8 wks p.o. Ethanol, Zn & lysine x 8 wks	1.8 to 47.2 µg/dL w Pb. Decreased to 34.2 µg/dL w Zn + lysine.	Ethanol reduced blood but not liver GSH beyond Pb alone. Zn + lysine partially restored ALAD, increased GSH, and reduced Pb in kidney.
Hashmi et al. (1989)	Rats	Pb acetate 1000 ppm x 6 weeks Fe-deficient or norm diet	—	Fe deficiency increased Pb in liver, kidney, spleen but increased femur Pb at 3 wks and decreased femur Pb at 6 weeks.
Tandon et al. (1993)	Rats	Pb acetate 400 µmol/kg i.p. x 1 Fe deficient & Fe-sufficient diets x 6 wk	—	Pb induced hepatic metallothionein (MT). Fe deficient diet + Pb restored kidney and intestinal MT from low levels caused by Fe def. Pb in liver & kidney enhanced by Fe def
Crowe and Morgan (1996)	Wistar rat pups	Pb acetate 2000 ppm x 15, 21, & 63 days Fe def & Fe suff diets	At 63 d Fe def-410 µg/dL Fe suff rats 170 µg/dL	Fe deficiency increased blood and kidney Pb but did not affect brain or liver Pb. Fe levels in brain and kidney were unaffected by Pb intoxication.
Singh et al. (1991)	Pregnant female albino rats	Pb acetate 250-2000 ppm from 15-20 d of gestation.	At 2000 ppm Pb, Fe def 220 µg/dL Fe suff 160 µg/dL	Fe def and Fe suff diets given to dams for 30 days. Fetuses removed on 21st day. At 2000 ppm, Pb in maternal blood, placenta, and fetus higher in Fe def. Max pathol changes in fetal kidney.
Shakoor et al. (2000)	Albino rats	Pb acetate 125 mg/kg x 90 d Al chloride 50-100 mg/kg x 90 d	—	Plasma creat 1.88 mg/dL in Pb-Rx'ed; 1.34 mg/dL in Pb + Al-Rx'ed Kidney Pb increased from 5.4 in ctrl to 220 µg/g in Pb-Rx'ed, decreased to 98.9 µg/g in Pb + Al.

CHAPTER 5 ANNEX

ANNEX TABLES AX5-8

Table AX5-8.1. Bone Growth in Lead-exposed Animals

Compound Dose/Concentration Duration Exposure Route	Species	Effects	Blood Level	Reference
Lead Acetate 41.7 mg Pb/l 83.3 mg Pb/l 166.6 mg Pb/l 12 to 16 weeks Drinking water	Rat	Lead level in bone of control animals Wk 0 = 1.3 ± 0.83 µg Pb/g; Wk 4 = 1.2 ± 0.99 µg Pb/g; Wk 8 = 1.3 ± 1.08 µg Pb/g; Wk 12 = 0.8 ± 0.13 µg Pb/g; Wk 16 = 1.3 ± 0.95 µg Pb/g Lead level in bone of animals receiving 41.7 mg Pb/l Wk 0 = 1.0 ± 0.50 µg Pb/g; Wk 4 = 5.9* ± 1.76 µg Pb/g; Wk 8 = 2.9* ± 1.15 µg Pb/g; Wk 12 = 6.2* ± 1.01 µg Pb/g; Wk 16 = 6.0* ± 0.75 µg Pb/g Lead level in bone of animals receiving 83.3 mg Pb/l Wk 0 = 2.0 ± 0.97 µg Pb/g; Wk 4 = 11.7* ± 3.56 µg Pb/g; Wk 8 = 8.8* ± 3.37 µg Pb/g; Wk 12 = 14.3* ± 4.29 µg Pb/g Lead level in bone of animals receiving 166.6 mg Pb/l Wk 0 = 0.9 ± 0.23 µg Pb/g; Wk 4 = 17.0* ± 3.89 µg Pb/g; Wk 8 = 35.7* ± 3.64 µg Pb/g; Wk 12 = 21.7* ± 5.11 µg Pb/g; *significantly higher than control animals at corresponding time point	Not given	Hac and Kruchniak (1996)
Lead aerosol 77, 249, or 1546 µg/m^3 for 50 to70 days Inhalation	Rat	16.9 ± 6.6 µg Pb/g bone taken up in animals exposed to 77 µg/m^3 for 70 days versus 0.2 ± 0.2 µg Pb/g in control animals; 15.9 ± 4.3 µg Pb/g bone in rats exposed to 249 µg/m^3 for 50 days; 158 ± 21 µg Pb/g bone in rats exposed to 1546 µg/m^3 for 50 days	Control: 2.6 µg/dL 77 µg/m^3: 11.5 µg/dL 249 µg/m^3: 24.1 µg/dL 1546 µg/m^3: 61.2 µg/dL	Grobler et al. (1991)
Lead acetate 250 ppm or 1000 ppm 7 weeks to females prior to mating, continuing through gestation and lactation Drinking water	Rat	Offspring body weight was depressed relative to controls during suckling (Day 11) and after weaning (Day 24) in high dose and continuously lead-exposed groups. Continuous lead exposure caused a greater decrease in offspring body weight than lead exposure only prior to or after parturition. Decreased tail length growth suggested possible effects of lead on tail vertebral bone growth.	Dams prior to mating: Control = 2.7 ± 0.6 µg/dL 250 ppm = 39.9 ± 3.5 µg/dL 1000 ppm = 73.5 ± 9.3 µg/dL	Hamilton and O'Flaherty (1994)

Table AX5-8.1 (cont'd). Bone Growth in Lead-exposed Animals

Compound Dose/Concentration Duration Exposure Route	Species	Effects	Blood Level	Reference
Lead acetate Hi Pb animals 5000 ppm for 6 months, reduced to 1000 ppm; Lo Pb animals 100 ppm Drinking Water	Rat	In male rats exposed to 100 ppm lead in drinking water and a low calcium diet for up to one year, bone density was significantly decreased after 12 months, while rats exposed to 5000 ppm lead had significantly decreased bone density after 3 months. Lead content of femurs was significantly elevated over the content of control rats at all time points (1, 3, 6, 9, 12 months). Trabecular bone from the low dose animals was significantly decreased from 3 months forward.	Low Pb (µg%): 1 month Control = 2 ± 1; Exp = 19 ± 10* 3 months Control = 2 ± 1; Exp = 29 ± 4* 6 months Control = 3 ± 1; Exp = 18 ± 2* 9 months Control = 1 ± 1; Exp = 17 ± 3* 12 months Control = 3 ± 1; Exp = 21 ± 3* Hi Pb (µg%): 1 month Control = 3 ± 1; Exp = 45 ± 13* 3 months Control = 3 ± 1; Exp = 90 ± 15* 6 months Control = 4 ± 1; Exp = 126 ± 10* 9 months Control = 4 ± 1; Exp = 80 ± 39* 12 months Control = 3 ± 1; Exp = 59 ± 18* *p < 0.001	Gruber et al. (1997)
Lead acetate 17 mg per kg of feed 50 days In diet	Rat	No differences in the length of the femurs, but the mean length of the 5th lumbar vertebra was significantly decreased. The mean length of the femur growth plate cartilage was also significantly decreased in lead-exposed animals.	Not given	Gonzalez-Riola et al. (1997)
Lead acetate 17 mg per kg of feed 50 days In diet	Rat	No differences in the length of the femurs, but the mean length of the 5th lumbar vertebra was significantly decreased. The mean length of the femur growth plate cartilage was also significantly decreased in lead-exposed animals.	Not given	Escribano et al. (1997)

Table AX5-8.1 (cont'd). Bone Growth in Lead-exposed Animals

Compound Dose/Concentration Duration Exposure Route	Species	Effects	Blood Level	Reference
Lead acetate 0.6% GD 5 to Adulthood (various) In drinking water	Rat	Early bone growth was significantly depressed in a dose-dependent fashion in pups of lead-exposed pups, with growth suppression in male offspring considerably greater than females. Significant decreases in plasma insulin-like growth factor and plasma sex steroids and increased pituitary growth hormone were also observed. Groups: DDW = Dams and pups received distilled deionized water entire study Ac/Ac = Dams and pups received acetic acid solution entire study Preg = Dams received 0.6% lead water from GD 5 to parturition Lact = Dams received 0.6% lead water during lactation only P + L = Dams received 0.6% lead water from GD 5 through lactation Postnatal = Dams and pups received 0.6% lead water from parturition through adulthood Pb/Pb = Dams and pups received 0.6% lead water from GD 5 through adulthood	Whole blood lead (µg/dL) in male/female offspring at age Day 85: DDW = 5.5 ± 2.0/6.8 ± 1.5; Ac/Ac = 1.9 ± 0.2/1.4 ± 0.3; Preg = 9.1 ± 0.7*/11.6 ± 4.6*; Lact = 3.3 ± 0.4/3.4 ± 0.8; P + L = 16.1 ± 2.3*/10.4 ± 1.8*; Postnatal = 226.0 ± 29.0*/292.0 ± 53.0*; Pb/Pb = 316.0 ±53.0*/264.0 ± 21.0* *p< 0.05 compared to Ac/Ac group.	Ronis et al. (1998a)
Lead acetate 0.05% to 0.45% GD 5 through sacrifice of pups at 21, 35, 55, and 85 days In drinking water	Rat	Early bone growth was significantly depressed in a dose-dependent fashion in pups of all lead-exposed groups, with growth suppression in male offspring considerably greater than females. Significant decreases in plasma insulin-like growth factor and plasma sex steroids and increased pituitary growth hormone were also observed. Between age 57 and 85 days growth rates were similar in control and lead-exposed pups, suggesting exposure at critical growth periods such as puberty and gender may account for differences in growth reported by various investigators.	Offspring: 0.05% Pb = 49 ± 6 µg/dL; 0.15% Pb = 126 ± 16 µg/dL; 0.45% Pb = 263 ± 28 µg/dL	Ronis et al. (1998b)
Lead nitrate 0.02% (125 ppm) GD5 to 1 day before sacrifice In drinking water	Rat	Exposure to 0.2% lead nitrate (125 ppm lead) did not significantly affect growth, though males weighed significantly less than females.	Rat Pups 5 days old: 43.3 ± 2.7 µg/dL 49 days old: 18.9 ± 0.7 µg/dL (females: 19.94 ± 0.8 µg/dL; males: 17.00 ± 1.1 µg/dL)	Camoratto et al. (1993)

Table AX5-8.1 (cont'd). Bone Growth in Lead-exposed Animals

Compound Dose/Concentration Duration Exposure Route	Species	Effects	Blood Level	Reference
Lead acetate 0.15% or 0.45% GD 4 until Day 55 In drinking water	Rat	A dose-dependent decrease in load to failure in tibia from lead-exposed (0.15% and 0.45% lead acetate in drinking water) male pups only. Hormone treatments (L-dopa, testosterone or dihydrotestosterone in males, or estradiol in females) failed to attenuate lead deficits during the pubertal period. Distraction osteogenesis experiments performed after stabilization of endocrine parameters (at 100 days of age) found decreased new endosteal bone formation and gap x-ray density in the distraction gaps of lead-exposed animals.	Offspring: 0.15% Pb = 67-192 µg/dL; 0.45% Pb = 120-388 µg/dL	Ronis et al. (2001)
Lead acetate 1000 ppm 22–26 days In drinking water	Rat	Lead disrupted mineralization during growth in demineralized bone matrix implanted subcutaneously into male rats. In the matrix that contained 200 micrograms lead/g of plaque tissue, alkaline phosphatase activity and cartilage mineralization were absent, though calcium deposition was enhanced. Separate experiments found enhanced calcification and decreased alkaline phosphatase activity in rats implanted with a control (no lead) matrix and given 1000 ppm lead in drinking water for 26 days.	Blood Pb (µg/dL) Control: Implantation Day 0 = 1.3 ± 0.6; Day 8 = 2.2 ± 0.9; Day 12 = 2.1 ± 0.7. Lead added to matrix: Implantation Day 0 = 1.5 ± 0.8; Day 8 = 5.7 ± 0.8[a,b]; Day 12 = 9.5 ± 0.5 [a,b]. Lead in drinking water: Implantation Day 0 = 129.8 ± 6.7 [a]; Day 8 = 100.6 ± 6.8 [a,b]; Day 12 = 96.4 ± 5.3 [a,b]. [a] Significant ($p \leq 0.05$) difference from control. [b] Significance ($p \leq 0.05$) difference from corresponding value at implantation (Day 0).	Hamilton and O'Flaherty (1995)

Abbreviations

Mg -	milligram	l -	liter
µg -	microgram	m^3 -	cubic meter
ppm -	parts per million	Exp	experimental group
GD -	gestational day	wk -	week
Pb -	lead	dL -	deciliter
g -	gram	% -	percent
µg% -	microgram percent		

Table AX5-8.2. Regulation of Bone Cell Function in Animals – Systemic Effects of Lead

Compound Dose/Concentration Duration Exposure Route	Species	Effects	Blood Level	Reference
Lead acetate 30 mg/kg Single IV injection	Rat	Groups of male rats were killed 0.5, 5, 15, and 30 min and 1, 2, 6, and 12 h after the single lead injection. Serum calcium and phosphorus levels both initially increased after lead injection with serum phosphorus reaching a maximum value (13.5 mg%) after 30 min and calcium (17 mg%) after 1 h. Calcium and phosphorus levels decreased after 1 h and returned to baseline levels after 12 h.	Not given	Kato et al. (1977)
Lead acetate 0.82% 1 week In diet	Rat	Ingestion of 0.82% lead in male rats fed either a low phosphorus or low calcium diet reduced plasma levels of 1,25-$(OH)_2$CC, while lead had no effect in rats fed either a high calcium diet or a normal phosphorus diet. Effect of lead on serum 1,25-$(OH)_2$CC levels in rats fed low P or normal P diet Dietary Phosphorus / Supplement / Serum 1,25-$(OH)_2$CC / µg/100mL 0.1% / Control / <10 pg/mL / 3 ± 1 0.1% / Cholecalciferol / 248 ± 7 pg/mL / 9 ± 8 0.1% / 0.82% Pb+Cholecalciferol / 94 ± 13 pg/mL / 352 ± 40 0.3% / Control / <10 pg/mL / <3 0.3% / Cholecalciferol / 285 ± 44 pg/mL / <3 0.3% / 0.82% Pb+Cholecalciferol / 245 ± 46 pg/mL / 284 ± 36 Effect of lead on serum 1,25-$(OH)_2$CC levels in rats fed low Ca or high Ca diet Dietary Calcium / Supplement / Serum 1,25-$(OH)_2$CC / µg/100mL 0.02% / Control / <10 pg/mL 0.02% / Cholecalciferol (50ng/day) / 754 ± 18 pg/mL 0.02% / 0.82% Pb+Cholecalciferol / 443 ± 79 pg/mL 1.2% / Control / <10 pg/mL / <3 1.2% / Cholecalciferol (50ng/day) / 285 ± 44 pg/mL / <3 1.2% / 0.82% Pb+Cholecalciferol / 245 ± 46 pg/mL / 284 ± 36		Smith et al. (1981)
Lead acetate 0.15% or 0.45% GD 4 until Day 55 In drinking water	Rat	No effects of lead on plasma concentrations of vitamin D metabolites, 25-OH D_3 or 1,25-$(OH_2)D_3$, in pubertal male rats exposed to either 0.15% or 0.45% lead acetate in drinking water and maintained on an adequate diet.	Offspring: 0.15% Pb = 67-192 µg/dL; 0.45% Pb = 120-388 µg/dL	Ronis et al. (2001)

Table AX5-8.2 (cont'd). Regulation of Bone Cell Function in Animals – Systemic Effects of Lead

Compound Dose/Concentration Duration Exposure Route	Species	Effects	Blood Level	Reference
$PbCl_2$ 0, 0.2, or 0.8% 1 or 2 weeks In diet	Chicks	Compared with control animals, lead exposure significantly increased intestinal calbindin protein and mRNA levels in addition to plasma 1,25-dihydroxyvitamin D concentration. The effect was present after 1 week of exposure and continued through the second week. In calcium-deficient animals increased plasma 1,25-dihydroxyvitamin D and calbindin protein and mRNA were significantly ($p < 0.05$) inhibited by lead exposure in a dose dependent fashion over the 2 week experimental period.	None given	Fullmer (1995)
$PbCl_2$ 0, 0.2, or 0.8% 1 or 2 weeks In diet	Chicks	Dose dependent increases in serum 1,25-$(OH_2)D_3$ levels (and Calbindin-D protein and mRNA) with increasing dietary lead exposure (0.1% to 0.8%) in experiments performed on Leghorn cockerel chicks fed an adequate calcium diet.	None given	Fullmer (1996)
Lead acetate 1% for 10 weeks or 0.001-1% for 24 weeks In drinking water	Rat	Short term administration of 1% lead resulted in significant increases in bone lead. Total serum calcium and ionized serum calcium were significantly decreased, as compared to controls. Circulating levels of 1,25-$(OH_2)D_3$ were also decreased, though the rats were maintained on a normal calcium diet (0.95%). In the long term study, a dose-dependent increase in parathyroid weight occurred with increasing exposure to lead in drinking water.	Short term (10 week) study: Control: < 0.02 µg/l Lead-exposed: > 5µg/l	Szabo et al. (1991)

Short term (10 weeks) exposure	Controls	Lead-exposed
Serum Calcium (mM) Ionized Calcium	2.42 ± 0.03	2.32 ± 0.02*
(mM) 1,25$(OH)_2D_3$ (pM)	1.25 ± 0.03	1.15 ± 0.03*
Parathyroid Weight (µg/gland)	232 ± 18.9	177 ± 10.8*
*p < 0.01	96 ± 34	178 ± 25*

Long term (24 weeks) exposure Pb in water	Normalized Parathyroid Weight (µg/g body wt)	1,25$(OH)_2D_3$ (pM)
0%	0.50 ± 0.06	241 ± 32
0.001%	0.72 ± 0.25	188 ± 27
0.01%	0.81 ± 0.28	163 ± 17
0.1%	0.94 ± 0.27	206 ± 24
1.0%	0.81 ± 0.29*	144 ± 33*
p < 0.01		

Table AX5-8.2 (cont'd). Regulation of Bone Cell Function in Animals – Systemic Effects of Lead

Compound Dose/Concentration Duration Exposure Route	Species	Effects	Blood Level	Reference
Lead nitrate 0.02% (125 ppm) GD5 to 1 day before sacrifice In drinking water	Rat	Basal release of growth hormone from control and lead-exposed pups at age 49 days was not significantly different. Growth hormone releasing factor-stimulated release of growth hormone from pituitaries of lead-exposed pups was smaller than the stimulated release of growth hormone from pituitaries of control animals (75% increase over baseline vs. 171% increase, respectively), but the difference did not achieve significance (P = 0.08). Growth hormone content of the pituitary glands was also not influenced by lead exposure.	Rat Pups 5 days old: 43.3 ± 2.7 µg/dL 49 days old: 18.9 ± 0.7 µg/dL (females: 19.94 ± 0.8 µg/dL; males: 17.00 ± 1.1 µg/dL)	Camoratto et al. (1993)
Lead acetate 0.05% to 0.45% GD 5 through sacrifice of pups at 21, 35, 55, and 85 days In drinking water	Rat	Pituitary GH content (µg/mg) at postnatal day 55: Control Male Pups = 56.6 ± 8.0; Female Pups = 85.6 ± 9.3 0.05% Pb Male Pups = 107.2 ± 10.5*; Female Pups = 116.2 ± 9.1 0.15% Pb Male Pups = 96.8 ± 5.0*; Female Pups = 105.1 ± 7.3 0.45% Pb Male Pups = 106.0 ± 9.8*; Female Pups = 157.0 ± 9.9* *significantly different from control, $p < 0.05$	Offspring: 0.05% Pb = 49 ± 6 µg/dL; 0.15% Pb = 126 ± 16 µg/dL; 0.45% Pb = 263 ± 28 µg/dL	Ronis et al. (1998b)

Abbreviations

mg -	milligram
h -	hour
1,25 -	$(OH)_2CC$ – 1,25-dihydroxycholecalcigerol
µg -	microgram
25 -	OH D_3- 25-hydroxycholecalciferol
$PbCl_2$	lead chloride
1,25 -	$(OH)_2$ D_3 – vitamin D_3
kg –	kilogram
mg% -	milligram percent
pg -	picogram
GD -	gestational day
mM	millimolar
Pb -	lead
pM -	picomolar
IV -	intravenous
% -	percent
mL -	milliliter
dL	deciliter
mRNA -	messenger ribonucleic acid
ppm -	parts per million
GH -	growth hormone
min -	minute

Table AX5-8.3. Bone Cell Cultures Utilized to Test Effects of Lead

Compound Dose/Concentration Duration Exposure Route	Species	Effects	Blood Level	Reference
Stable "Pb" 5 mg/mL in drinking water given during gestation. On GD 18, 50 µCi ^{210}Pb given IV to pregnant dams	Rat (Fetal Bone Organ Culture)	PTH (3885 IU/mg bone) enhanced cell-mediated release of ^{210}Pb from bone. Release of ^{210}Pb was accompanied by proportional loss of stable lead and calcium from treated bones. Time Release of ^{210}Pb (EM/CM ratio) 0 min 1.00 10 min 0.82 ± 0.05 2 hr 1.12 ± 0.04 6 hr 1.59 ± 0.08* 24 hr 3.69 ± 0.15* 48 hr 3.75 ± 0.09* 48 hr 0.78 ± 0.14* (in presence of 30 mU/mL salmon calcitonin) *Different from 1.00, $p < 0.01$.	Not given/Not applicable	Rosen and Wexler (1977)
^{210}Pb nitrate 6.5 to 65 µM 5 min to 2 h In medium	Mice (bone cell isolation from calvaria)	Uptake of ^{210}Pb by OC cells rapid. OC cells have greater avidity for lead compared to OB cells. OC cell uptake of lead almost linear vs. little increase in lead uptake by OB cells with increasing Pb concentrations in media. 15-30% release of ^{210}Pb label occurred in OC cells over 2 h time period. Physiological concentrations of PTH resulted in marked increase in ^{210}Pb and ^{45}Ca uptake by OC cells. ^{210}Pb uptake linear over PTH concentrations of 50 to 250 ng/mL). Media concentrations of lead ≥ 26 µM enhanced calcium uptake by cells.	Not applicable	Rosen (1983)
^{210}Pb nitrate 5 µM 20 hours In medium	Mice (osteoclastic bone cell isolation from calvaria)	Three readily exchangeable kinetic pools of intracellular lead identified, with the majority (approximately 78%) associated with the mitochondrial complex.	Not applicable	Pounds and Rosen (1986)
Lead acetate 0 to 50 µM 20 h In medium	Mice (osteoclastic bone cell isolation from calvaria)	Cultures were labeled with ^{45}Ca (25 µCi/mL) for 2 or 24 h and kinetic parameters were examined by analysis of ^{45}Ca washout curves. In kinetic analysis using dual-label (1-2 µCi/mL ^{210}Pb and 25 µCi/mL ^{45}Ca) wash out curves, the Ca:Pb ratios of the rate constants were approximately 1:1, suggesting similar cellular metabolism.	Not applicable	Rosen and Pounds (1988)

Table AX5-8.3 (cont'd). Bone Cell Cultures Utilized to Test Effects of Lead

Compound Dose/Concentration Duration Exposure Route	Species	Effects	Blood Level	Reference
Lead acetate and ^{210}Pb label 0-100 µM 20 hours In medium	Mice (osteoclastic bone cell isolation from calvaria) and Rat Osteosarcoma Cells (ROS 17/2.8)	Concentrations as high as 100 µM did not cause toxicity in either cell culture. There was a slight decrease in growth of ROS cells at 5 µM lead concentration and a 50% decrease in growth at 25 µM lead at day 9. ^{210}Pb washout experiments with both cell cultures indicated similar steady-state lead kinetics and intracellular lead metabolism. Both cell cultures exhibited one large, slowly exchanging pool of lead, indicative of the mitochondrial pool.	Not applicable	Long et al. (1990a)
Lead acetate 5 or 25 µM Up to 5 hours In medium	Rat Osteosarcoma Cells (ROS 17/2.8)	Used ^{19}F NMR in combination with 1,2-bis(2-amino-5-fluorophenoxy)ethane-N,N,N',N'-tetraacetic acid (5F-BAPTA) to distinguish and measure concentrations of Pb^{2+} and Ca^{2+} in aqueous solution. Basal concentration of $[Ca^{2+}]_i$ was 128 ± 24 nM. Treatment of cells with 5 and 25 µM Pb^{2+} produced sustained 50% and 120% increases in $[Ca^{2+}]_i$, respectively, over a 5 hour exposure period. At a medium concentration of 25 µM Pb^{2+} a measurable entry of Pb^{2+}into the cells ($[Pb^{2+}]_i$ of 29 ± 8 pM) was noted.	Not applicable	Schanne et al. (1989)
Lead nitrate 5 µM 20 minutes In medium	Rat (osteoblastic bone cell isolation from calvaria)	Lead (5 µM) linearly raised the emission ratio of FURA-2 loaded cells 2-fold within 20 minutes of application, most likely due to increase in $[Pb^{2+}]_i$ rather than increase in $[Ca^{2+}]_i$. Intracellular calcium increased even in the absence of extracellular calcium.	Not applicable	Schirrmacher et al. (1998)
Pb^{2+} 5 or 12.5 µM Up to 100 minutes In medium	Rat (osteoblastic bone cell isolation from calvaria)	5 or 12.5 µM Pb^{2+} applied simultaneously with re-added calcium reduced immediate CRAC to 70% or 37% of control value, respectively. During CRAC a large influx of Pb^{2+} occurred, leading to a 2.7-fold faster increase in the FURA-2 excitation ratio. These effects were exclusive of any inhibitory action of Pb^{2+}on calcium ATPase activity.	Not applicable	Wiemann et al. (1999)

Table AX5-8.3 (cont'd). Bone Cell Cultures Utilized to Test Effects of Lead

Compound Dose/Concentration Duration Exposure Route	Species	Effects	Blood Level	Reference
Lead nitrate 0-150 μM Up to 72 hours In medium	Mice (bone cell isolation from parietal bones)	Pb^{2+} concentrations of 50 μM and above stimulated release of hydroxyproline and previously incorporated ^{45}Ca from organ culture. This did not occur in bone inactivated by freezing and thawing. Eel calcitonin, bafilomycin A_1, and scopadulcic acid B significantly inhibited Pb mediated ^{45}Ca release. There was a high correlation between ^{45}Ca and PGE_2 release ($p < 0.001$), inferring Pb-induced bone resorption mediated by PGE_2. This was further supported by the significant depression of Pb-stimulated ^{45}Ca release that occurred with concurrent exposure to 10 μM of either indomethacin or flurbiprofen, both inhibitors of cyclooxygenase.	Not applicable	Miyahara et al. (1995)
Lead acetate 0-25 μM 48 hours In medium	Rat Osteosarcoma Cells (ROS 17/2.8)	Osteocalcin production in cells treated with 100 pg 1,25-dihydroxyvitamin D_3/mL of medium and 0 μM Pb^{2+} for 16, 24, or 36 h was 20.1 ± 2.1, 23.5 ± 3.4, 26.1 ± 2.5 in cell digests, and 87.2 ± 3.3, 91.6 ± 6.7, 95.1 ± 5.2 in the medium, respectively. The presence of 25 μM Pb^{2+} in the medium, reduced osteocalcin levels to as low as 30% of control levels. Cells treated with 0, 5, 10, or 25 μM lead acetate for 24 h, followed by an additional 24 h exposure to 0 or 100 pg of 1,25-dihydroxyvitamin D_3 and continued Pb^{2+} exposure, resulted in a concentration-dependent reduction of 1,25-dihydroxyvitamin D_3-stimulated osteocalcin secretion. 10 μM Pb resulted in medium osteocalcin levels similar to control levels, however, 25 μM Pb resulted in about a 30% decrease. Cellular osteocalcin levels were unaffected.	Not applicable	Long et al. (1990b)
Lead glutamate 4.5×10^{-5} to 4.5×10^{-7} M 2, 4, or 6 days In medium	Rat Osteosarcoma Cells (ROS 17/2.8)	In the presence of serum in the cultures, concentrations of Pb^{2+} less than 4.5×10^{-5} M had no effect on cell proliferation. In the absence of serum, 4.5×10^{-7} M Pb^{2+} increased proliferation at Day 4 and 4.5×10^{-6} M Pb^{2+} inhibited proliferation at Day 6. Lead exposure for 48 h (4.5×10^{-6} M) significantly ($p < 0.01$) increased total protein production in cells and media of cultures labeled with [^{3}H] proline, but did not increase collagen production. Protein synthesis and osteonectin were enhanced in cells following Pb^{2+} exposure.	Not applicable	Sauk et al. (1992)

Table AX5-8.3 (cont'd). Bone Cell Cultures Utilized to Test Effects of Lead

Compound Dose/Concentration Duration Exposure Route	Species	Effects	Blood Level	Reference
Lead glutamate 4.5 x 10^{-5}M -10^{-7}M 1,3, or 5 days incubation In medium	Human Dental Pulp Cells	All concentrations significantly increased cell proliferation on Day 1, 3 and 5 of exposure in serum free conditions. Lead exposure resulted in dose-dependent decrease in intracellular protein and procollagen I production over 5 days. In presence of serum only, 4.5 x 10^{-5}M Pb^{2+} significantly increased protein production, however, at that same concentration lead significantly decreased osteocalcin production (i.e. reduced the level of osteocalcin by 55% at 12 hours).	Not applicable	Thaweboon et al. (2002)
Lead glutamate 5-20 µM 48 hours In medium	Rat Osteosarcoma Cells (ROS 17/2.8)	Cells treated with 0, 5, 10, or 20 µM lead acetate for 24 h, followed by an additional 24 h exposure to 0 or 100 pg of 1,25-dihydroxyvitamin D_3 and continuted Pb^{2+} exposure, resulted in a significant ($p < 0.05$ or less) reduction of osteocalcin secretion, both in the presence and absence of 1,25-dihydroxyvitamin D_3 at all Pb^{2+} concentrations. This effect is not mediated by PKC.	Not applicable	Guity et al. (2002)
Lead 0.5 to 5 µM 40 min In medium	Rat Osteosarcoma Cells (ROS 17/2.8)	1 and 5 µM Pb^{2+} significantly increased $[Ca^{2+}]_i$ in the absence of 1,25-dihydroxyvitamin D_3 and significantly reduced the peak elevation in $[Ca^{2+}]_i$ induced by 1,25-dihydroxyvitamin D_3. Simultaneous treatment of previously unexposed cells to $Pb^{2+}$and 1,25-dihydroxyvitamin D_3 produced little reduction in the 1,25-dihydroxyvitamin D_3-induced ^{45}Ca uptake, while 40 min of treatment with Pb^{2+} before addition of 1,25-dihydroxyvitamin D_3 significantly reduced the 1,25-dihydroxyvitamin D_3-induced increase in ^{45}Ca influx.	Not applicable	Schanne et al. (1992)
Lead nitrate 5 X 10^{-4} to 5 X 10^{-15} M 24 h In medium	Rat Osteosarcoma Cells (ROS 17/2.8)	Osteocalcin secretion significantly reduced below control values by culture with 1 µM Pb^{2+} in the presence or absence of added 1,25-dihydroxyvitamin D_3 or 1,25-dihydroxyvitamin D_3 and IGF-I. Inhibition of osteocalcin secretion was almost complete in either hormone-stimulated or basal cultures with the addition of 100 µM Pb^{2+}. Cellular alkaline phosphatase activity paralleled those of osteocalcin, though there was no response to IGF-I alone or in combination with 1,25-dihydroxyvitamin D_3. Pb^{2+}at 10^{-15}, 10^{-12}, and 10^{-9} to 10^{-7}M did not influence DNA contents of cell cultures, but 1 µM significantly ($p < 0.05$) inhibited basal cultures and those with IGF-I + D3. Cell cultures exposed to 1,25-dihydroxyvitamin D_3 and Pb^{2+} were inhibited at 10 µM Pb^{2+}.	Not applicable	Angle et al. (1990)

Table AX5-8.3 (cont'd). Bone Cell Cultures Utilized to Test Effects of Lead

Compound Dose/Concentration Duration Exposure Route	Species	Effects	Blood Level	Reference
Lead acetate 2 to 200 µM 72 h In medium	Rat Osteosarcoma Cells (ROS 17/2.8)	Lead (2 to 200 µM) had no effect on cell number or DNA and protein synthesis. Alkaline phosphatase activity was significantly reduced ($p < 0.001$) by lead in a dose- and time-dependent manner. Pb Concentration — Alkaline Phosphatase Inhibition 2 µM — 10.0 ± 1.1% 20 µM — 22.0 ± 6.4% 200 µM — 57.8 ± 8.8% Reductions in alkaline phosphatase mRNA levels mirrored Pb^{2+} -induced inhibition of enzyme activity.	Not applicable	Klein and Wiren (1993)
Unidentified Pb^{2+} Various incubation times Not applicable	Bovine (Bovine-derived osteocalcin)	Binding studies of Ca^{2+} to osteocalcin suggested a single binding site with a dissociation constant (Kd) of 7 ± 2 µM for Ca-osteocalcin. Competitive displacement experiments by addition of Pb^{2+} indicated the Kd for Pb-osteocalcin is 1.6 ± 0.42 nM, approximately 3 orders of magnitude higher.	Not applicable	Dowd et al. (1994)
Unidentified Pb^{2+} Various incubation times Not applicable	Bovine (Bovine-derived osteocalcin)	Circular dichroism indicated Pb^{2+} binding induced a structural change in osteocalcin similar to that found in Ca^{2+} binding, but at 2 orders of magnitude lower concentration. Pb^{2+} has 4 orders of magnitude tighter binding to osteocalcin (Kd = 0.085 µM) than Ca^{2+} (Kd = 1.25 mM). Hydroxyapatite binding assays showed similar increased adsorption of Pb^{2+} and Ca^{2+} to hydroxyapatite, but Pb^{2+} adsorption occurred at a concentration 2-3 orders lower than Ca^{2+}.	Not applicable	Dowd et al. (2001)
Lead acetate 10 µM 2 h In medium	Rat Osteosarcoma Cells (ROS 17/2.8)	Pb^{2+} treatment reduced the unidirectional rate of ATP synthesis (P_i to ATP) by a factor of 6 or more ($\Delta M/M_o$: Control = 0.18 ± 0.04, $Pb^{2+} < 0.03$). Intracellular free Mg^{2+} concentration decreased 21% after 2 h of 10 µM Pb^{2+} treatment (0.29 ± 0.02 mM prior to Pb^{2+} treatment and 0.23 ± 0.02 mM after 2 h of Pb^{2+} treatment, $p < 0.05$).	Not applicable	Dowd et al. (1990)
Lead acetate 5 or 25 µM Up to 24 h In medium	Rat Osteosarcoma Cells (ROS 17/2.8)	5 µM Pb^{2+} significantly altered effect of EGF on intracellular calcium metabolism. In cells treated with 5 µM Pb^{2+} and 50 ng/mL EGF, there was a 50% increase in total cell calcium over cells treated with 50 ng/mL EGF alone.	Not applicable	Long and Rosen (1992)

Table AX5-8.3 (cont'd). Bone Cell Cultures Utilized to Test Effects of Lead

Compound Dose/Concentration Duration Exposure Route	Species	Effects	Blood Level	Reference
Lead acetate 5 or 25 µM 20 h In medium	Rat Osteosarcoma Cells (ROS 17/2.8)	Treatment with 400 ng/mL culture medium for 1 h or with 25 µM Pb^{2+} for 20 h increased total cell calcium: Treatment — Cell Calcium Control — 7.56 ± 1.05 nmol/mg protein PTH (400 ng/mL) — 23.28 ± 1.40* nmol/mg protein Pb (25 µM) — 11.37 ± 0.57* nmol/mg protein PTH + Pb — 37.88 ± 4.21* nmol/mg protein * $p \leq 0.05$ from control	Not applicable	Long et al. (1992)
Lead acetate 10^{-11} to 10^{-7} M 3 min In medium	Rat Osteosarcoma Cells (ROS 17/2.8)	Treatment of ROS cells with Pb at 1 or 5 µM concentrations produced a rise in $[Ca^{2+}]_i$ to 170 nM and 230 nM, respectively, over the basal level of 125 nM. An elevation in $[Ca^{2+}]_i$ to 210 nM occurred during treatment with an activator of PKC, phorbol 12-myristate 13-acetate (10 µM). Pretreatment with a selective inhibitor of PKC, calphostin C, did not change basal $[Ca^{2+}]_i$, but prevented the Pb-induced rise in $[Ca^{2+}]_i$. Free Pb^{2+}activated PKC in a range from 10^{-11} to 10^{-7} M, with a Kcat (activation constant) of 1.1 X 10^{-10}M and a maximum velocity (Vmax) of 1.08 nmol/mg/min compared with Ca activation of PKC over a range of 10^{-8} to 10^{-3} M, with a Kcat of 3.6 X 10^{-7}M, and a Vmax of 1.12 nmol/mg/min.	Not applicable	Schanne et al. (1997)
Lead acetate 0.5 to 60 µM 24 to 48 h In medium	Human Osteosarcoma Cells (HOS TE 85) and Rat Osteosarcoma Cells (ROS 17/2.8)	HOS TE 85 Cells Inhibition of proliferation (IC_{50}) = 4 µM lead Cytotoxicity = 20 µM lead ROS 17/2.8 Cells Inhibition of proliferation (IC_{50}) = 6 µM lead Cytotoxicity = 20 µM lead Highest lead concentration in both cell types found in mitochondrial fraction.	Not applicable	Angle et al. (1993)

Table AX5-8.3 (cont'd). Bone Cell Cultures Utilized to Test Effects of Lead

Compound Dose/Concentration Duration Exposure Route	Species	Effects	Blood Level	Reference
Lead acetate or lead chloride 0.1 to 200 μM 24 h to 6 d In medium	Chick growth plate chondrocytes	Growth plate chondrocytes were exposed to 3 or 30 μM for up to 6 days. Maximal inhibition of cell proliferation as measured by thymidine incorporation occurred after a 3-day exposure to lead. A similar 40% inhibition was found at both concentrations. Higher concentrations (up to 100 μM) did not produce further inhibition. In cultures treated for 24 h, lead produced a dose-dependent inhibition of alkaline phosphatase, with 10 μM producing maximal inhibition (40-50% inhibition). Effects of lead on proteoglycan synthesis were not found until after 48 h of exposure, with maximal effect after 72 h of exposure (twofold, 30 μM). Lead exposure (10 to 200 μM) for 24 h produced a dose-dependent inhibition of both type II and type X collagen synthesis.	Not applicable	Hicks et al. (1996)
Lead acetate 0.1 to 30 μM 24 h In medium	Chicken growth plate and sternal chondrocytes	A dose-dependent inhibition of thymidine incorporation into growth plate chondrocytes was found with exposure to 1-30 μM lead for 24 h. A maximal 60% reduction occurred at 30 μM. Lead blunted the stimulatory effects on thymidine incorporation produced by TGF-β1 (24% reduction) and PTHrP (19% reduction), however, this effect was less than with lead alone. Lead (1 and 10 μM) increased type X collagen in growth plate chondrocytes approximately 5.0-fold and 6.0-fold in TGF-β1 treated cultures and 4.2-fold and 5.1-fold in PTHrP treated cultures when compared with controls, respectively. Lead exposure alone reduced type X collagen expression by 70-80%.	Not applicable	Zuscik et al. (2002)

Table AX5-8.3 (cont'd). Bone Cell Cultures Utilized to Test Effects of Lead

Compound Dose/Concentration Duration Exposure Route	Species	Effects	Blood Level	Reference

Abbreviations

Pb - lead
µCi - microCurie
IU - international units
hr - hour
OB - osteoblast
5F-BAPTA - 1,2-bis(2-amino-5-fluorophenoxy)ethane-N,N,N',N'-tetraacetic acid
$[Pb^{2+}]_i$-free intracellular lead
FURA-2 - 1-[6-Amino-2-(5-carboxy-2-oxazolyl)-5-benzofuranyloxy]-2-(2-amino-5-methylphenoxy) ethane-N,N,N',N'-tetraacetic acid
M - molar
DNA - deoxyribonucleic acid
ΔM - decrease in magnetization of intracellular P_i upon prolonged saturation of gamma-phosphate of ATP
mM - millimolar
Kcat - activation constant
IC_{50} - inhibitory concentration 50%
mg - milligram
^{210}Pb - lead-210 radionuclide
EM - experimental medium
mU - milliunits
^{45}Ca - calcium-45 radionuclide
CRAC - calcium release activated calcium reflux
pg - picogram
mRNA - messenger ribonucleic acid
ng - nanogram
Vmax - maximum velocity
TGF-β1 - transforming growth factor-beta 1
mL - milliliter
IV - intravenous
CM - control medium
µM - micromolar
ng - nanogram
$[Ca^{2+}]_i$ -free intracellular calcium
PGE_2 – prostaglandin E_2
PKC - protein kinase C
Kd – dissociation constant
nmol - nanomole
HOS TE 85 cells - human osteosarcoma cells
PTHrP - parathyroid hormone-related protein
GD - gestational day
PTH - parathyroid hormone
min - minute
OC - osteoclast
ROS 17/2.8 -rat osteosarcoma cells
nM - nanomolar
h - hour
IGF-I- insulin growth factor – I
ATP - adenosine triphosphate
EGF - epidermal growth factor

Table AX5-8.4. Bone Lead as a Potential Source of Toxicity in Altered Metabolic Conditions

Compound Dose/Concentration Duration Exposure Route	Species	Effects	Blood Level	Reference
Lead acetate 200 µg/mL 105 days prior to mating or 105 days prior to mating and during gestation and lactation (160 days) In drinking water	Mice	Results suggested very little lead was transferred from mother to fetus during gestation, however, lead transferred in milk and retained by the pups accounted for 3% of the maternal body burden of those mice exposed to lead prior to mating only. The amount of lead retained in these pups exceeded that retained in the mothers, suggesting lactation effectively transfers lead burden from mother to suckling offspring. Transfer of lead from mothers was significantly higher when lead was supplied continuously in drinking water, rather than terminated prior to mating.	Not given	Keller and Doherty (1980a)
Lead acetate 12 mM 8 weeks prior to mating and during gestation In drinking water	Rat	Considerably higher lactational transfer of lead from rat dams compared to placental transfer was reported. Continuous exposure of rat dams to lead until day 15 of lactation resulted in milk lead levels 2.5 times higher than in whole blood, while termination of maternal lead exposure at parturition yielded equivalent blood and milk levels of lead, principally from lead mobilized from maternal bone.	Concentration (µg/l) in whole blood at day 15 of lactation: Controls = 14 ± 4; Lead-exposed until parturition = 320 ± 55; Lead-exposed until day 15 of lactation = 1260 ± 171* *$p < 0.001$ compared with dams at parturition.	Palminger Hallén et al. (1995)
Lead acetate 100 ppm (A) Exposure for 158 ± 2 days from 21 days of age to midlactation; (B) Exposure 144 ± 2 days from day 21 up to delivery; (C) Exposure only during lactation; (D, E, and F) groups of non-pregnant rats exposed for periods equivalent to groups A, B and C, respectively. In drinking water	Rat	In rats exposed to lead 144 days prior to lactation (B), the process of lactation itself elevated blood lead and decreased bone lead, indicating mobilization of lead from bone as there was no external source of lead during the lactation process. Rats exposed to lead for 158 days (A)(144 days prior to lactation and 14 days during lactation) also experienced elevated blood lead levels and loss of lead from bone. Lead exposure only during the 14 days of lactation was found to significantly increase intestinal absorption and deposition (17 fold increase) of lead into bone compared to non-pregnant rats, suggesting enhanced absorption of lead takes place during lactation. The highest concentration of lead in bone was found in non-pregnant, non-lactating control animals, with significantly decreased bone lead in lactating rats secondary to bone mobilization and transfer via milk to suckling offspring.	Concentration (µg/dL) in whole blood at day 14 of lactation or equivalent: Group A = 31.2 ± 1.1; Group B = 28.0 ± 1.7; Group D = 27.3 ± 2.2; Group E = 24.7 ± 1.2	Maldonado-Vega et al. (1996)

Table AX5-8.4 (cont'd). Bone Lead as a Potential Source of Toxicity in Altered Metabolic Conditions

Compound Dose/Concentration Duration Exposure Route	Species	Effects	Blood Level	Reference
Lead acetate 100 ppm (A) Exposure for 158 ± 2 days from 21 days through 14 days of lactation; (B) Nonpregnant control Group A; (C) Exposure 144 ± 2 days from day 21 up to delivery; (D) Nonpregnant control Group C; (E) Lactating rats not exposed to Pb; (F) Nonpregnant rats not exposed to Pb. In drinking water	Rat	When dietary calcium was reduced from the normal 1% to 0.05%, bone calcium concentration decreased by 15% and bone lead concentration decreased by 30% during the first 14 days of lactation. In non-lactating rats on the 0.05% calcium diet, there were also decreases in bone calcium, but no incremental bone resorption nor lead efflux from bone, suggesting the efflux from bone during lactation was related to bone resorption. Enhancement of calcium (2.5%) in the diet of lactating rats increased calcium concentration in bone by 21%, but did not decrease bone resorption, resulting in a 28% decrease in bone lead concentration and concomitant rise in systemic toxicity.	Concentration (µg/dL) in whole blood at day 14 of lactation or equivalent: Group B = 26.1 ± 2.1, Group A = 32.2 ± 2.7*; Group D = 23.8 ± 2.1, Group C = 28.2 ± 2.2*; Groups E and F = 5.1 ± 0.4. * $p < 0.01$, compared to appropriate control	Maldonado-Vega et al. (2002)
Lead acetate 250 mg/mL Beginning at 5 weeks of age, rats exposed to lead for 5 weeks, followed by no additional exposure. In drinking water	Rat	Demonstrated adverse effects in rat offspring born to females whose exposure to lead ended well before pregnancy. Five week-old-female rats given lead acetate in drinking water (250 mg/mL) for five weeks, followed by a one month period without lead exposure before mating. To test the influence of dietary calcium on lead absorption and accumulation, some pregnant rats were fed diets deficient in calcium (0.1%) while others were maintained on a normal calcium (0.5%) diet. All lead-exposed dams and pups had elevated blood lead levels, however pups born to dams fed the diet deficient in calcium during pregnancy had higher blood and organ lead concentrations compared to pups from dams fed the normal diet. Pups born to lead-exposed dams had lower mean birth weights and birth lengths than pups born to non-lead-exposed control dams ($p < 0.0001$), even after confounders such as litter size, pup sex, and dam weight gain were taken into account.	Blood lead concentration of pups (µM): Low calcium/no Pb = 0.137 ± 0.030^{C}; Low calcium/Pb = 1.160 ± 0.053^{A}; Normal calcium/No Pb = 0.032 ± 0.003^{C}; Normal calcium/Pb = 0.771 ± 0.056^{B}. Values that are not marked by the same letter are significantly different ($p<0.05$).	Han et al. (2000)

Table AX5-8.4 (cont'd). Bone Lead as a Potential Source of Toxicity in Altered Metabolic Conditions

Compound Dose/Concentration Duration Exposure Route	Species	Effects	Blood Level	Reference
Lead acetate 1500 µg/Common Pb/kg/day approximately 10 years, replaced by a ^{204}Pb-enriched dose (50 days), then ^{206}Pb-enriched dose (50 days), and finally a ^{207}Pb-enriched dose (50 days, with reduced concentration) Orally, in gelatin capsule	Nonhuman Primate	Sequential doses of lead mixes enriched in stable isotopes (^{204}Pb, ^{206}Pb, and ^{207}Pb) were administered to a female cynomolgus monkey (*Macaca fascicularis*) that had been chronically administered a common lead isotope mix. The stable isotope mixes served as a marker of recent, exogenous lead exposure, while the chronically administered common lead served as a marker of endogenous (principally bone) lead. From thermal ionization mass spectrometry analysis of the lead isotopic ratios of blood and bone biopsies collected at each isotope change, and using end-member unmixing equations, it was determined that administration of the first isotope label allowed measurement of the contribution of historic bone stores to blood lead. Exposure to subsequent isotopic labels allowed measurements of the contribution from historic bone lead stores and the recently administered enriched isotopes that incorporated into bone. In general the contribution from the historic bone lead (common lead) to blood lead level was constant (approximately 20%), accentuated with spikes in total blood lead due to the current administration of the stable isotopes. After cessation of each sequential administration, the concentration of the signature dose rapidly decreased.	Total blood lead range: 31.2 to 62.3 µg/100g.	Inskip et al. (1996)
Lead acetate 1300 to 1500 µg/Common Pb/kg/day approximately 10 years, replaced by a ^{204}Pb-enriched dose (47 or 281 days), then ^{206}Pb-enriched dose (50 or 105 days), and finally a ^{207}Pb-enriched dose (50 days, with 650 µg concentration in only one primate) Orally, in gelatin capsule	Nonhuman Primate	Initial attempts to apply a single bone physiologically based model of lead kinetics were unsuccessful until adequate explanation of these rapid drops in stable isotopes in the blood were incorporated. Revisions were added to account for rapid turnover of the trabecular bone compartment and slower turnover rates of cortical bone compartment, an acceptable model evolved. From this model it was reported that historic bone lead from 11 years of continuous exposure contributes approximately 17% of the blood lead concentration at lead concentration over 50 µg/dL, reinforcing the concept that the length of lead exposure and the rates of past and current lead exposures help determine the fractional contribution of bone lead to total blood lead levels. The turnover rate for cortical (approximately 88% of total bone by volume) bone in the adult cynomolgus monkey was estimated by the model to be approximately 4.5% per year, while the turnover rate for trabecular bone was estimated to be 33% per year.	Various	O'Flaherty et al. (1998)

Table AX5-8.4 (cont'd). Bone Lead as a Potential Source of Toxicity in Altered Metabolic Conditions

Compound Dose/Concentration Duration Exposure Route	Species	Effects	Blood Level	Reference
Lead acetate 1100 to 1300 µg/Common Pb/kg/day approximately 14 years, replaced by a ^{204}Pb-enriched dose, ^{206}Pb-enriched dose, and/or finally a ^{207}Pb-enriched dose of varied durations and concentrations. Orally, in gelatin capsule	Nonhuman Primate	Using the method of sequential stable isotope administration examined flux of lead from maternal bone during pregnancy of 5 female cynomolgus monkeys. Blood lead levels in maternal blood attributable to lead from mobilized bone were reported to drop 29 to 56% below prepregnancy baseline levels during the first trimester of pregnancy. This was ascribed to the known increase in maternal fluid volume, specific organ enlargement (e.g. mammary glands, uterus, placenta), and increased metabolic activity that occurs during pregnancy. During the second and third trimesters, when there is a rapid growth in the fetal skeleton and compensatory demand for calcium from the maternal blood, the lead levels increased up to 44% over pre-pregnancy levels. With the exception of one monkey, blood lead concentrations in the fetus corresponded to those found in the mothers, both in total lead concentration and proportion of lead attributable to each isotopic signature dose (common = 22.1% vs. 23.7%, ^{204}Pb = 6.9% vs. 7.4%, and ^{206}Pb = 71.0% vs. 68.9%, respectively). Between 7 and 25% of lead found in fetal bone originated from maternal bone, with the balance derived from oral dosing of the mothers with isotope during pregnancy. In offspring from a low lead exposure control monkey (blood lead <5 µg/100 g) approximately 39% of lead found in fetal bone was of maternal origin, suggesting enhanced transfer and retention of lead under low lead conditions	Various, with total blood lead as high as approximately 65 µg/100g	Franklin et al. (1997)
Lead acetate 250 mg/l Exposure began either at 5, 10, or 15 weeks of age and continued for a total of 5 weeks. Drinking water	Rat	Exposed rats for five weeks to 250 mg/l lead as acetate in drinking water beginning at 5 weeks of age (young child), 10 weeks of age (mid-adolescence), or 15 weeks of age (young adult), followed by a 4 week period of without lead exposure. An additional group of rats were exposed to lead beginning at 5 weeks, but examined following an 8 or 20 week period after cessation of lead. Significantly lower blood and bone lead concentrations were associated with greater age at the start of lead exposure and increased interval since the end of exposure. Young rats beginning exposure to lead at 5 weeks and examined 20 weeks after cessation of exposure still, however, had bone lead concentrations higher than those found in older rats only 4 weeks after cessation of exposure.	Lead concentration (µM) 4 weeks after cessation of lead exposure: Exposure started at 5 weeks of age = 1.39 ± 0.09; Exposure started at 10 weeks of age = 1.18 ± 0.12; Exposure started at 15 weeks of age = 0.82 ± 0.05.	Han et al. (1997)

Table AX5-8.4 (cont'd). Bone Lead as a Potential Source of Toxicity in Altered Metabolic Conditions

Compound Dose/Concentration Duration Exposure Route	Species	Effects	Blood Level	Reference
Lead acetate 50 ppm 11 months Drinking water	Rat	Studied differences in tissue distribution of lead in adult and old rats. Adult (8 months old) and old (16 months old) rats were exposed to 50 ppm lead acetate in drinking water for 11 months at which time the experiment was completed. Bone (femur) lead levels in older rats were found to be less than those in younger rats, however, blood lead levels were higher in the older rats. Brain lead concentration in the older rats exposed to lead were significantly higher, and brain weight significantly less than the brain lead concentration and weights of unexposed older control rats or adult rats exposed to lead, suggesting a potential detrimental effect. Authors suggested that a possibility for the observed differences in tissue concentrations of lead was due to changes in the capacity of bone to store lead with advanced age.	Approximate median values after 6 months of exposure: Adult rats : 23 µg/dL Old rats: 31 µg/dL After 11 months of exposure: Adult rats: 16 µg/dL Old rats: 31 µg/dL	Cory-Slechta et al. (1989)
Lead acetate 0, 2, or 10 mg/kg/day 9.5 months Drinking water	Rat	Examined kinetic and biochemical responses of young (21 day old), adult (8 months old), and old (16 months old) rats exposed to lead at 0, 2, or 10 mg lead acetate/kg/day over a 9.5 month experimental period. Results suggested older rats may have increased vulnerability to lead due to increased exposure of tissues to lead and greater sensitivity of the tissues to the effects of lead.	Various from approximately 1 µg/dL up to 45 µg/dL	Cory-Slechta (1990)
Lead acetate 7 years total Drinking water	Nonhuman primate	In studies of bone lead metabolism in a geriatric, female nonhuman primates exposed to lead approximately 10 years previously, there were no significant changes in bone lead level over a 10 month observation period as measured by ^{109}CD K X-ray fluorescence. The mean half-life of lead in bone of these animals was found to be 3.0 ± 1.0 years, consistent with data found in humans, while the endogenous exposure level due to mobilized lead was 0.09 ± 0.02 µg/dL blood.	Historic concentrations during exposure: 44 to 89 µg/100 mL.	McNeill et al. (1997)
Lead (type unidentified) occurring naturally in diet (0.258 ng/mg dry wt) and water (5.45 ppb). Exposure from age 1 month up to 958 days. Drinking water and diet	Mice	The lead content of femurs increased by 83% (values ranged from 0.192 to 1.78 ng Pb/mg dry wt), no significant relationship was found between lead and bone density, bone collagen, or loss of calcium from bone. The results suggest against low levels of bone lead contributing to the osteopenia observed normally in C57BL/6J mice.	None given	Massie and Aiello (1992)

Table AX5-8.4. (cont'd). Bone Lead as a Potential Source of Toxicity in Altered Metabolic Conditions

Compound Dose/Concentration Duration Exposure Route	Species	Effects	Blood Level	Reference
Lead acetate 250 mg/l Exposure for 5 weeks Drinking water	Rat	Rats were exposed to lead for 5 weeks, followed by a 4 week washout period without lead to allow primarily accumulation in the skeleton. Rats were then randomly assigned to a weight maintenance group (WM), a moderate weight loss (MWL) group (70% of maintenance diet), or a substantial weight loss (SWL) group (40% of maintenance diet) for a four week period. At the end of this experimental period the blood and bone levels of lead did not differ between groups, however, the amount and concentration of lead in the liver increased significantly. Femur: WM 826 ± 70; MWL 735 ± 53; SWL 935 ± 84 Spinal Column Bone: WM 702 ± 67; MWL 643 ± 59; SWL 796 ± 59	WM = 1.25 ± 0.10 µM; MWL = 1.16 ± 0.10 µM; WM = 1.32 ± 0.10 µM;	Han et al. (1996)
Lead acetate 250 µg/l 14 days Drinking water	Rat	Study was undertaken to determine the effect of weight loss and exercise on the distribution of lead. Weight loss secondary to dietary restriction was a critical factor elevating organ lead levels and, contrary to prior study (Han et al. (1996)), elevated blood levels of lead. No significant difference in organ or blood lead concentrations were reported between the exercise vs. no exercise groups.	Graphs indicate concentrations ranging from 0.20 to 2.00 µM.	Han et al. (1999)

Abbreviation

µg – microgram
mL – milliliter
% - percent
mM - millimolar
l – liter
ppm - parts per million
dL – deciliter
mg - milligram
µM – micromolar
Pb – lead
kg – kilogram
g - gram
^{204}Pb, ^{206}Pb, ^{207}Pb - Stable isotopes of lead 204, 206, 207, respectively
wt – weight
ppb - parts per billion

Table AX5-8.5. Uptake of Lead by Teeth

Compound Dose/Concentration Duration Exposure Route	Species	Effects	Blood Level	Reference
Lead acetate 1 µg/kg body weight Single IP injection	Rat	Uptake of lead label into incisors of suckling rats: 0.7% of injected dose in 4 incisors of suckling rat after 24 h, 1.43% after 192h. 0.6% of injected dose in 4 incisors of adult after 24 h, 0.88% after 192h.	Mean percent of dose after time: Suckling: 3.04% after 24h 1.71% after 72h 1.52% after 144h 1.18% after 192h Adult: 6.40% after 24h 3.41% after 24h 1.92% after 24h 1.04% after 72h 0.72% after 144h 0.48% after 192h	Momcilovic and Kostial (1974)
Lead aerosol 77, 249, or 1546 µg/m^3 for 50 to70 days Inhalation	Rat	11 micrograms Pb/g incisor taken up in animals exposed to 77 µg/m^3 for 70 days versus 0.8µg Pb/g in control animals 13.8 µg Pb/g incisor in rats exposed to 249 µg/m^3 for 50 days 153µg Pb/g incisor in rats exposed to 1546 µg/m^3 for 50 days	Control: 2.6 µg/dL 77 µg/m^3: 11.5 µg/dL 249 µg/m^3: 24.1 µg/dL 1546 µg/m^3: 61.2 µg/dL	Grobler et al. (1991)
Lead acetate 0, 3, or10 ppm During pregnancy and 21 days of lactation Drinking water	Rat	Lead concentration in teeth of offspring: 0 ppm group – Incisors (1.3 ppm), 1st molars (0.3 ppm) 3 ppm group – Incisors (1.4 ppm), 1st molars (2.7 ppm) 10 ppm group – Incisors (13.3 ppm), 1st molars (11.4 ppm)	Not given	Grobler et al. (1985)

Abbreviations

µg – microgram
kg – kilogram
IP – intraperitoneal
% - percent
h – hour
m^3 - cubic meter
Pb – lead
g - gram
ppm - parts per million

Table AX5-8.6. Effects of Lead on Enamel and Dentin Formation

Compound Dose/Concentration Duration Exposure Route	Species	Effects	Blood Level	Reference
Pb "salt" 0.075 mM/100g , 0.15 mM/100g or 1.5 mM/100g Single, SC injection	Rat	0.075 mM dose, no disruption of dentin and enamel. 0.15 mM dose, mild mineralization disruption of dentin and enamel. 1.5 mM dose, mild to moderate disruption of dentin and enamel.	Not given	Eisenmann and Yaeger (1969)
Pb acetate 30 mg/kg Single, IV injection	Rat	Rapid rise in serum calcium and phosphorus after injection. Formation of a "lead line" in growing dentin within 6 hours after injection.	Not given	Appleton (1991)
Pb acetate 3 mg/kg Single, IV injection	Rat	Production of a hypomineralized band in dentin	Not given	Appleton (1992)
Pb acetate 0 mg/l, 34 mg/l, or 170 mg/l 70 days Drinking water	Rat	Increased in relative amount of protein in enamel matrix. Significant ($p<0.05$) decrease in microhardness values of groups exposed to lead in regions of maturation enamel, but not fully mature enamel. Delay in enamel mineralization in animals exposed to lead.	0 mg/l group: 0 ppm 34 mg/l group: 18.1 ppm 170 mg/l group: 113.3 ppm	Gerlach et al. (2002)
Pb acetate 40 mg/kg Single, IP injection	Rat	Significantly ($p<0.05$) reduced eruption rates at various time points (days 8, 14, 16, 22, 24, 28) under hypofunctional conditions.	Days after injection 0 d: 48 µg/dL 10 d: 37 µg/dL 20 d: 28 µg/dL 30 d: 16 µg/dL (Values estimated from graph)	Gerlach et al. (2000b)

Abbreviations

Pb – lead
mM – millimolar
g – gram
SC - subcutaneous
mg – milligram
kg – kilogram
IV – intravenous
l - liter
ppm - parts per million
IP – intraperitoneal
µg – microgram
dL - deciliter

Table AX5-8.7. Effects of Lead on Dental Pulp Cells

Compound Dose/Concentration Duration Exposure Route	Species	Effects	Blood Level	Reference
Pb glutamate 4.5 x 10^{-5}M -10^{-7}M 1,3, or 5 days incubation	Human Dental Pulp Cells	All concentrations significantly increased cell proliferation on Day 1, 3 and 5 of exposure in serum free conditions. Lead exposure resulted in dose-dependent decrease in intracellular protein and procollagen I production over 5 days. In presence of serum only 4.5 x 10^{-5}M significantly increased protein production. Lead significantly decreased osteocalcin production.	Not applicable	Thaweboon et al. (2002)

Abbreviations

Pb – lead
M – molar

Table AX5-8.8. Effects of Lead on Teeth – Dental Caries

Compound Dose/Concentration Duration Exposure Route	Species	Effects	Blood Level	Reference
Lead acetate 0.5 mEq 84 d males 98 d females Drinking water	Hamster	Significant increase in dental caries in male hamsters only (85 mean molar caries score control vs. 118 for lead exposed). No significant difference in dental caries in female hamsters (68 mean molar caries score control vs. 85 for lead exposed).	Not given	Wisotzky and Hein (1958)
Lead acetate 34 ppm Pre- and perinatal Drinking water	Rat	Lead exposure resulted in an almost 40% increase in the prevalence of caries and nearly 30% decrease in stimulated parotid salivary gland function.	Control: < 5 µg/dL 34 ppm Pb: 48 ± 13 µg/dL	Watson et al. (1997)
Lead acetate 10 or 25 ppm lead 3 weeks Drinking water	Rat	When 15 ppm fluoride was concurrently given in diet, lead did not increase prevalence of caries.	Not given	Tabchoury et al. (1999)

Abbreviations

mEq – milliequivalents
d – days
ppm - parts per million
% – percent
µg – microgram
dL – deciliter

CHAPTER 5 ANNEX

ANNEX TABLES AX5-9

Table AX5-9.1. Studies on Lead Exposure and Immune Effects in Humans

Nature of Exposure	Dose or Blood Lead Levels (BLLs)	Sample Population	Reported Effects	Reference
Environmental	10.1-48.2 µg/L (BLL)	2nd grade children living near industrial waste incinerator or other industries causing pollution	Increased blood lead concentration associated w/ increased IgE, especially above 28 µg/dL Also decreased T-cells, cytotoxic T-cells, and B-cells (non-linear relation)	Karmaus et al. (2005)
Occupational	22 µg/dL: <30 years old 23.0 µg/dL: 30-39 years old 24.1 µg/dL: ≥40 years old	Employees of lead storage battery factories in Korea 554 Men 52 Women	Serum IgE higher when BLL >30 µg/dL – Correlation of BLL with serum IgE For employees less than 30 years old, IL-4 was lower when BLL >30 µg/dL	Heo et al. (2004)
Environmental	3.47 – 49.19 µg/dL	Children 6-11 years of age 30 girls 35 boys Proximity to smelter	Indirect (PHA) macrophage activation NO production negatively associated with BLL With proximity closest to smelter monocytes had increased superoxide anion production by indirect and direct activation (positive correlation with BLL – stronger for boys than girls)	Pineda-Zavaleta et al. (2004)
Environmental	2.56 – 43.69 µg/dL (mean 9.52 µg/dL)	38 preschool children (3-6 years of age); 35 controls	Percent of $CD4^+$ and $CD4^+CD+$ cells decreased while $CD8^+$ increased	Zhao et al. (2004)
Occupational	Range of 10.0-400.9 µg/dL Mean=88.3 µg/dL Controls all below 10 µg/dL	Male lead-exposed workers	PHA-mitogen response decreased; and IFN-gamma production increased. No effect on NK cytotox.	Mishra et al. (2003)
Environmental	2.56 - 43.69 µg/dL (BLL mean of 9.52 µg/dL)	96 females 121 males (3-6 years old)	IgG and IgM lower in high BLL group (≥9.52 µg/dL) IgE greater in high BLL group ($P < 0.10$) No difference among males but females exposed to higher lead had significant decreases in IgG and IgM and increased in IgE Correlation of BLL and serum IgE $r = 0.48$; $P = 0.002$	Sun et al. (2003)
Occupational	10-20 year exposure (original BLL mean 60 µg/dL; at time of study BLL mean = 30 µg/dL)	30 lead workers from battery manufacturing plant (43 males and 21 females)	Increased percentage of monocytes while percentage of B-cells, numbers of lymphocytes, monocytes, and granulocytes decreased	Sune et al (2003)
Occupational	74.8 ± 17.8 µg/dL vs. 16.7 ± 5.0 µg/dL for controls	25 male storage battery workers exposed >6 months; age 33 ± 8.5 years	Decreased blood hemoglobin, $TCD4^+$ cells, IgG, IgM, C3 and C4 compliment proteins. Increased zinc protoporphyrin. Impaired neutrophil chemotaxis and random migration	Basaran and Undeger (2000)

Table AX5-9.1 (Cont'd). Studies on Lead Exposure and Immune Effects in Humans

Nature of Exposure	Dose or Blood Lead Levels (BLLs)	Sample Population	Reported Effects	Reference
Environmental	1.7-16.1 µg/dL (Range in <3 yr old group)	1561 children and adults in high lead community 480 controls	6-35 months: increased IgA, IgG, IgM, number and proportion of B lymphocytes decreased proportion of T-lymphocytes especially true when BLL > 15 µg/dL >3 years of age – no differences	Sarasua et al. (2000)
Epidemiological study	Blood leads from 1-45 µg/dL	Urban Children population in Missouri; 56% male 279 children 9 months-6 yr. of age	Correlation of blood lead levels and serum IgE levels in Missouri children	Lutz, et al. (1999)
Occupational	BLL=39 Range 15-55 µg/dL	145 lead exposed workers 84 controls	No major effects; only subtle effects Elev. B cells elevated CD4+/CD45RA+ cells Decr. Serum IgG	Pinkerton et al. (1998)
Occupational	Lead workers with BLL between 7-50 µg/dL; mean 19 µg/dL	71 male chemical plant workers vs. 29 controls	T cell populations, Naïve T cells correlated positively with PBB levels. Memory T cells reduced with lead.	Sata et al. (1998)
Occupational	Exposed—Range of 38-100 µg/dL mean = 74.8 µg/dL; Controls 11-30 µg/dL mean = 16.7 µg/dL (high controls!)	25 Male battery plant workers vs. 25 controls	Absolute and relative numbers of CD4+ T cells reduced in exposed group. IgG, IgM C3 and C4 serum levels all lower in workers.	Undeger et al. (1996)
Occupational	BLL 12-80.0 µg/dL	33 male workers in a storage battery plant	No changes in serum Igs of PHA response of PBMC	Queiroz et al. (1994)
Occupational	Males high BLL ≥25 µg/dL lower BLL <25 µg/dL control BLL ≤10 µg/dL	51 Firearms instructors (high and lower) vs. controls	T cell phenotypes and response—lead reduced relative CD3+ cells and relative and absolute CD4+ cells also reduced PHA (high lead)and PWM mitogen responses, reduced MLR also(high lead)	Fischbein et al. (1993)
Occupational	>60 µg/dL for group showing best IgE effect	2 groups of male workers occupationally exposed	IgE positively correlated with BLL	Horiguchi et al. (1992)
Occupational	BLL 14.8-91.4 µg/dL	39 male workers of storage battery plant (4 year mean exposure)	Impaired neutrophil migration Impaired nitroblue tetrazolium positive neutrophils Greater for those exposed up to 1 year than those with longer exposure "safe" levels of lead can still cause immunosuppression	Queiroz et al. (1993)
In Vitro	207-1035 µg/L	Human lymphocytes from adults 25-44 years of age	Lead associated with greater IgG production after stimulation with PWM – not dose dependent	Borella and Giardino (1991)
Occupational	33.2 µg/dL in lead-exposed group 2.7 µg/dL in controls	10 Male workers in scrap metal refinery vs. 10 controls	PMN chemotaxis reduced to 2 different chemoattractants	Valentino et al. (1991)

Table AX5-9.1 (Cont'd). Studies on Lead Exposure and Immune Effects in Humans

Nature of Exposure	Dose or Blood Lead Levels (BLLs)	Sample Population	Reported Effects	Reference
Occupational	10 lead exposure workers vs. controls worker BLLs of 41-50 µg/dL no controls >19 µg/dL		ConA-generated suppressor cell production—increased, Some other cellular parameters unchanged	Cohen et al. (1989)
Occupational	Blood leads 64 µg/dL Range 21-90	39 male workers in lead exposed group	PHA response of lymphocytes from workers decreased	Alomran and Shleamoon (1988)
Occupational	Comparison of workers with 25-53 µg/dL vs. controls with 8-17 µg/dL	Workers exposure to lead	No change in serum Ig levels PHA response of cells or NK activity	Kimber et al. (1986b)
Environmental	Near smelter BLLs varied seasonally 25-45 µg/dL Control area BLLs varied seasonally 10-22 µg/dL	Boys and girls ~11.5 years old living near lead smelting plant	Higher BLL associated with: decreased Δ-amino levulinic acid dehydrogetase Decreased IgM and secretory IgA Inversely related to IgG	Wagnerova et al. (1986)
Occupational	Workers (18-85.85.2 µg/dL BLL) controls (6.6-20.8 µg/dL BLL)	73 workers vs. 53 controls	Negative correlation of BLL and serum IgG and C3. Positive correlation of BLL and saliva IgA	Ewers et al. (1982)
Environmental	12 Afr.- American children BLLs 41-51µg/dL; 7 controls BLLs 14-30 µg/dL	12 African American preschool children vs. 7 controls	No difference in anti-tetanus antibody levels or in complement levels	Reigart and Graber (1976)

Table AX5-9.2. Effect of Lead on Antibody Forming Cells (AFC) (In Vitro Stimulation)

Species	Strain/Gender	Age	Effect	In Vivo/ Ex Vivo	Lead Dose/ Concentration	Duration of Exposure	Reference
Mouse	Various	Adult	↑AFC	No	10 µM	5 days	McCabe and Lawrence (1991)
Mouse	Various	Adult	AFC No change	Yes	10 mM in water	8 weeks	Mudziuski et al. (1986)
Mouse	CBA/J females	Adult	↑AFC primary response	No	100 µM	5 days	Warner and Lawrence (1986)
Mouse	BDF_1 females	Adult	↑AFC - T dependent antigen AFC - T independent antigen, no change		50 µg lead acetate in water	3 weeks	Blakley and Archer (1981)
Mouse	CBA/J females	Adult	↑AFC	Yes	0.08 mM and 0.4 mM	4 weeks	Lawrence (1981a)
Mouse	CBA/J	Adult	↑AFC	No	10^{-5} M	5 days	Lawrence (1981b)
Mouse	CBA/J females	Adult	↑AFC	No	10^{-4} M	1 hr preincubation	Lawrence (1981c)
Mouse	Swiss males	Adult	↓AFC	Yes	0.5 ppm tetraethyl lead	3 weeks	Blakley et al. (1980)
Mouse	Swiss	Adult	↓AFC	Yes	1300 ppm	10 weeks	Koller and Roan (1980)
Rat	SD	Neonate–Juvenile	↓AFC (IgM)	Yes	25 ppm and 50 ppm	3 weeks prenatal and 6 weeks postnatal	Luster et al (1978)
Mouse	Swiss	Adult	↑AFC – IgM ↓AFC – IgG	Yes	4 mg i.p. or oral	Single dose	Koller et al. (1976)
Mouse	Swiss	Adult	↓AFC – IgM ↓AFC – IgG	Yes	13.75 ppm – 1,375 ppm	8 weeks	Koller and Kovacic (1974)

Table AX5-9.3. Studies Reporting Lead-Induced Suppression of Delayed Type Hypersensitivity and Related Responses

Species	Age	Strain/Gender	Route	Lowest Effective Dose	Duration of Exposure	Reference
Rat	Embryo	SD females	Oral to Dam	250 ppm (BLL at 4 wk = 6.75 µg/dL)	5 weeks	Chen et al. (2004)
Chicken	Embryo	Cornell K females	*in ovo*	200 µg	Single injection E12	Lee et al. (2002)
Rat	Fetal	CD females	Oral to Dam	500 ppm	6 days	Bunn et al. (2001c)
Rat	Embryo – fetal	F344 and CD females	Oral to Dam	250 ppm	3 weeks	Bunn et al. (2001b)
Rat	Embryo – fetal	F344 females	Oral to Dam	250 ppm (BLL = 34.8µg/dL at birth)	3 weeks	Bunn et al. (2001a)
Chicken	Embryo	Cornell K females	*in ovo*	200 µg (BLL = 11 µg/dL)	Single injection E12	Lee et al. (2001)
Mouse	Adult	BALB/c females	Oral	512 ppm (BLL = 87 µg/dL)	3 weeks	McCabe et al. (1999)
Rat	Embryo- fetal	F344 females	Oral to Dam	250 ppm lead acetate	5 weeks (2 before, 3 during gestation)	Chen et al. (1999)
Rat	Embryo- fetal	F344 females	Oral to Dam	250 ppm lead acetate	5 weeks (2 before, 3 during gestation)	Miller et al. (1998)
Goat	Adult	Females	Gastric intubation	50 mg/kg lead acetate	6 weeks	Haneef et al. (1995)
Rat	Adult	Wistar males	Oral	6.3 m mol kg^{-1}	8 weeks	Kumar et al. (1994)
Mouse	Adult	Swiss	s.c.	0.5 mg/kg/day	Shortest = 3 days just prior to challenge	Laschi-Loquerie et al. (1984)
Rat	Neonatal/ Juvenile	CD females	Oral	25 ppm lead acetate (BLL= 29.3 µg/dL)	6 weeks	Faith et al. (1979)
Mouse	Adult	BALB/c	i.p.	0.025 mg lead acetate	30 days	Muller et al. (1977)

Table AX5-9.4. Effect of Lead on Allogeneic and Syngeneic Mixed Lymphocyte Responses (MLR)

Species	Strain/Gender	Age	Proliferation Effects	In Vivo/ Ex Vivo	Lead Dose/Concentration	Duration of Exposure	References
Mouse	C57Bl/6 and BALB/c	Adult	↑Allo-MLR	No	0.1 µM	4 days	McCabe et al. (2001)
Rat	Lewis males	Adult	↑Allo-MLR ↑Syngeneic-MLR	No	50 ppm lead acetate	4 days	Razani-Boroujerdi et al. (1999)
Mouse	CBA/J females	Adult	↑Allo-MLR	No	$10^{-6} – 10^{-4}$ M	5 days	Lawrence (1981b)
Mouse	CBA/J females	Adult	↑Allo-MLR	Yes	0.08 mM and 0.4 mM	4 weeks	Lawrence (1981a)
Mouse	DBA/2J males	Adult	Allo-MLR no significant change	Yes	13, 130 and 1300 ppm	10 weeks	Koller and Roan (1980a)

Table AX5-9.5. Effect of Lead on Mitogen-Induced Lymphoid Proliferation

Species	Strain/ Gender	Age	Proliferation Effects	In Vivo/ Ex Vivo	Lead Dose/ Concentration	Duration of Exposure	References
Human	-	Adult	↓PHA	Yes	Not available	Occupational	Mishra et al. (2003)
Mouse	TO males	Adult	↓ConA ↓LPS	Yes	1 mg/kg daily	2 weeks	Fernandez – Carbezudo et al. (2003)
Mouse	Several	Adult	PHA stimulation No change	No	25 μM	3 days	McCabe et al., 2001
Rat	Lewis and F344 males	Adult	↑ConA ↑LPS	No	25 ppm	3 days	Razamni – Boroujerdi et al. (1999)
Mouse	CBA/J	Adult	LPS No change	No	10 μM	3 days	McCabe and Lawrence (1990)
Rat	AP strain males	Adult	PHA No change	Yes	100 ppm and 1,000 ppm	2-20 weeks	Kimber et al. (1986)a
Mouse	CBA/J females	Adult	ConA LPS No change	No	10^{-4} M	2 days	Warner and Lawrence (1986)
Mouse	BDF1 females	Adult	↑ConA ↑PHA ↑Staph A enterotoxin LPS no change	Yes	0-1,000 ppm	3 weeks	Blakley and Archer (1982)
Rat	SD males	Adult	↑ConA ↑PHA ↑LPS	Yes	1% lead acetate in diet	2 weeks	Bendich et al. (1981)
Mouse	CBA/J females	Adult	PHA no change ↓LPS (high doses only)	Yes	10 mM	4 weeks	Lawrence (1981a)
Mouse	CBA/J females	Adult	ConA, PHA no change ↑LPS	No	10^{-6} – 10^{-4} M	2.5 days	Lawrence (1981)b
Mouse	CBA/J females	Adult	ConA, PHA no change ↑LPS	No	10^{-6} – 10^{-4} M	2-5 days	Lawrence (1981)c
Mouse	C57 Bl/6 males	Adult	↓PHA ↓ConA LPS No change	Yes	1,300 ppm	8 weeks	Neilan et al. (1980)
Rat	SD females	Neonatal – Juveniles	↓PHA ↓ConA	Yes	25 ppm	6 weeks	Faith et al. (1979)
Mouse	BALB/c	Adult	↑LPS	No	10^{-5} – 10^{-3} M	3 days	Gallagher et al. (1979)
Mouse	Swiss males	Adult	↓PHA ↓PWM	Yes	2,000 ppm	30 days	Gaworski and Sharma (1978)

Table AX5-9.5 (cont'd). Effect of Lead on Mitogen-Induced Lymphoid Proliferation

Species	Strain/ Gender	Age	Proliferation Effects	In Vivo/ Ex Vivo	Lead Dose/ Concentration	Duration of Exposure	References
Mouse	Swiss males	Adult	↑PHA ↑PWM	No	0.1 mM – 1.0 mM	2-3 days	Gaworski and Sharma (1978)
Mouse	CBA/J	Adult	↑LPS	Yes	13 ppm	18 months	Koller et al. (1977)
Mouse	BALB/c	Adult	↑LPS	No	10^{-5} – 10^{-3} M	2-3 days	Shenker et al. (1977)

Table AX5.9.6. Pattern of Lead-Induced Macrophage Immunotoxicity

Species	Strain/ Gender	Age	Function	In Vivo/ Ex Vivo	Lowest Effective Dose	Duration of Exposure	References
Nitric Oxide							
Human	Both genders	Juvenile	↓NO	Yes	NK		Pineda-Zavaleta et al. (2004)
Rat	CD males	Embryo	↓NO	Yes	500 ppm	6 days	Bunn et al., 2001c
Chicken	Cornell K strain females	Embryo	↓NO	Yes	10 µg	One injection (E5)	Lee et al., 2001
Mouse	BALB/c females	Adult	↓NO	No	20 µg/mL one lower dose ↑NO	2 hrs	Krocova et al. (2000)
Chicken	HD-11 cell line	-	↓NO	No	4.5 µg	18 hrs	Chen et al. (1997)
Mouse	CBA/J females	Adult	↓NO	No	1.0 µg	4 days	Tian & Lawrence (1996)
Mouse	CBA/J females	Adult	↓NO	No	0.625 µM	4 days	Tian & Lawrence, 1995
Reactive Oxygen Intermediates							
Human	Associated in males	Juvenile	↑ROI	Yes	NK	NK	Pineda-Zavaleta et al. (2004)
Rat	Not indicated	NK	↑ROI	No	240 µM	3 hrs	Shabani & Rabbani (2000)
Mouse	BALB/c females	Adult	↑ROI	Yes	1.5 mg/kg diet	30 days	Baykov et al. (1996)
Rabbit	New Zealand white males	Adult	↑ROI	Yes	31 µg/m^3 inhaled	3 days	Zelikoff et al. (1993)

CHAPTER 5 ANNEX

ANNEX TABLES AX5-10

Table AX5-10.1. Hepatic Drug Metabolism

Concentration	Duration	Species	Effects[a]	Reference
Triethyl Pb chloride, 0-3.0 mg/kg b. wt. In vitro, 0.0-3.0 mM triethyl Pb	2 days Not specified	In vitro, rat microsomes In vivo, rat microsomes	Triethyl Pb increased microsomal N-oxygenation in vivo and decreased microsomal C oxygenation by in vitro treatment. Either treatment thus gave rise to an increase in the N-oxygenation/C-oxygenation ratio, which may lead to tumor potentiation.	Odenbro and Arhenius (1984)
5 or 10 µmol/100g b. wt. Pb nitrate; i.v.	36 h	Male Fischer 344 rats	Lead decreases phase I components (liver microsomal cyt.P-450), and increases Phase II components (GST, DT diaphorase etc). Liver cytosol in treated animals had a polypeptide that cross-reacted with GSTP.	Roomi et al. (1986)
5, 10, 50 mg Pb acetate kg^{-1} b. wt.	Multiple durations (15 days, 2 and 3 months)	Female albino rats	Over all induction of cyt-p - 450 and b5 in liver, long-term increase in liver GST and GSH.	Nehru and Kaushal (1992)
100 µmol/kg; i.v. Pb acetate	24 h	Male Fischer 344 rats	Decrease in total CYP amount, selective inhibition of CYP1A2 and decrease in the expression at m-RNA and protein level, induction of placental form of glutathione s-transferase (GST-P).	Degawa et al. (1994)
100 µmol/kg Pb nitrate; i.v.	9 h before or 6 h after 2-methoxy-4-aminoazobenzene (2-Meo-AAB)	Male Fischer 344 rats	Male fisher rats treated with different metal ions ─Pb nitrate, nickel chloride, cobalt chloride or cadmium chloride exhibited decreased total CYP amount in liver microsomes. However, only Pb reduced the levels of the mRNA and protein of CYP 1A2 induced with 2-methoxy-4-aminoazobenzene (2-Meo-AAB) and decreased the microsomal activity (Per CYP), Pb also induced placental form of Glutathione, a marker enzyme for preneoplastic lesion.	Degawa et al. (1995)
100 µmol/kg; i.v. Pb acetate	24 h	Male F 344 rats	Inhibition of CYP1A mRNA(s) by Pb nitrate is by aromatic amines, not by aryl hydrocarbons.	Degawa et al. (1996)
100 µmoles /kg; i.v.	Multiple durations (3, 6, 12, 24, and 36 h)	Male Wistar rats	Stimulation of TNFα preceding hepatocyte DNA synthesis indicates a role for it in liver cell proliferation. Lead nitrate enhances sensitivity to bacterial LPS, in hepatocytes.	Shinozuka et al. (1994)

Table AX5-10.1 (cont'd). Hepatic Drug Metabolism

Concentration	Duration	Species	Effects[a]	Reference
Single 0.33 mg/kg-1 Pb nitrate	Multiple durations	Male Wistar rats	Lead confers protection against the CCL4 induced hepatotoxicity as evident by marked reduction in serum Alanine aminotransferase (AST) and aspartate aminotransferase (AST) and this protection is not associated with the mitotic response of Pb.	Calabrese et al. (1995)
Lead acetate, 75 mg of Pb^{2+}/kg, intraperitonial	Multiple analyses up to 30 h	C57BL/6 male mice	The decrease in P-450 as a result of Pb poisoning occurs at two levels. (1) A mechanism unrelated to heme, where Pb interferes with P-450 in 2 ways. (2) A mechanism dependent on heme, in which Pb inhibits heme synthesis.	Jover (1996)
0-10^{-6} M Pb nitrate	3 days	Fish hepatoma cell line PLHC-1	Effect of heavy metals Cu(II), Cd(II), Co(II), Ni(II), Pb(II), and Zn(II), on Cytochrome induction (CYP1A) induction response and Ethoxy resorufin-o-deethylase (EROD) activity. All metals had a more pronounced effect on EROD activity than Cyp1 A protein. The rank order of the metal inhibition on EROD is Cd(II) > Ni(II) > Cu(II) > Co(II) = Zn(II), Pb(II), Cd(II) and (Cu). May affect Cyp1 A system of the fish liver at low concentrations through the direct inhibition of CYP 1A enzyme activity.	Bruschweiler et al. (1996)
DT Diaphorase activity 0-125 mg/kg Pb acetate, Pb nitrate Time course experiments 100 mg/kg Pb acetate, i.p.	24 h - 120 h	Male Wistar rats	Lead acetate and nitrate induce DT diaphorase activity which is inhibited significantly by Dil a calcium antagonist, showing that these changes are mediated by intracellular calcium changes. Lead acetate induces DT diaphorase activity with out thymus atrophy and hence was suggested to be a monofunctional inducer as against the Methyl cholanthrene induced DT diaphorase activity	Arizono et al. (1996)
Cell viability assays 0-30 μM, for all other As , Pb, Hg, 5μM, Cd, 1 μM	24 h in general and for EROD assays by PAHs 24 -72 h	Primary human hepatocytes	The effect of metals on PAH induced CYP1A and 1A2 as probed by Ethoxy resorufin o- deethylase activity has demonstrated, metals - Arsenic, Pb, mercury and cadmium decreased CYP1A1/ A2 expression by polycyclic aromatic hydrocarbons depending on the dose, metal and the PAH. Arsenic was most effective, followed by Pb, cadmium, and mercury. Cell viability was decreased by 20-28% by metals.	Vakharia (2001)

Table AX5-10.1 (cont'd). Hepatic Drug Metabolism

Concentration	Duration	Species	Effects[a]	Reference
10-100 µM, in vitro	24 h	Murine hepatoma cell line.	Effect of heavy metals on Aryl hydrocarbon regulated genes –metals alone did not induce a significant change in the cyp1a1 activity and protein levels but increased its m-RNA expression. AHR ligand - mediated induction of cyp1a1 activity and protein was observed by all the metals. Pre and post translational modulation in this regulation have been implicated. These results demonstrate that the heavy metals differentially modulate the constitutive and the inducible expression of AHR regulated genes.	Korashy and El-Kadi (2004)
5 and 10 µmoles/100g of b.wt, single i.p	—	Wistar Rat	Lead nitrate induced the expression of Placental form of Glutathione transferase along with liver cell proliferation. The biochemical lesions induced by Pb under these conditions were similar to that of hepatic nodules.	Roomi et al. (1987)
100 µmoles/100 g b. wt. Pb nitrate, single injection, i.v.	Animals were sacrificed at 1, 2, 3, 4, and 15 days	Wistar rats	Acute Pb treatment results in induced activity of Gamma- glutamyl transpeptidase, induced GSTP, a typical marker of pre neoplastic lesion in most hepatocytes. Lead also inhibited liver adenylate cyclase activity 24 h post exposure.	Columbano et al. (1988)
100 mg/kg i.p., single exposure	Multiple analyses 0-96 h	Male DDY strain mice	Lead decreased Glutathione content and decreased Glutathione s-transferase activity that is independent of Glutathione levels.	Nakagawa et al. (1991)
100 µmol/kg body wt, i.v	70 h	Male Sprague Dawley rats	Acute Pb nitrate treatment caused a significant increase of GST activity in liver and kidney. While in liver the activity increase is mainly due to isozyme GST 7-7, in kidney it is through the induction of all the isozymes.	planas- Bhone and Elizalde (1992)
100 µmol/kg b. wt., intra cardiac	Multiple time point analyses starting 6 days to 5 months.	Male and female Wistar rats	Intracardiac administration of Pb acetate results in elevation of glutathione transferase (GST) in Kupffer cells, the early response to GST.Yp was observed in sinusoidal cells and had a later patchy response in the expression of GST Yp Yp in hepatocytes	Boyce and Mantel (1993)
10 µmol/100g b. wt., Pb nitrate, i.v. Single dose	Analyses at multiple time points 0-10 days	Male Fischer 344 young adult rats.	Glutathione transferase P1-1 is induced significantly by a single intravenous dose of Pb nitrate through increased transcription and modulations at post transcription and translational levels.	Koo et al. (1994)

Table AX5-10.1 (cont'd). Hepatic Drug Metabolism

Concentration	Duration	Species	Effects[a]	Reference
Lead nitrate, 100 µm/kg i.p, 3 times every 24 h Lead acetate 100 µm/kg.	48 h 0.5-24 h	Transgenic rats with 5 different constructs having GST-P and/or chloromphenical acetyl transferase coding sequence.	GSTP (placental GST), is regulated by Pb at transcriptional level. GST-P enhancer (GPEI), is an essential cis- element required for the activation of the GST-P gene by Pb and is involved in the activation regardless of the trans-activators involved. GPEI element consists of two AP-1 binding sites. Activation of GST-P gene by Pb is mediated in major part by enhancer GPEI, which may involve AP-1 activation partially.	Suzuki et al. (1996)
10 nM Pb nitrate	24 h before transfection with ECAT deletion mutant, every 24 h there after till 48 h after transfection	NRK Kidney fibroblasts	Lead induces GST-P in NRK normal rat kidney fibroblast cell line.	
10mg Triethyl Pb, i.p. single dose	Analyses at multiple durations (3, 4, 7, 10, or 14 days)	Fischer 344 rats	Decreased liver Glutathione s-transferase (GST) activity and lower levels of several hepatic GST Increase in quinone reductase activity by day 14 in liver.	Daggett et al. (1997)
114mg Pb acetate/kg b. wt. i.p	Single (0.5-12 h group) or multiple (72 h and 7 d group) exposure	Sprague Dawley	Pb exposure resulted in hepatic Glutathione (GSH) depletion and increased malondialdehyde (MDA) production.	Dagget et al. (1998)
A. 1.5-3.0 mg/kg wt Triethyl Pb (TEL) i.p.	2 exposures for 48 h	Female Wistar	Pretreatment of rats did not affect the liver microsomal Oestradiol-17β metabolism or the content of cytochrome P-450 and cytochrome b5.	Odenbro and Rafter (1988)
B. 0.05-0.5, TEL to liver microsomal fractions	30 min incubations	Liver microsomes from female Wistar rats	TEL at 0.05 mM significantly reduced 17β-hydroxy steroid oxidation and at concentration of 0.05 mM decreased 16α-hydroxylation	

Table AX5-10.1 (cont'd). Hepatic Drug Metabolism

Concentration	Duration	Species	Effects[a]	Reference
50mg/kg, intragastric	8 weeks	Male Albino Wistar rats	Accentuation of liver membrane lipid peroxidation. significant inhibition of liver antioxidant enzymes. Reduced ratio of reduced glutathione(GSH) to oxidized glutathione (GSSG),	Sandhir and Gill (1995)

b. wt.	body weight	Cu	Copper	TNFα	Tumor necrosis factor
CYP	Cytochrome P-450	Cd	Cadmium		
GSH	Glutathione	Al	Aluminum		
GSSG	Oxidized glutathione	Zn	Zinc		
TEL	Triethyl lead	Pb	Lead		
CCL	Carbon tetrachloride	Ni	Nickel		
GSTP	Placental glutathione transferase				
MDA	Malondialdehyde				
ALA	Alanine aminotransferase				
PAH	Polycyclic aromatic hydrocarbons				
LPS	Lipopolysaccharides				

Table AX5-10.2. Biochemical and Molecular Perturbations in Lead-induced Liver Tissue

Concentration	Duration	Species	Effects[a]	Reference
Lead - diethyl dithiocarbomate complex, Pb (DTC) 2, or lead acetate 0.033- 10 μM	0.5 - 20 h	Primary hepatocytes	Effect of interactions between lead and diethyl dithiocarbomate (DTC) on the enzyme δ amino levulinic acid dehydratase in primary hepatocytes. Lipophilic Pb (DTC)2 caused a more rapid and stronger inhibition of ALAD activity than lead acetate. Lead uptake is higher and more rapid with Pb (DTC) 2 than lead acetate. This increased inhibition of ALAD activity by Pb (DTC) 2 might be due to facilitated cellular transport in the complexed form resulting in higher cellular concentrations of lead.	Oskarsson and Lindahl (1989)
—	—	Primary rat hepatocytes	DTC decreases cellular effects of Pb and Cd despite unchanged/ even slightly increased concentrations of the metals. Hepatic ALAD was significantly inhibited in cells treated with Pb Ac and Pb (DTC)2.	Hellstrom-Lindahl and Oskarsson (1990)
—	—	DBA and C57 mice	DBA mice(with a duplication of the ALAD gene accumulated twice the amount of lead in their blood and had higher lead levels in liver and kidney than mice with the single copy of the gene (C57), exposed to the same oral doses of the lead during adult hood. Blood Zinc protoporphyrin (ZPP) increased with lead exposure in C57 mice and were not affected in DBA mice	Claudio et al. (1996)
100 μmol/kg b. wt. i.v single dose	Single dose, analyses performed 12, 24, 48, 72, 96 and 168 h	Male Wistar Albino Rats	First in vivo report showing association between lead induced liver hyperplasia, Glucose - 6 - phosphate levels, and cholesterol synthesis. Lead treatment increased hepatic de novo synthesis of cholesterol as evident by increased cholesterol esters and increase of G-6-PD to possibly supply the reduced equivalents for de novo synthesis of cholesterol. Changes in these biochemical parameters were accompanied by liver hyperplasia.	Dessi et al. (1984)
Lead nitrate, Single dose 100 μmol/kg b. wt.	0 – 168 h	Male Wistar rats	Lead nitrate induces hepatic cell proliferation followed by reabsorption of excess tissue with in 10-14 days. The proliferation was correlated with hepatic denovo synthesis of cholesterol, stimulation of hexose monophosphate shunt pathway and alterations in serum lipo proteins.	Pani et al. (1984)
Lead nitrate	—	Wistar rats	Lead nitrate induces multiple molecular forms of Glucose-6- phosphate dehydrogenase with an increase of band 3 and a concomitant increase of band 1, shifting from the pattern induced by fasting with an increase in band 1.	Batetta et al. (1990)

Table AX5-10.2 (cont'd). Biochemical and Molecular Perturbations in Lead-induced Liver Tissue

Concentration	Duration	Species	Effects[a]	Reference
Lead nitrate, single i.v. 10 μM/ 100g b.wt.	Multiple time points 24-72 h and 20 days	Male Wistar rats	Lead nitrate exposure results in complete loss of liver glycogen between 24 and 48 h, which was replenished and was found in excess in treated liver hepatocytes by 20 days. Glycogen synthase and glycogen phosphorylase activities were diminished by 24 h and return to normal values by day 20. The pentose phosphate enzymes were upregulated, which coincided highly with the increase in mitotic rate. Overall lead nitrate induces drastic alterations in hepatic carbohydrate metabolism along with increased hepatic cell proliferation.	Hacker et al. (1990)
—	—	Rats	Lead acetate induced mitotic response much more effectively in renal epithelial cells than liver cells (675 fold less).	Calabrese and Baldwin et al. (1992)
10 or 20 mg/kg as lead acetate, subcutaneous	Once a wk for 5 wks.	Occupationally exposed workers Rats	Lead induces lipid peroxidation in serum of manual workers, while blood superoxide dismutase (SOD) activity decreased. Similar phenomenon was observed with rats that were subcutaneously injected with lead acetate. At higher than 20 μM concentration, lead in untreated microsomes increased NADPH dependent lipid peroxidation.	Ito et al. (1985)
100 μM/kg b. wt lead nitrate, i.v	36 h post exposure	Male Wistar Albino rats	Endogenous source of newly synthesized cholesterol together with increase of HMP shunt enzyme activities is essential for hepatic cell proliferation by lead nitrate	Dessi et al. (1990)
2000 ppm lead acetate in diet.	3 wks	Arbor Acres male Chicks	Liver non protein sulphahydryl (NPSH) and glutathione (GSH) were increased upon lead exposure. The concentrations of liver glutamate, glycine, and methionine were also elevated upon lead exposure.	Mc Gowan and Donaldson et al. (1987)
0- 4000 ppm lead acetate, oral	21 days	Arbor Acre broiler chicks	Lead increases tissue peroxidation via a relative increase of 20:4 fatty acids. Decrease in the hepatic ratio of 18:2/20:4 might be specific to lead toxicity	Donald and Leeming, (1984)

Table AX5-10.2 (cont'd). Biochemical and Molecular Perturbations in Lead-induced Liver Tissue

Concentration	Duration	Species	Effects[a]	Reference
Sodium Vanadate, 30 mg/kg subcutaneous in mice 30 mg/kg b.wt, i.p. in rats 0.5 mM	Acute studies, 24 h	Male Swiss-Webster mice Male Sprague Dawley Rat	Sodium orthovandate increases lipid peroxidation in kidneys of mice and rats. Malondialdehyde (MDA) formation increased 100%, with in 1 h. after injection. In both rat and mice, no significant increase in lipid peroxidation was observed in brain, heart, lung, and spleen. Chronic exposure to vanadium, through maternal milk and drinking water for 10 weeks increased MDA formation and lipid peroxidation in kidneys.	Donaldson et al. (1985)
Vanadium sulphate in drinking water for chronic treatment	Chronic studies 10 wks			
250 -2000 ppm lead acetate in diet	19 days	Arbor Acre broiler chicks	Dietary Pb consistently increased liver arachidonic acid, the arachidonate/linoleate ratio and hepatic non-protein sulfhydryl concentration. Hepatic microsomal fatty acid elongation activity was decreased by Pb. over all these results demonstrate that changes in the precursors and mechanisms involved with eicosanoid metabolism are not always reflected in tissue concentrations of leukotriens and prostaglandin.	Knowles and Donaldson et al. (1990)
1.25-20.00 mg/L lead nitrate, oral	30 days	Fresh water fish	Lead accumulation in the liver and other tissue increased in a dose dependent manner up to 5mg/L, exposure to sublethal concentration (5 ppm) of lead reduced the total lipids, phospholipids, and cholesterol levels in the liver and ovary. Lead nitrate may affect the fecundity of fish by altered lipid metabolism.	Tulasi et al. (1992)
250 mg/L of lead as lead acetate, oral	5 weeks of exposure followed by 4 weeks of recovery	Weanling female SD rats	Effect of weight loss on body burden of lead - Weight loss increases the quantity and concentration of lead in the liver even in the absence of continued exposure	Han et al. (1996)
35-70 mg, lead intra gastric	One or two times a wk/7 wks.	Male Buffalo rats	Decrease in plasma cholesterol, & HDL fraction, increase in serum triglyceride, atrophy of the elastic fibers in the aorta.	Skoczynska et al. (1993)

CYP	Cytochrome P-450	ZPP	Zinc protoporphyrin
ALAD	Reduced Glutathione	HMP	Hexose monophosphate shunt pathway
GSH	Aminolevulinic acid dehydratase	b. wt.	body weight

Table AX5-10.3. Effect of Lead Exposure on Hepatic Cholesterol Metabolism

Concentration	Duration	Species	Effects[a]	Reference
100 µmol/kg body wt, i.v. lead nitrate	Multiple durations 0, 3, 6, 12, 24, and 48 h	Male Sprague Dwaley (SD) Rats.	Lead nitrate, activates the expression of the SREBP-2 and CYP 51 gene with out decreasing the serum cholesterol level.	Kojima et al. (2002)
100 µmol/kg body wt, i.v. lead nitrate	Multiple durations 0, 1, 3, 6, 12, 18, 24, 48, and 72 h	Male Sprague Dwaley (SD) rats	Lead nitrate effects on hepatic enzymes involved in cholesterol homeostasis--- Demonstrated for the first time sterol independent gene regulation of cholesterol synthesis in lead nitrate treatment	Kojima et al. (2004)
0.05 mg/kg body wt/day. lead acetate, subcutaneous, with or without cadmium acetate 0.025 mg lead acetate/kg body wt/day	preexposure for 5–7 days, gestation through lactation.	female Charles Foster rats	Lead and cadmium accumulated in the livers of metal treated pregnant and lactating rats. Hepatic steroid metabolizing enzyme 17-β-hydroxy steroid oxidoreductase and UDP glucaronyl transferase were decreased and the hepatic Cytochrome P-450 content was reduced by the metal exposure. Lead and cadmium alter liver biochemical parameters, however, combined exposure had no intensifying effect on liver parameters. When administered together on similar concentration basis, the major effects are mediated by cadmium.	Pillai and Gupta (2004)
300 mg/L lead acetate, oral	Gestation through lactation analyses done at day 12 and day 21 post natal.	Female Wistar Rats	In neonates, decrease in liver Hb, iron, alkaline and acid phosphatase levels. Protein, DNA and lipid total amounts were reduced and hepatic glycogen content was reduced. Lead intoxication of mothers in gestation and lactation results in alterations in the hepatic system in neonates and pups.	Corpas et al. (2002)

[a] CYP = Cytochrome P-450
b. wt. = body weight

Table AX5-10.4. Lead, Oxidative Stress, and Chelation Therapy

Concentration and Compound	Duration	Species	Effects	Reference
Lead acetate, 50 mg/kg b.wt, intragastric	8 wks	Male Albino Wistar rats	Lead induces accentuation of membrane lipid peroxidation in liver by the changes (decrease) in the activities of several antioxidant enzymes such as SOD, Catalase, GPx and Glutathione reductase. Lead exposure also caused a reduction in GSH/GSSG ratio (reduced to oxidized Glutathione).	Sandir and Gill (1995)
2,000 ppm, lead acetate, Diet	5 wks	Male Fisher 344 rats	Effect of lead on lipid peroxidation in young vs. adult rats– Liver GSSG and malondialdehydehyde levels were significantly higher in young rats than adult rats. Blood lead levels were higher in young exposed animals as compared to adults. In young, lead exposed animals, lead induced oxidative stress was more pronounced particularly in liver tissue.	Aykin-Burns et al. (2003)
0.1-1.0 µM		Rat liver hepatocytes. Normal and LAN loaded	Lipid peroxidation as indicated by Malondialdehyde accumulation upon exposure to various redox-sensitive metals in cultured rat hepatocytes and hepatocytes loaded with α-linolenic acid indicated that - Al, Cr and Manganese, Ni, lead and tin did not effectively induce lipid peroxidation in these cells. – The induction was the highest in ferrous iron treated cells compared to other metals (Cu, Cd, V, Ni).	Furono et al. (1996)
$FeSO^4$, VCl_3, $CuSO^4$, $CdCl_2$, $CoCl_2$, $AlCl_3$, $CrCl_3$, $MnCl_2$, $NiSO^4$, $Pb(NO_3)_2$, $SnCl_2$, culture medium	9 h			
LAN - bovine serum complex 0.8 mM in culture medium	Additional 12 h incubation		With any metal, the induction was higher in α-linolenic acid treated cells. Iron and V induced cell injury in LAN loaded cells was prevented by addition of DPPD. Cd was a weak inducer of lipid peroxidation under these conditions	
5 mg kg−1, lead acetate, i.p., single dose followed by therapy with chelating agents	Analyses after 6 days of treatment DMSA, Mi-ADMSA at multiple times (0.5, 24 hr, 4th and 5th day after lead treat	Wistar 6 day old suckling rats	Treatment with DMSA and Mi- ADMSA showed Mi-ADMSA to be more effective in reducing the skeletal, kidney and brain content of lead. However there was no difference in reducing the liver lead content between the two compounds.	Blanusha et al. (1995)

Table AX5-10.4 (cont'd). Lead, Oxidative Stress, and Chelation Therapy

Concentration and Compound	Duration	Species	Effects	Reference
550 ppm lead acetate, oral DMSA treatment.	(A) Pb for 35 + 21 days (B) Pb 35 days and Pb & DMSA for 21 days (C) Pb 35 days and DMSA for 21 days (D) Acedified Di H_2O for 35 days and Di water for 21 days	6-7 Wk old male Sprague-Dawley rats	DMSA reversed the hematological effects of Pb, decreased the blood, brain , bone, kidney and liver concentration and produced marked lead diuresis, even when challenged with ongoing Pb exposure.	Pappas et al. (1995)
Lead acetate Dose to achieve blood lead levels of 35-40 µg/dL. Biweekly dose adjustments, oral followed by treatment with chelator.	1 year, chelator for two successive 19 day period following lead exposure.	Infant rhesus monkeys	Specific emphasis on the beneficial effects of succimer treatment to cessation of lead exposure. These data demonstrated that succimer efficiently reduces blood Pb levels which does not persist beyond the completion of treatment. They also demonstrate the relative benefit of eliminating lead exposures , which serves to underscore the importance of primary prevention of lead exposure. Neither DMSA treatment nor the cessation of lead exposure were beneficial in reducing skeletal lead levels.	Smith et al. (2000)
5 mg Pb kg−1, i.p lead acetate followed by chelators for various time points.	Analyses at Day 5	Suckling Wistar rats	Meso - DMSA is the treatment of choice for acute lead poisoning in infants compared to EDTA and Rac-DMSA.	Kostial et al. (1999)
50 mg/kg lead as lead nitrate, i.p, two injections, 16h apart 50% Ethanol, 0.5 mL, two injections, 16 h apart	24 h	Male Albino rats	S- Adenosyl methionine confers protection against alterations in several parameters (ALAD, GSH, MDA) indicative of lipid peroxidation in blood, liver and brain in lead and acute lead and ethanol exposed animals as well as the organ concentration of lead.	Flora and Seth (1999)
0.1% lead acetate in drinking water with and without Sodium Molybdate, i.p	4 weeks	Male Albino rats	Sodium molybdate significantly protected the uptake of lead in blood, liver and kidney. The treatment with molybdate also restored the lead induced inhibited activity of blood δ-aminolevulinic acid dehydratase and the elevation of blood Zn protoporphyrin , hepatic lipid peroxidation and serum ceruloplasmin.	Flora et al. (1993)

Table AX5-10.4 (cont'd). Lead, Oxidative Stress, and Chelation Therapy

Concentration and Compound	Duration	Species	Effects	Reference
20 mg/kg lead acetate, i.p.	3 days treatment	Male Albino rats	Significant lead induced inhibition of hepatic heme synthesis associated with decline of mixed function oxidases, depletion in anti oxidants such as vitamin C. Oral supplementation with vitamin C confers protection against toxic insult by reversing these parameters	Vij et al. (1998)
1.5 mg/per bird /day	30 days	Broiler chicken	Lead - induced inhibition of 5' mono deiodinase (5'- D) activity in chickens appeared to be mediated through the lipid peroxidative process.	Chaurasia et al. (1998)
10 mg/4mL/kg, lead acetate, with or without ethanol, lysine or Zinc, oral	5 days a week for 8 weeks	Male Albino Rats	Simultaneous administration of lysine and Zinc reduced tissue accumulation of lead and lead -induced biochemical alterations irrespective of exposure to lead alone or lead and ethanol.	Tandon et al. (1997)
1300 ppm lead acetate in drinking water	5 weeks	C57BL/6 mice	Pb treatment resulted in depletion of GSH, increased GSSG and promoted Malondialdehyde (MDA) production in both liver and brain samples. DMSA or N-acetyl cysteine (NAC) treatment resulted in reversion of these observations. DMSA treatment resulted in reduced lead levels in blood, liver and brain, where as treatment with NAC did not reduce these levels.	Ercal (1996)
2000 ppm of lead acetate in drinking water	5 weeks followed by treatment with succimer DMSA, or thiol agent NAC	Fisher 344 male rats	Lead induces oxidative stress in RBC and these biochemical alterations are reversed by both a thiol antioxidant (NAC) as well as a chelating agent DMSA.	Gurer et al. (1998)
500 μM lead acetate in cells 2000 ppm of lead acetate in drinking water	Cells-20 h Animals 5 weeks followed by treatment with α-lipoic acid	Male fisher rats and Chinese hamster ovary cells	Lead induces oxidative stress. α-lipoic acid (LA)treatment significantly increased thiol capacity of cells and animals via. increasing glutathione levels and reducing Malondialdehyde levels, increased cell survival. LA was not effective against reducing blood or tissue lead levels.	Gurer et al. (1999)

Table AX5-10.4 (cont'd). Lead, Oxidative Stress, and Chelation Therapy

Concentration and Compound	Duration	Species	Effects	Reference
0-500 µM lead acetate 2000 ppm of lead acetate in drinking water for 5 weeks Taurine 1.1 kg/day	6 h 5 weeks 6th week	CHO cells and Fischer 344 rats.	Antioxidant Taurine reversed the abnormalities associated with lipid peroxidation parameters such as increased. Malondialdehyde formation and decreased Glutathione and enhanced CHO cell survival. However, was not effective in reducing cell and tissue lead burden in CHO cells and lead exposed Fischer rats.	Gurer et al. (2001)
1 mg Pb2+/kg B.wt , i.p. lead acetate	4 wks, treatment with various antioxidant in the 5th wk	IVRI 2 CQ rats	Lead exposure resulted in increased lipid peroxidation, with tissue specific changes in liver. Treatment of exposed rats with ascorbic acid and α-tocopherol lowered the lipid peroxidation.	Patra et al. (2001)
Lead as acetate, 400 mgPb2+/mL, drinking water 0.5 mg/mL L-methionine	10 days 4 wks post-lead exposure	Kunming mice	L- methionine has an ameliorative effect on lead toxicity–Methionine reduced the decrease in Hb content and depressed body growth caused by lead. Treatment with dietary methione along with lead decreased the MDA formation as opposed to lead, moderately reversed the decreased iron content of the organs and decreased organ lead content.	Xie et al. (2001)
100 µM/kg b.wt. lead acetate, intramuscular, single	3 and 24 h	Male Albino rats	Lead exposure resulted in significant increases in acid and alkaline phosphatases, serum GOT and GPT, elevated liver and kidney lipid peroxidation and decreased antioxidant enzymes at 3 and 24 h after exposure. Selenium administration prior to lead exposure produced pronounced prophylactic effects against lead exposure by enhancing endogenous anti oxidant capacity.	Othman and El Missiry (1998)
100 µg/ lead acetate, intra gastric, oral and intraperitoneal, treated with or with out thiamin (25, 50 mg/kg b.wt) and or Ca EDTA (50 mg/kg B.wt	3 days	CD-1 mice	Two times more whole body lead was retained by intraperitoneal injection as compared to intragastric administration. Thiamin treatment increased the whole body retention of both intragastric and intraperitoneal lead by about 10%. Calcium EDTA either alone or in combination with thiamin reduced the whole body retention of lead by about 14% regardless of the route of exposure. Regardless of the route Ca EDTA in the combined treatment reduced the relative retention of lead in both in liver and kidney. These studies indicate the combination treatment with thiamin and Ca EDTA alters the distribution and retention of lead in a manner which might have therapeutic application.	Kim et al. (1992)

Table AX5-10.4 (cont'd). Lead, Oxidative Stress, and Chelation Therapy

Concentration and Compound	Duration	Species	Effects	Reference
2000 ppm lead acetate, oral	4 wks, 5 days of treatment with antioxidant or chelators	Male Wistar albino rats	Treatment with all the chelators reduced hepatic GSH and reduced GSSG levels. Significant beneficial role of Alpha-lipoic acid (LA), in recovering the altered biochemical parameters, however showed no chelating properties in lessening body lead burden either from blood, liver, or kidney. Most beneficial effects against lead poisoning was observed with combined treatment of lipoic acid and either DMSA (meso 2,3 - dimercaptosuccinic acid) or MiADMSA (Mono isoamyl DMSA).	Pande and Flora (2002)
0.1% lead as acetate in drinking water DMSA - 50 mg/kg, i.p./day MiADMSA50 mg/kg, i.p./day Vitamin E 5 mg/kg and vitamin C 25 mg/kg/ day, i.v. and oral	3 months 5 days post-lead exposure	Male Wistar rats	Single or combined administration of vitamin C, α-tocopherol and the chelators DMSA and Mi ADMSA against the Parameters of lead induced oxidative stress–thiol chelators and the vitamins could bring the blood ALAD to normal levels, most significantly by combined administration of Mi ADMSA with vitamin C. Vitamin C and E were effective against reducing oxidized glutathione (GSSG), and thibarbituric acid reactive substance(TBARS) and increasing catalase activity. MiADMSA and DMSA with vitamin C were effective in increasing hepatic GSH levels. In summary combined treatment regimens with thiol chelators and vitamins seem very effective in reducing the lead induced Oxidative stress.	Flora et al. (2003)
500 mg/kg lead acetate daily, oral treatment with chelators	Multiple durations (2, 4, and 6 wks)	Male Albino rats	Impact of combined administration of vitamin C and Sylimarin on lead toxicity. Combined treatment of lead-exposed animals with vitamin C and Silymarin showed marked improvement of the adverse biochemical, molecular and histopathological signs associated with lead toxicity.	Shalan et al. (2005)
Lead as acetate0.2% in drinking water LA 25 mg/kg b.wt and DMSA 20 mg/kg b.wt	5 wks followed by a 6th wk administration of LA and or DMSA	Male Albino rats	Lead treatment for 5 weeks resulted hepatic enzymes alanine transaminase, aspartate transaminase, and alkaline phosphatase, increased lipid peroxidation, and decreased hepatic anti oxidant enzymes. LA or DMSA alone, partially abrogated these effects, however, in combination completely reversed the lipid oxidative damage.	Sivaprasad et al. (2004)

b. wt.	body weight	Cr	Cromium	$CuSO^4$	Copper sulphate
[a]CYP	Cytochrome P-450	V	Vanadium	$CrCl_3$	Cromium chloride
SOD	Super oxide dismutase	Pb	Lead	$MnCl_2$	Manganese chloride
GSH	Glutathione	NAC	N acetyl cysteine	$NiSO^4$	Nickel sulphate
GSH/GSSG Ratio	Reduced Glutathione/Oxidized Glutathione	$FeSO^4$	Ferrous sulphate	$CoCl_2$	Cobalt chloride
MDA	Malondialdehyde	$AlCl_3$	Aluminum chloride	LAN	α Linolenic acid
Al	Aluminum	VCl_3	Vanadium chloride	DPPD	DPPD,*N-N* Diphenyl –p-phynylene-diamine
As	Arsenic	$CdCl_2$,	Cadmium chloride		

Table AX5-10.5. Lead-induced Liver Hyperplasia: Mediators and Molecular Mechanisms

Concentration	Duration	Species	Effects[a]	Reference
—	—	Rat	Apoptosis plays a major role in the regressive phase of lead nitrate induced hepatic hyperplasia as detected by the apoptotic bodies by in situ end labeling and H&E sections of the hepatic tissue. H&E scores mostly cells in apoptosis phase II, ISEL (in situ end labeling) scores for cells in phase I. Combination of these two methods is suggested for the better understanding of the extent and nature of apoptotic process in liver cells treated with chemicals.	Nakajima et al. (1995)
A. Lead nitrate, 100 µM/kg b.wt, intra-gastric B. Diethyl nitrosoamine 200 mg/kg b.wt, i.p.	3 and 15 days	Male Wistar Albino rats	Mitogenic stimuli (3 days lead nitrate treatment) and complete regression (15 days after the treatment), affected the apoptosis differentially. Influence of apoptosis Vs necrosis on the growth of hepatocytes initiated by diethyl nitrosamine followed by lead nitrate treatment indicated that the regenerative response elicited by a necrogenic dose of CCL4 promoted GSTP (Placental glutathione), a pre-neoplastic marker positive cells as against the lead nitrate that induced the apoptosis.	Columbano et al. (1996)
0-100 µM Pb sulphate, Pb monoxide, Pb chloride and Pb acetate up to 1 mM, culture media.	Multiple time points ranging from 24 h up to 7 days.	REL liver cells	Lead compounds showed a dose and time related effect on REL liver cell proliferation with varying potencies specific to the different lead salts. Pb acetate was the most effective and Pb monoxide, the least effective. On 1 hr treatment none of the compounds tested affected the intracellular communication.	Apostoli et al. (2000)
Choline 1g/kg/day in drinking water	0, 20 and 24 h	Male and female rats , partial hepatectomy	PKC isozymes during liver cell regeneration— PKC δ showed a pronounced increase 20h after partial hepatectomy. α, β, and Zeta at 24 h corresponding with S-phase. Sexual dimorphism matching with sexual differences in DNA synthesis was evident. Administration of choline was able to modulate the protein kinase C isozyme pattern in females in relation to DNA synthesis and c-myc expression. Taken together the data positively implicates α, β, and Zeta in growth after partial hepatectomy and δ in negative regulation.	Tessitore et al. (1995)
Lead nitrate, 75 µM/kg b.wt, single i.v.	6 h – 4 wks	Adult male Albino rats	Lead induced significant increase in liver weight. Increased 3H Thymidine incorporation. Lead induces extensive hypomethylation in treated rat livers. Site-specific effect on methylation was confirmed at Hpa II, Msp I, Hae III.	Kanduc et al. (1991)
75 µmol/kg b. wt. Lead nitrate in adult and 20 µg/mL in the young, i.v.; single dose	Analyses at 72 h	Male Wistar Albino Rats.	Effect of lead nitrate on the 5- methyl deoxy cytidine (5-mdcyd) content and the HpaII, MSPI, Hae III restriction patterns of hepatic DNA from young, middle aged and senescent rats. The results indicated that the methylation pattern of genomic DNA changed significantly with age and the methylation patterns were differentially affected in all the three populations.	Kanduc & Prisco (1992)

Table AX5-10.5 (cont'd). Lead-induced Liver Hyperplasia: Mediators and Molecular Mechanisms

Concentration	Duration	Species	Effects[a]	Reference
10 µmol/100 g body weight lead nitrate, i.v.	Multiple analyses up to 40 h	Male Wistar Rats, hepatocytes from partial hepatectomy and lead nitrate treatment.	The kinetics of DNA synthesis and expression of Proto oncogenes in partially hepatectamized liver cells and lead nitrate treated hepatic cells indicated peak DNA synthesis after 24 h in the formal and after 36 h in the later case. Both proliferative stimuli induced c-fos, c-myc and c-Ha Ras expression. Induced c-myc expression persisted for up to 40h during the lead nitrate- induced liver cell proliferation. Lead induces hepatic hyperplasia through changes in proto-oncogene expression.	Coni et al. (1989)
100 µmol/kg, b. wt. lead nitrate, i.v.	Analyses at multiple time points 0.25 – 24 h	Male Wistar Albino Rats	proliferative stimuli by means of lead nitrate exposure resulted in increased expression of c-jun m-RNA where as compensatory regeneration in partially hepatectamized cells occurred through increased expression of c-fos and c-jun. Different mitogenic stimuli induced differential expression of these protooncogenes, in addition had a different profile than cells from partial hepatectomy despite the cell cycle timings being the same in some cases.	Coni et al. (1993)
100 µmol/kg b. wt.	8 h	Male Sprague Dawley rats	In rat liver, in addition to a few hepatocytes four types of non parenchymal cells namely, fibroblasts, macrophages, bile ducts and periductular cells proliferate in response to lead nitrate treatment. This growth is not related to adaptive response secondary to parenchymal enlargement. However, such growth in parenchymal cells seems dormant and does not play a functional role in adult liver epithelial growth.	Rijhsinghani et al. (1992)
100 µmol/kg b. wt., i.v.	Multiple analyses time points, 1-120 h	Male Wistar rats	Both mRNA levels and enzyme activity of DNA polymerase β markedly increased before and/or during DNA synthesis in proliferating hepatocytes in lead nitrate treated and partially hepatectomized rats. 5 fold increase in the enzyme activity was observed 8 h after lead nitrate administration. In the regenerative liver cells a 3 fold increase was observed 24-48h after partial hepatectomy.	Menegazzi et al. (1992)
100 µ mol/kg b. wt., i.v. lead nitrate	Analyses at multiple time points 8 h to 15 days	Male Wistar rats	Lead nitrate induced Poly (ADP-ribose) polymerase mRNA 24 hr after exposure. A 2 fold increase in the mRNA levels of the enzyme occurred two days after the exposure. Such changes were also observed in hepatic cells from livers of partial hepatectomy. These changes preceded the increase in DNA synthesis and remained high during the time of extensive DNA synthesis.	Menegazzi et al. (1990)

Table AX5-10.5 (cont'd). Lead-induced Liver Hyperplasia: Mediators and Molecular Mechanisms

Concentration	Duration	Species	Effects[a]	Reference
30 mg/kg b. wt. lead nitrate	Multiple time points up to 8 days	Adult male and female rats	Lead nitrate induced liver hyperplasia exhibited sexual dimorphism where mitogenic action was less effective and was delayed in females as compared with males. Pre administration with choline partially filled these sexual differences.	Tessitore et al. (1994)
30 mg/kg b. wt. lead nitrate	Multiple time point up to 60 h	Adult male and female rats	Lead nitrate induced liver hyperplasia exhibited sexual dimorphism. Pre administration with choline partially filled these sexual differences. Significant down regulation of PKC β and PKC α activities occurred during lead induced proliferation	Tessitore et al. (1994)
100 µmol/kg b. wt. lead nitrate, i.v., single dose.	Multiple time point analyses ranging from 12 - 168 h	Male Wistar Rats	Effect of lead nitrate on protein kinase C (PKC) activity. A single dose of lead nitrate resulted in enhanced activity of PKC in the purified particulate fraction of the rat liver, reached its peak activity by 24 h which lasted for 48 h. This was accompanied by increased frequency of mitotic cells. These results indicate, lead nitrate induced PKC activity may play a role in liver cell proliferation.	Liu et al. (1997)
A. Mitosis – Lead nitrate- 100 µM/kg, i.v. Ethylene dibromide 100 mg/kg , intra gastric Cyproterone acetate, 60 mg/kg intra gastric. B. Hepatocyte nodules diethyl nitrosamine 200 mg/kg	30' - 3 h	Adult male Wistar rats	Liver cell proliferation by enhanced DNA synthesis was observed with the mitogens Cyproterone acetate, ethylene dibromide, and lead nitrate as early as 30 mints after treatment and persisted even after 5 days of treatment by lead nitrate administration. hepatocytes isolated from pre neoplastic liver nodules have also exhibited enhanced cell proliferation.	Coni (1991)
Lead nitrate, single i.v. 100 µm/ kg b.wt LPS- 12.5 µg/rat, post Pb nitrate treatment.	Multiple analyses at 3, 6, 12, 24 and 36 h	Male Wistar rats	Stimulation of hepatocyte cell proliferation by lead nitrate was not accompanied by changes in liver levels of Hepatocyte growth factor (HGF), Transforming growth factor-α (TGF-α), or TGF-β1 m-RNA. Lead nitrate treatment resulted in the enhancement of Tumor necrosis factor α at a time preceding the onset of hepatocyte DNA synthesis, indicating its role in lead induced hepatic cell proliferation. The survival of lead nitrate treated rats decreased significantly with an after treatment of LPS (lipopolysaccharide).	Shinozuka et al. (1994)

Table AX5-10.5 (cont'd). Lead-induced Liver Hyperplasia: Mediators and Molecular Mechanisms

Concentration	Duration	Species	Effects[a]	Reference
15 mg/kg b. wt. lead acetate	Pb+ LPS group analyzed after 14 h and the rest after 24 h after lead administration	Male Sprague Dawley rat	Lead augments the lethality of endotoxin lipopolysaccharide (LPS) in rats and enhances liver injury, which is further enhanced by TNF. Lead + LPS treatment increased both serum TNF concentrations and TNF area as compared to LPS alone. simultaneous administration of lead with either LPS or TNF, serum aspartate transaminase, alanine transaminase, alkaline phosphatase, glutamyl trans peptidase and plasma triglyceride levels were markedly increased	Honchel et al. (1990)
Lead nitrate 100 µM/kg b. wt. i.v. single dose	Multiple time points of analyses extending up to 48 h after treatment	Male Wistar rats	Lead nitrate and ethylene bromide induce liver cell proliferation via induction of TNFα. Dexa methasone, a known TNF inhibitor, decreases TNF expression and liver cell proliferation by these mitogens. These studies support the fact that TNF might mediate hepatic cell proliferation by lead nitrate and ethylene bromide.	Ledda-Columbano et al. (1994)
100 µmol/kg b. wt lead nitrate, single, i.v.	Multiple time points of analyses up to 48 h		Lead nitrate (LN) treatment resulted in increased Brdu incorporation of hepatocytes and non parenchymal cells at 12 h after treatment and reached the peak index at 36 h. Rats given a single iv of recombinant TNFα enhanced proliferation in non parenchymal cells after 24 h, the labeling of hepatocytes at 36 h. NAF, Nafenopin another mitogen which does not induce liver TNFα, increased the number of labeled hepatocytes without increasing the labeling of non parenchymal cells indicating that only lead nitrate induced proliferation is mediated by TNFα and these mitogens initiate proliferation in different cells based on their capacity to stimulate TNFα production.	Shinozuka et al. (1996)
100 µmol/kg b. wt. single i.v.,	Multiple time points of analyses up to 80 h	Male Sprague Dawley rats	Lead nitrate induces liver cell proliferation in rats without accompanying liver cell necrosis. This proliferation involves enhanced TNF mRNA and levels but not hepatocyte growth factor. The role of TNF in lead nitrate induced liver cell proliferation is supported by the inhibition of TNF and reduced hepatocyte proliferation by several TNF inhibitors.	Kubo et al. (1995)
100 µmol/kg b. wt. i.v. single dose	Multiple time points of analyses up to 24 h	Male Wistar rats	Lead nitrate induced liver cell proliferation involves TNF α production, enhanced NF-κB activation increased hepatic levels of iNos mRNA as opposed to other mitogens such as Cyproterone acetate or Nafenopine.	Menegazzi et al. (1997)
100 µ mol/kg b. wt. i.v., single dose	Multiple time point analyses up to 96 h	Male Sprague Dawley rats	The role of neurotrophins, the nerve growth factor (NGF), the brain derived neurotrophic factor (BDNF) and neurotrophin -3 (NT-3) in lead nitrate treated liver cells was studied. LN, treatment resulted in increased in the levels of NGF, BDNF and NT-3. The increase in neurotrophin receptors and the gene expression were correlate with liver weights. This study demonstrates that lead nitrate induced hyperplasia may be mediated by neurotrophins.	Nemoto et al. (2000)

Table AX5-10.5 (cont'd). Lead-induced Liver Hyperplasia: Mediators and Molecular Mechanisms

Concentration	Duration	Species	Effects[a]	Reference
Multiple doses 0-50 µM, culture medium	Multiple time points up to 24 h	Hepatocytes from Adult male Swiss-mice, primary	Interaction between Pb and cytokines in hepatotoxicity– Pb potentiated cytokine - induced oxidative stress by decreasing GSH and increased efflux of Oxidized glutathione (GSSG). Combined treatment resulted in a decline in intra cellular ATP concentration	Sieg & Billing (1997)
50 µM lead acetate, culture medium	24 h	Rat hepatocyte and Kupffer cell and granulocyte co-cultures	Lead stimulates intercellular signaling between Kupffer cells and hepatocytes which increased synergistically at low lipopolysaccharide levels. These signals promote proteolytic hepatocyte killing in combination with a direct cellular interaction between the granulocytes and hepatocytes.	Milosevic and Maier (2000)
10 µM/110 g b. wt., single i.v.	Multiple time point analyses up to 5 days	Adult male Wistar Rats	Lead nitrate induced hepatocyte apoptosis was prevented by pre- treatment with gadolinium chloride, a Kupffer cell toxicant – Role for Kuffer cell in hepatocyte apoptosis	Pagliara, et al. (2003a)
10 µmol/100 g b. wt. single i.v.	Multiple time points up to 9 days	Male Wistar rats	Lead nitrate-induced liver hyperplasia in rats results in a significant increase in the expression of aceyl glycoprotein receptor (ASGP-R) during the involutive phase of lead nitrate induced hyperplasia in rat-liver, which coincided with the massive death by apoptosis of the same cells. A significant rise in the galactose-specific receptors was also observed 3 days after the treatment. These studies demonstrate that carbohydrate receptors regulate lead nitrate induced liver cell apoptosis.	Dini et al. (1993)
10 mmol/100g, lead nitrate, i.v.	Multiple time points	Male Wistar rats	Demonstration of the expression of carbohydrate receptors on Kupffer cells. Lead nitrate induced apoptosis in Kupffer cells and internalization of apoptotic cells (Phagocytes) is mediated by both Mannose and Galactose receptors.	Ruzittu et al. (1999)

Table AX5-10.5 (cont'd). Lead-induced Liver Hyperplasia: Mediators and Molecular Mechanisms

Concentration	Duration	Species	Effects[a]	Reference
Pb (No3)2, , i.v. 100µM/110 g .b. wt	1, 3, and 5 days.	In vivo Adult male Wistar rats	Hepatic apoptosis induced by lead nitrate in vivo is abolished by gadolium chloride, a Kupffer cell toxicant that suppresses Kupffer cell activity and reduces to half the apoptotic rate. Lead nitrate treatment also deprives the hepatic cells from reduced glutathione and this process is reversed by Gadolium chloride. Lead nitrate induces apoptosis in Kupffer cells, and HepG2 cells in vitro.	Pagliara (2003b)
$GdCl_3$ 0.75 mg/100 g. b. wt, i.v.	2, 4, or 24 h before lead nitrate injection.			
In vitro, 10 mM lead nitrate	Analyses at multiple time points up to 24 h in Hep G2 cells and at 24 and 48 h in Kupffer cells	Hep G2 cells		
Multiple concentrations varying from 300 nM–10µM, up to 100 µM in certain in vitro expts	1, 2, 4 and 6 days	Hepatoma cell line, H4- II-C3	Acute effect of lead on glucocorticoid regulation of Tyrosine aminotransferase (TAT) in hepatoma cells –Lead treatment does not significantly alter initial glucocorticoid receptor number or ligand binding. Lead may perturb PKC mediated phosphorylations in the glucocorticoid-TAT signal transduction system. Lead also may be increasing the turnover of TAT by actions at transcription, translation and /or post translation.	Heiman and Toner (1995)
0–10 µM lead acetate in the culture medium	24 & 48 h	H4-IIE - C3 hepatoma cell culture model	In HTC cells glucocarticoid signal transduction pathways involve calcium-mediated events and PKC isoforms , lead exposure interferes with calcium mediated events and aberrant modulation of PKC activities and may contribute to the over all toxicity of lead.	Tonner and Heiman, (1997)

b. wt. = body weight

Table AX5-10.6. Effect of Lead Exposure on Liver Heme Synthesis

Concentration	Duration	Species	Effects[a]	Reference
75 mg Pb/kg b. wt., i.p.	Multiple time point analyses 0-30 h	C57 BL/6 mice	Lead poisoning decreases P-450 as a consequence of two different mechanisms, a mechanism unrelated to heme where P-450 transcription is inhibited (reduces the synthesis and activity), and a second mechanism where by inhibition of heme synthesis occurs decreasing the heme saturation of P450 and/or apo-P450 content.	Jover et al. (1996)
10^{-5} ppm lead nitrate	Multiple analyses up to 24 h	RLC-GA1 Rat liver cell line	Lead increases heme synthesis in RLC-GA1 in rat liver cell line, when measured by the amount of ^{59}Fe incorporated into heme fraction. Increased incorporation of ^{59}Fe into the heme fraction of the lead treated cells was the result of increased uptake of iron ^{59}Fe into the heme fraction of lead treated cells. Cellular degradation of lead was not significantly affected by lead.	Lake and Gerschenson (1978)
A. Triethyl lead-3.5 &8.0 mg/kg b.wt. Lead nitrate 3.5, 25 and 100 mg/kg Single Subcutaneous	Multiple analyses up to 28 days	Adult male Fischer rats	Triethyl lead chloride has a similar potency to inorganic lead nitrate in inhibiting ALAD both in vitro and in vivo. Liver and blood ALAD have similar sensitivities to lead compounds. Inhibition is reduced in the presence of Zn.	Bondy (1986)
B. In vitro, 10^{-3}-10^{-9} M triethyl lead or lead nitrate	30 minutes			
5 µM lead acetate or lead diethyldithiocarbomate lead uptake studies 0.33-10 µM	Multiple analyses from 0 – 20 h	Rat primary hepatocyte cultures	Effect of lead and diethyl dithiocarbomates on rat primary hepatocytes as studied with lead acetate and or lead- diethyldithiocarbomate complex (Pb DTC_{2l}) labeled with ^{203}Pb indicated that (Pb DTC_{2l}) complex caused a more rapid and stronger inhibition of ALAD activity than lead acetate. Uptake of lead was rapid and higher with the complex than lead acetate. The complex also inhibited the ALAD activity in vitro when incubated with purified ALAD enzyme.	Oskarsson et al. (1989)
Per OS eqimolar doses (17 µM Me/kg) of $SnCl^2$ or Pb $(CH_3COO)_2$ every day	5 days.	Female rabbits	Lead decreased liver and bone marrow ALAD, but had no change in the Aminolevulinic acid synthetase (ALA-S) and increased erythrocyte free protoporphyrin.	Zareba and Chmielnicka (1992)

Table AX5-10.6 (cont'd). Effect of Lead Exposure on Liver Heme Synthesis

Concentration	Duration	Species	Effects[a]	Reference
Lead 500 ppm in drinking water	14 days	Male ddY mice	Urinary excretion of β- Aminoisobutyric acid (ABA) and δ-aminolevulinic acid (ALA) increased significantly in mice exposed to lead in drinking water for 14 days. The degree of increasing excretion for ALA was higher than urinary ABA. Liver and kidney ALA dehydratase was inhibited, while ALA synthatase was not affected.	Tomokuni et al. (1991)
0.5 or 2.4 μM lead acetate in culture medium	Analyses at multiple time points, 0 -28 days	Hepatocyte cultures on 3T3 cells	Hepatocyte cultures on 3T3 cells produce and excrete porphyrins for 28 days. Lead exposure for 4 weeks alters cell morphology and produces cytotoxic effects that could be monitored by altered porphyrin excretion.	Quintanilla-Vega et al. (1995)
500 ppm lead in drinking water	Rat exposure 62 days Human occupational exposure 0.3–38 yrs.	A. .Male Wistar rats B. Lead smelt workers, males	Lead exposure significantly increases the urinary ALA (Aminolevulinic acid) and Coproporphyrins (CP-III>CP-I in rats and exposed workers. Urinary 5-hydroxy indole acetic acid was not influenced by lead exposure.	Ichiba and Tomokuni (1987)
A. Cu deficient diet-1 mg/kg Cu in the diet B. Moderately deficient- 2 mg/kg C. High Zn diet 60 mg/kg b. wt.	4 wks	Weanling Sprague Dawley rats	High Zn in the diet reduces plasma copper, but not plasma ceruloplasmin activity or the recovery of plasma copper or ceruloplasmin activity after oral copper sulphate of Cu-deficient rats. High dietary Zn also modifies the response of plasma SOD activity to dietary copper , but does not influence RBC SOD activity	Panemangalore and Bebe (1996)
1200 mg/kg b. wt. lead acetate in diet, Sub acute toxic studies 400 mg/lead	4 wks	Broiler chickens	Liver porphyrin levels increased during lead toxicosis. Concurrent administration of selenium or monensin in the feed further enhances this process.	Khan & Szarek (1994)
0-100 μM lead acetate in the culture medium	19 h	Primary Rat and chick embryo hepatocyte cultures.	Formation of Zn protoporphyrins in cultured hepatocytes– Lead did not specifically increase Zinc protoporphyrin accumulation or alter iron availability in cultured hepatocytes.	Jacobs et al. (1998)

Table AX5-10.6 (cont'd). Effect of Lead Exposure on Liver Heme Synthesis

Concentration	Duration	Species	Effects[a]	Reference
Lead acetate, 160 mg/L, semi liquid diet, oral	8 weeks	Male Wistar rats	Rats exposed to lead had a higher blood and liver lead, increased erythrocytic protoporphyrin. Lead exposure also resulted in hypoactivity of aminolevulinate dehydrase. Rats exposed to ethanol and lead had altered abnormalities in heme similar to animals exposed to lead alone. Hepatic levels of Zn decreased significantly only in animals exposed to both. Hepatic GSH, urinary ALA and porphyrin levels were maintained similarly in all the groups Transferrin bound iron uptake by Pb was also inhibited by lead at higher concentrations such as 4 μM.	Santos (1999)
Lead acetate, 0.0625 μM- 32 μM, in vitro	10 minutes pre incubation and 20 minute incubation	Rabbit reticulocytes	The effect of lead on ferrous iron transport is similar between lead chloride, acetate, and nitrate and reversible. Uptake of ferrous iron into all (heme, cytosolic and stromal fractions) was inhibited by low concentrations of lead. 50% inhibition in the uptake by cytosol occurred at 1 μM lead.	Qin and Morgan (1990)
1, 5, or 10 mg/kg b. wt. lead acetate or nitrate, i.p. 10^{-4} M Pb acetate for Hep G2 cells	3 days	A. Transgenic mice carrying chimeric human TF gene	These studies present evidence for the modulation of the synthesis of human transferrin by lead. In transgenic mouse with chimeric human chloromphenical acetyl transferase lead regulates human Transferrin (TF) transgenes at the m RNA level. Liver catalase (CAT) enzyme activity, CAT protein, and TF-CAT m-RNA levels were all suppressed. Lead did not alter other liver proteins, mouse TF and Albumin.	Adrian et al. (1993)
		B. Hep G2 cells	Pb suppressed synthesis of Transferrin protein in cultured human hepatoma Hep G_2 cells.	
10 mg lead/kg b. wt. as lead acetate , i.p., single injection 10 and 100 μM lead acetate	Analyses at multiple time points up to 72 h	Transgenic mice and Hep G 2 cells	Lead suppresses human transferrin synthesis by a mechanism different from acute phase response. Common proteins such as C3 and albumin associated with acute phase response were not altered by lead. Lead acetate suppresses ^{35}S - transferrin protein synthesis and m-RNA levels in Hep G2 cells and transgenic mice, while LPS altered only protein levels.	Huckins et al. (1997)

b. wt = body weight

Table AX5-10.7. Lead and In Vitro Cytotoxicity in Intestinal Cells

Compound and Concentration	Duration	Species	Effects[a]	Reference
$HgCl_2$, $CdCl_2$, Ti_2SO_4, $Pb(NO_3)_2$ – concentration not given clearly, Butathionine, up to 1 mM Glutathione 1 mM N- Acetyl cysteine, 1 mM	Cell proliferation assays 48 h Glutathione depletion assays 48 h Sulphahydryl repletion studies.	I-407, Intestinal epithelial cell line.	Rank order cytotoxicity of various metal salts in I-407 intestinal epithelial cells in terms of LC_{50} values- $HgCl_2$ (32 µM) > $CdCl_2$ (53 µM), $CuCl_2$ (156 µM) > Ti_2SO_4 (377 µM)>$Pb(NO_3)_2$ (1.99 mM) Role of Glutathione, in the cytotoxicity of these metals by the assessment of GSH depletion by Butathionine sulfoxamine pretreatment at non cytotoxic concentration increased the toxicity of $HgCl_2$ (5.7-fold), and $CuCl_2$ (1.44-fold). Administration of glutathione, with either $HgCl_2$ or $CdCl_2$ did not protect the cells against the toxicity. *N*-acetyl cysteine reduced the cytotoxicity of mercury.	Keogh et al. (1993)

Table AX5-10.8. Lead and Intestinal Uptake - Effect on Ultrastructure, Motility, Transport, and Miscellaneous

Compound and Concentration	Duration	Species	Effects[a]	Authors
Lead acetate, 0.1%, in drinking water.	Multiple analyses at 2, 30, and 60 days after lead exposure.	Male Wistar rats	Small intestinal goblet cells are involved in lead detoxification. Lead treatment for 30 days produces characteristic goblet cells in the intestine and lead appears in conjunction with goblet cell membrane. Prolonged exposure to lead more than 30 days caused silver sulphide deposition (indicative of heavy metal deposition) in the mucus droplets of cytoplasmic goblet cells.	Tomczok et al. (1988)
100 mg/lead acetate/kg. b. wt.	Multiple analyses at 2, 30 and 60 days	Male Wistar rats	Lead poisoning changes the ultra structure of intestine. 30 day lead exposed rat intestinal enterocytes showed numerous, small rough-membraned vesicles and prominent, dilated golgi complexes, in their cytoplasm. By 60th day, lead-exposed rats had a vacuolated cytoplasm and prominent golgi filled with vacuoles.	Tomczok et al. (1991)
Added lead concentration in the milk – 0-80 µg/ml	—	Adult & Infant rats (16 days) Fresh or frozen rat or Avian milk	90% of Pb in rat and bovine milk was found associated with caseine micelles regardless of whether the milk is labeled in vitro or in vivo with ^{203} Pb. Similarly lead in infant milk formula was also predominantly associated with casein, however, to a much lower extent than rat and bovine milk formulae. Lead tracer studies indicated that in infant rats, as the milk traversed through the intestine, in the collected luminal fluid, Pb was primarily associated with casein curd and remained as a non precipitable, non-dialyzable fraction as it moved to the small intestine, indicating that Pb remains with protein fraction as it traverses through the stomach and small intestine. fraction	Beach and Henning (1988)
Pb as lead acetate, for 0.5-10.0 µM, Zn as Zn acetate 0, 5, 10 or 50 µM Temperature variation Expts, 5 µM Pb, and incubated for 10 mints at 4, 22, or 37 °C	5, 10, 30 or 60 mints, Simultaneously with lead for 10 minutes Incubation time 10 minutes	IEC-6 normal rat intestinal epithelial cells.	Pb uptake by IEC-6 cells depends on the extracellular Pb concentration. Pb transport in IEC-6 cells is time and temperature dependent, involves sulphahydryl groups, and is decreased by the presence of Zn.	Dekaney et al. (1997)

Table AX5-10.8 (cont'd). Lead and Intestinal Uptake - Effect on Ultrastructure, Motility, Transport, and Miscellaneous

Compound and Concentration	Duration	Species	Effects[a]	Authors
OECD (Organisation for Economic Co-operation and Development) medium was artificially contaminated at 1, 3, 5, or 10 times the Dutch intervention value of 530 mg/Pb/kg dry wt. Lead containing medium was presented at the apical surface of the cells in 2 ml DMEM/chyme. Neutral red uptake studies had DMEM/chime with low 5µM and high 50µM lead content	Cell viability studies—24 h incubation. Lead transport studies, 1, 3, 5 and 24 h		Transport of bioaccessible lead across the intestinal epithelium—In Coco- 2 cells exposed to artificial chime, with in 24 hrs. App. 27% of the lead was associated with the cells and 3% were transported across the cell monolayer. Lead associated with cells showed a linear relationship with the lead available in the system. Results indicate that only a fraction of the bioavailable lead is transported across the intestinal epithelium. On the basis of lead speciation in chime, It could be attributed that dissociation of labile lead species, such as lead phosphate, and lead bile complexes and subsequent transport of the released free metal ions flow toward the intestinal membrane.	Oomen et al. (2003)
44 mg/kg/day lead as 53 mmol/L lead acetate	4 weeks	Rat	Lead exposure significantly decreases the amplitude of contraction in rat duodenum.	Karmakar and Anand (1989)
2.5 mg/ml lead acetate in drinking water	55 days	Colonic segments taken from chronically exposed guinea pigs	Colonic propulsive activity as measured by the velocity of the displacement of the balloon, from the oral to the aboral end, did not get affected significantly by lead treatment.	Rizzi et al (1989)
100 µM lead nitrate, in vitro	Duration not specified	Muscle – myenteric plexus preparations of distal ileum of controlled animals	Moderate decrease of electrically induced cholinergic contractions.	

Table AX5-10.8 (cont'd). Lead and Intestinal Uptake - Effect on Ultrastructure, Motility, Transport, and Miscellaneous

Compound and Concentration	Duration	Species	Effects[a]	Authors
40 µM-240 µM Tri ethyl lead added in a cumulative manner in vitro to mid-ileal portion.	7.5 sec – 2 minutes	Swiss mice JVI1 ileum	1. Peristaltic contractile activity of ileum as measured as a change in period duration and force amplitude indicated that tri ethyl lead (TEL) concentrations of < 40 µM had no obvious effects on these parameters. 2. In the concentration range between 40 µM -120 µM, tri ethyl lead affected the rhythm of contraction in a concentration dependent manner with elongation in period and reduction in force amplitude. 3. At concentrations above 120 µM, TEL induced irreversible dramatic changes in the ileal contractile activity.	Shraideh et al. (1999)

Table AX5-10.9. Lead, Calcium, and Vitamin D Interactions, and Intestinal Enzymes

Compound and Concentration	Duration	Species	Effects[a]	Authors
Ca – 0.5% in diet (low Calcium) 1.2% in diet (high calcium) Pb – 0.8% in the diet as Pb chloride.	10 days	White Leghorn Cockerels	Dietary lead affects intestinal Ca absorption in two different ways depending on the dietary Ca status. A. In chicks fed low Ca diet (0.05%), ingested lead inhibited intestinal ^{47}Ca absorption, intestinal Calbindin D, and alkaline phosphatase synthesis in a dose dependent fashion. B. In normal calcium diets (1.2%) lead exposure had no bearing on the intestinal Ca absorption, or Calbindin D, or Alkaline phosphatase synthesis and in fact elevated their levels at higher lead concentrations. These results indicate that the primary effects of lead in both cases, occur at or prior to intestinal protein synthesis involving Cholecalciferol endocrine system.	Fullmer and Rosen (1990)
Ca – 0.1% or 1.2% in the diet with Lead – 0.1 – 0.8% as Lead chloride in the diet	1 or 2 weeks	Leghorn Cockerels	- Dietary Ca deficiency, initially (1^{st} week) stimulates Ca absorption and Calbindin D levels regardless of dietary Pb intake. - At 2 weeks, this response is reversed by lead. - Intestinal lead absorption was enhanced by Ca deficiency initially and was inhibited by prolonged dietary lead intake. - Intestinal Pb absorption was increased in adequate Ca situation, but only after 2 weeks at the lower levels of dietary Pb.	Fullmer (1991)
Ca – 0.1-1.2% Pb – 0.8%	2 weeks	White Leghorn Cockerels	Interactions between dietary lead and Ca-influence on serum vitamin D levels. – Lead ingestion and Ca deficiency alone or in combination generally increased serum 1,25 (OH)2 D levels over the most of the range of dietary lead and Ca. – In severe Ca deficiency, Pb ingestion resulted in significant decreases in hormone concentration. – Similarities in response profiles for 1,25 (OH)2 D, intestinal Ca absorption and Calbindin- D suggested major interactions between lead and calcium mediated changes via circulating1,25(OH)2 D concentration.	Fullmer (1997)

Table AX5-10.9 (cont'd). Lead, Calcium, and Vitamin D Interactions, and Intestinal Enzymes

Compound and Concentration	Duration	Species	Effects[a]	Authors
Lead, Alkaline phosphatase and Ca^{2+} ATPase 2.0 – 10.0 mM Lead, Sucrase 0.5 mM – 6.0 mM Lead, γ-glutamyl transpeptidase1.0-10 mM Lead, Acetyl choline esterase 10.00-35.00 mM	Incubation time-not specified	Male Albino rats	Lead inhibited the activity of several intestinal brush border enzymes such as Ca^{2+}- ATPase, Sucrase, γ-glutamyl – transpeptidase and acetyl choline esterase with the exception of alkaline phosphatase. Inhibition of Ca^{2+}- ATPase was competitive and that of the other enzymes is by non-competitive means.	Gupta et al. (1994)
Oral lead in Similac or apple juice adjusted for attainment of blood lead levels 35 - 40 µg/dL. Succimer 30 mg/kg/day ^{204} Pb 24.5 nM followed by ^{206} Pb 352 nM, Single dose	Administered from 8th day post partum, until age 26 weeks. Two successive 19 days at age 53 weeks and 65 weeks. Administered immediately before chelation	Female infant Rhesus Monkeys	Effect of oral succimer chelation on the Gastro intestinal absorption and the whole body retention of lead— Radio isotope Pb tracer technique--- Succimer significantly reduced Gastro intestinal absorption of lead and increased urinary excretion of lead. The initial decrease in whole body lead by 10% was over come when majority of administered tracer was retained in the body after 5 days of treatment	Cremin et al. (2001)

CHAPTER 5 ANNEX

ANNEX TABLES AX5-11

Table AX5-11.1. Lead-binding Proteins

Source	Organ	Species	Molecular Weight	Protein Properties	Inducible	Separation Technique
Goyer (1968a)	Kidney	Rat		Intranuclear lead inclusion bodies	Yes	
Goyer (1970a,b)	Kidney	Rat		Lead is concentrated in the intranuclear inclusion body	Yes	
Choie and Richter (1972)	Kidney	Rat		Initial inclusion bodies in cytoplasm	Yes	
Moore et al. (1973)	Kidney	Rat		Protein in inclusion bodies is acidic, with high levels of aspartic a, glutamic a, glycine & cystine	Yes	
Moore & Goyer (1974)	Kidney	Rat	Inclusion body protein is 27.5 kDa		Yes	Acrylamide gel electrophoresis
Shelton and Egle (1982)	Kidney	Rat	Inclusion body is 32 kDa with pI of 6.3	Named p32/6.3	Yes	Two-dimensional gel electrophoresis
Egle and Shelton (1986)	Brain	Rat, mouse, dog, guinea pig, and chicken		p32/6.3 found	No (?)	
Oskarsson et al. (1982)	Kidney cytosol & brain	Rat	11.5 and 63 kDa		No	^{203}Pb binding followed by Sephadex G-75 or G-200 chromatography, then SDS-PAGE
Mistry et al. (1984)	Kidney cytosol	Rat	11.5 kDa, 63 kDa, > 200 kDa	Respective Kd values: 13, 40 123 nM	No	^{203}Pb binding followed by Sepharose-6B column chromatography
Fowler and DuVal (1991)	Kidney cytosol	Rat		Cleavage product of alpha-2 microglobulin	No	Chromatography followed by reverse phase HPLC, then production of antibodies. Kd 10-8 M

Table AX5-11.1 (cont'd). Lead-binding Proteins

Source	Organ	Species	Molecular Weight	Protein Properties	Inducible	Separation Technique
Smith et al. (1994, 1998)	Kidney cortex	Human	9 kDa and 5 kDa	ACBP and thymosin B4	No	Sephadex G-75 fractions < 30 kDa, Sephadex A-25, then HPLC
Goering et al. (1986)	Brain	Rat	12 kDa		No	Labeled (^{203}Pb) cytosol applied to Sephadex G-75
DuVal & Fowler (1989)	Brain	Rat	23 kDa	Glutamic a, aspartic a, cysteine. Not MT	No	Labeled cytosol applied to Sephadex G-75, DEAE, followed by SDS-PAGE
Fowler et al. (1993)	Kidney and brain	Monkey	Brain lead-binding protein larger than kidney	aspartic a, glutamic a, glycine, serine	No	Sephadex G-75 and DEAE
Quintanilla-Vega et al. (1995)	Brain	Human	5 kDa and 20 kDa	Thymosin B4 and unidentified protein	No	Sephadex G-75, A-25 DEAE, reversed phase HPLC
Raghavan & Gonick (1977)	RBC	Human lead-workers	10 kDa		Yes	^{210}Pb binding, Sephadex G-75, followed by SDS-PAGE
Raghavan et al. (1980)	RBC	Human lead-workers	10 kDa	Lead-binding protein absent in controls, low in symptomatic, high in asymptomatic	Yes	^{210}Pb binding, Sephadex G-75
Raghavan et al. (1981)	RBC	Human lead workers	10 kDa	Lead in membrane fraction correlates inversely with Na-K-ATPase	Yes	^{210}Pb binding, Sephadex G-75
Gonick et al. (1985)	RBC	Human lead workers	12 kDa, pI 5.3, and 30 kDa	Glycine, histidine, aspartic a, leucine	Yes	Sephadex G-75, HPLC, isoelectric focusing, SDS-PAGE
Ong and Lee, (1980)	RBC	Normal human	67 kDa	Thought to be hemoglobin	Yes	^{203}Pb binding, Sephadex G-75
Lolin and O'Gorman, (1988)	RBC	Human lead workers	Low molecular weight	Lead-binding protein correlates with restored ALAD	Yes	Sephadex G-75, lead measured by atomic absorption

Table AX5-11.1 (cont'd). Lead-binding Proteins

Source	Organ	Species	Molecular Weight	Protein Properties	Inducible	Separation Technique
Church et al. (1993a)	RBC	Human lead workers, one asymptomatic and one symptomatic	6-7 kDa	First pt had 67% of RBC Pb bound to protein. Second pt had 22% of RBC Pb in protein	Yes	RBC hemolysate filtered through Amicon YM 30 membrane. Superose 12 column. Lead quantitated by A.A.
Church et al. (1993b)	RBC	Human lead workers	5, 7 and 12 kDa, pI 4.7-4.9	30 % cysteine Thought to be MT on basis of greater UV abs at 254 nm than 280 nm	Yes	Superose 12, Amicon YM 30, Amicon YM 2, HPLC
Xie et al. (1998)	RBC	Human lead workers	240 ~ 260 kDa, < 30 kDa	High M.Wt. Peak identified as ALAD. Low M.Wt. peak seen after adding lead in vitro	Yes	Bio-gel A column. Pb determined by A.A.
Goering & Fowler, (1987a)	Kidney	Rat		Pre-treatment with zinc before injecting ^{203}Pb leads to zinc-thionein binding Pb	No	^{203}Pb binding, Sephadex G-75
Goering & Fowler, (1987b)	Kidney and liver	Rat		Pre-Rx with Zn or Cd induces Zn or Zn, Cd-MT. The MT decreases Pb inhibition of ALAD	No	^{203}Pb binding, Sephadex G-75
Qu et al. (2002); Waalkes et al. (2004)	Kidney	MT-null phenotypic mice		Pb-exposed MT-null developed no Pb inclusion bodies, accumulated less renal Pb than WT		

CHAPTER 6 ANNEX

ANNEX TABLES AX6-2

Table AX6-2.1. Analytical Methods for Determining Lead in Blood, Urine, and Hair

Sample Matrix	Preparation Method	Analytical Method	Sample Detection Limit	Accuracy (percent recovery)	Reference
Blood	Wet ashing with acid mixtures; residue dissolution in dilute $HClO_4$	ASV with mercury-graphite electrode (NIOSH Method 195)	40 µg/L	95–105	NIOSH (1977b)
Blood	Wet ashing with HNO_3; residue dissolution in dilute HNO_3	GFAAS (NIOSH Method 214)	100 µg/L	No data	NIOSH (1977e)
Blood	Dilution with Triton X-100®; addition of nitric acid and diammonium phosphate	GFAAS	2.4 µg/L	93–105	Aguilera de Benzo et al. (1989)
Blood	Dilution of sample with ammonium solution containing Triton X-100	ICP/MS	15 µg/L	96–111	Delves and Campbell (1988)
Blood	Dilution of sample in 0.2% Triton X-100 and water	GFAAS	≈15 µg/L	97–150	Que Hee et al. (1985)
Blood	Wet ashing, dilution	ICP-MS	0.1 ppb	94–100	Zhang et al. (1997)
		GFAAS	4 ppb	90–108	
Blood and urine	Mixing of urine sample with HNO_3; filtration, chelation of lead in whole blood or filtered urine with APDC, extraction with MIBK	AAS (NIOSH Method 8003)	0.05 µg/g (blood); 50 µg/L (urine)	99 (±10.8%)	NIOSH (1994)
Blood and urine	Wet ashing of sample with HNO_3, complexation with diphenylthio-carbazone, and extraction with chloroform	Spectrophotometry (NIOSH Method 102)	30 µg/L (blood); 12 µg/L (urine)	97 97	NIOSH (1977a)
Blood and urine	^{206}Pb addition and sample acid digestion; lead copre-cipitation by addition of $Ba(NO_3)_2$, followed by electrodeposition on platinum wire	IDMS	No data	98–99	Manton and Cook (1984)
Blood and tissue	Digestion of sample with $HNO_3/HClO_4/H_2SO_4$; heat	ICP-AES (Method 8005)	0.01 µg/g (blood); 0.2 µg/g (tissue)	113	NIOSH (1984)

Table AX6-2.1 (cont'd). Analytical Methods for Determining Lead in Blood, Urine, and Hair

Sample Matrix	Preparation Method	Analytical Method	Sample Detection Limit	Accuracy (percent recovery)	Reference
Blood	Addition of 50 µL of blood into reagent, mixing, and transferring to sensor strip (commercial test kit)	Gold electrode sensor	1.4 µg/dL	No data	ESA (1998)
Urine	Collect 50 mL urine sample and add 5 mL concentrated HNO_3 as preservative; filter samples through cellulose membrane, adjust pH to 8, ash filters and resins in low temperature oxygen plasma for 6 hours	ICP-AES (Method 8310)	5 µg/L	100	NIOSH (1994)
Serum, blood, and urine	Filtration of sample if needed; blood requires digestion in a Parr bomb; dilution of serum or urine with acid or water	ICP-AES	10–50 µg/L	85 (serum) >80 (urine, blood)	Que Hee and Boyle (1988)
Urine	Wet ashing of sample with acid mixture and dissolution in dilute $HClO_4$	ASV with mercury-graphite electrode (Method 200)	4 µg/L	90–110	NIOSH (1977c)
Hair	Cleaning of sample with acetone/ methanol; digestion with acid mixture and heat; diammonium phosphate addition as matrix modifier	GFAAS	0.16 µg/g	99	Wilhelm et al. (1989)
Hair	Cleaning with lauryl sulfate and water; digestion with heated nitric acid	ICP-AES	1 µg/g	No data	DiPietro et al. (1989)
Hair	Cleaning with water; digestion with heated nitric acid and H_2O_2	ET-AAS	<0.026 µg/g	>90	Drash et al. (1997)
Hair	Cleaning with acetone/water	XRF	0.5 µg/g	No data	Gerhardsson et al. (1995a)

AAS, atomic absorption spectroscopy; APDC, ammonium pyrrolidine dithiocarbamate; ASV, anode stripping voltammetry; $Ba(NO_3)_2$, barium nitrate; ET-AAS, electro-thermal atomic absorption spectrometry; GFAAS, graphite furnace atomic absorption spectroscopy; H_2O_2, hydrogen peroxide; H_2SO_4, sulfuric acid; $HClO_4$, perchloric acid; HNO_3, nitric acid; ICP-AES, inductively coupled plasma/atomic emission spectroscopy; ICP-MS, inductively coupled plasma-mass spectrometry; IDMS, isotope dilution mass spectrometry; MIBK, methyl isobutyl ketone; NIOSH, National Institute for Occupational Safety and Health; ^{206}Pb, lead 206; XRF, X-ray fluorescence.

Table AX6-2.2. Summary of Selected Measurements of Blood Lead Levels in Humans

Reference, Study Location, and Period	Study Description	Blood Lead Measurement	Comment
United States			
CDC (2005) U.S. 1999-2002	Design: national survey (NHANES IV) stratified, multistage probability cluster design Subjects: children and adults (≥1 yrs, n = 16, 915) in general population Biomarker measured: blood lead Analysis: ICP-MS	Units: µg/dL Geometric mean (95% CI) **Age (yr) 1999-2000 2001-2002** 1-5: 1.66 (1.60, 1.72) 1.45 (1.39, 1.40) n: 7, 970 8, 945 6-11: 1.51 (1.36, 1.66) 1.25 (1.14, 1.36) n: 905 1,044 12-19: 1.10 (1.04, 1.17) 0.94 (0.90, 0.99) n: 2, 135 2, 231 ≥20: 1.75 (1.68, 1.81) 1.56 (1.49, 1.62) n: 4, 207 4, 772 Males: 2.01 (1.93, 2.09) 1.78 (1.71, 1.86) n: 3, 913 4, 339 Females: 1.37 (1.32, 1.43) 1.19 (1.14, 1.25) n: 4,057 4, 606	Data from NHANES IV Phase 1 (1999-2000) and 2 (2001-2002).
Brody et al. (1994) Pirckle et al. (1998) U.S. 1988-1994	Design: national survey (NHANES III) stratified, multistage probability cluster design. Subjects: children and adults (≥1 yrs, n = 29, 843) in general population Biomarker measured: blood lead Analysis: GFAAS	Units: µg/dL Geometric mean (95% CI) **Age (yr) 1988-1991 1991-1994** 1-5: 3.6 (3.3, 4.0) 2.7 (2.5, 3.0) n: 2, 234 2, 392 6-11: 2.5 (2.2, 2.7) 1.9 (1.8, 2.1) n: 1, 587 1, 345 12-19: 1.6 (1.4, 1.9) 1.5 (1.4, 1.7) n: 1, 376 1, 615 20-49: 2.6 (2.5, 2.8) 2.1 (2.0, 2.2) n: 4, 320 4, 716 50-69 4.0 (3.8, 4.2) 3.1 (2.9, 3.2) n: 2,071 2,026 ≥70 4.0 (3.7, 4.3) 3.4 (3.3, 3.6) n: 1, 613 1, 548 *Males:* 3.7 (3.5, 3.9) 2.8 (2.6, 2.9) n: 6,051 6, 258 *Females:* 2.1 (2.0, 2.2) 1.9 (1.8, 2.) n: 6,068 7, 384	Comparison of data from NHANES III Phase 1 (1988-1991) and Phase 2 (1991-1994) indicated declining blood lead concentrations in children.

Table AX6-2.2 (cont'd). Summary of Selected Measurements of Blood Lead Levels in Humans

Reference, Study Location, and Period	Study Description	Blood Lead Measurement	Comment
United States (cont'd)			
Nash et al. (2003) U.S. 1988-1994	Design: national survey (NHANES III) stratified, multistage probability cluster design Subjects: women(n = 2, 575), age range: 40-59 yrs, in general population Biomarker measured: blood lead Analysis: GFAAS	Units: µg/dL Geometric mean (95% CI, n) Premenopausal: 1.9 (1.7, 2.0, 1, 222) Surgically menopausal: 2.7 (2.4, 3.2, 139) Naturally menopausal: 2.9 (2.5, 3.2, 653)	Geometric mean blood lead concentrations were significantly lower in premenopausal women. Increasing blood lead concentrations were significantly associated with decreased bone mineral density.
Pirckle et al. (1994) U.S. 1976-1980	Design: national survey (NHANES II, III) stratified, multistage probability cluster design Subjects: children and adults (≥1 yrs, n = 29, 843) in general population Biomarker measured: blood lead Analysis: GFAAS	Units: µg/dL Geometric mean (95% CI) **Age (yr) / 1976-1980 / 1988-1991** 1-5: 15.0 (14.2, 15.8) / 3.6 (3.3, 4.0) n: 2, 271 / 2, 234 6-19: 11.7 (11.2, 12.4) / 1.9 (1.7, 2.2) n: 2,024 / 2, 963 20-74: 13.1 (12.7, 13.7) / 3.0 (2.8, 3.2) n: 5, 537 / 6, 922 *Males:* 15.0 (14.5, 15.5) / 3.7 (3.5, 3.9) n: 4, 895 / 6,051 *Females:* 11.1 (10.6, 11.5) / 2.1 (2.0, 2.2) n: 4, 937 / 6,068	Comparison of data from NHANES II (1976-1980) and Phase 1 of NHANES III (1988-1991) indicated declining blood lead concentrations in U.S. population.
Symanski and Hertz-Picciotto (1995) U.S. 1982-1984	Design: national survey (HHANES) multistage-area probability sample Subjects: adults, females (n = 3, 137), age range 20-60 yrs, in general Hispanic population Biomarker measured: blood lead Analysis: GFAAS	Units: µg/dL Arithmetic mean (SE, n) All Premenopausal: 7.5 (0.07, 1, 984) Menopausal: 8.9 (0.11, 1, 152) Mexican-American: Premenopausal: 7.2 (0.13, 1, 219) Menopausal: 8.4 (0.20, 624)	Mean difference between premenopausal and postmenopausal (≤4 yrs) was 1.4 µg/dL (95% CI: 0.20, 2.7).

Table AX6-2.2 (cont'd). Summary of Selected Measurements of Blood Lead Levels in Humans

Reference, Study Location, and Period	Study Description	Blood Lead Measurement					Comments
United States (cont'd)							
Yassin et al. (2004) U.S. 1988-1994	Design: national survey (NHANES III) stratified, multistage probability cluster design Subjects: adults (n = 11, 126) in general population, age range: 18-64 yr), stratified by occupational category Biomarker measured: blood lead Analysis: GFAAS	Units: µg/dL Occupation Vehicle mechanics Food service workers Management, professional technical, and sales workers Personal service workers Agricultural workers Production workers: machine operators, material movers, etc. Laborers other than in construction Transportation workers Mechanics other than vehicle mechanics Construction trades people Construction laborers Health service workers All	GM 4.80 2.00 2.13 2.48 2.76 2.88 3.47 3.49 3.50 3.66 4.44 1.76 2.42	GSD 3.88 2.69 4.05 4.52 4.02 4.24 3.36 5.19 4.91 4.64 7.84 2.24 6.93	Maximum 28.1 27.0 39.4 25.9 23.4 52.9 21.8 22.3 16.6 16.9 36.0 22.4 52.9	n 169 700 4, 768 1, 130 498 1, 876 137 530 227 470 122 499 11, 126	
Mexico							
Hernandez-Avila et al. (2002) Mexico 1993-1995	Design: cross-sectional Subjects: adults females (n = 903) in general population, age range: 36-70 yr) Biomarker measured: blood lead Analysis: GFAAS	Units: µg/dL Arithmetic mean (SD, n) Premenopausal: 10.63 (5.46, 463) Menopausal: 11.39 (2.65, 437) Surgically menopausal: 10.23 (4.92, 115) Naturally menopausal: 11.30 (5.88, 322)					Mean difference between premenopausal and menopausal; was 0.76 µg/dL (95% CI: 0.224, 1.48).

GFAAS, graphite furnace atomic absorption spectroscopy; ICP-MS, inductively coupled plasma-mass spectrometry; NR, not reported.

Table AX6-2.3. Bone Lead Measurements in Cadavers

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States			
Wittmers et al. (1988) Minnesota 1976-82	Lead in tibia, skull, iliac crest, rib, and vertebrae. 81 Caucasian males and 53 male cadavers ranging in age from 0 to 98 yr. Ashing, nitric acid, AAS.	Mean and SEM (μg/g bone ash) >75 yr: Tibia 29.0 ±3.4 (n = 28), ilium 17.0 ± 2.6 (n = 29), rib 20.5 ± 2.4 (n = 31), vertebra 18.8 ± 2.6 (n = 30), skull 26.1 ± 3.2 (n = 28) 51-75 yr: Tibia 24.2 ± 2.3 (n = 38), ilium 19.2 ± 2.4 (n = 15), rib 22.3 ± 2.6 (n = 40), vertebra 22.4 ± 2.6 (n = 41), skull 22.8 ± 2.9 (n = 29) 36-50 yr: Tibia 16.6 ±4.1 (n = 14), ilium 9.9 ± 1.6 (n = 15), rib 9.7 ± 1.7 (n = 15), vertebra 11.9 ± 2.1 (n = 15), skull 15.2 ± 3.3 (n = 15) 21-35 yr: Tibia 5.9 ±1.2 (n = 18), ilium 5.3 ± 1.2 (n = 16), rib 5.0 ± 1.2 (n = 18), vertebra 6.3 ± 1.3 (n = 17), skull 4.9 ± 1.1 (n = 17) 14-20 yr: Tibia 2.3 ±1.0 (n = 13), ilium 2.3 ± 0.9 (n = 13), rib 2.9 ± 1.4 (n = 12), vertebra 3.8 ± 1.4 (n = 12), skull 3.2 ± 1.7 (n = 10) 0-2 yr: Tibia 0.3 ±0.2 (n = 11), ilium 0.0 ± 0.0 (n = 11), rib 0.7 ± 0.4 (n = 12), vertebra 0.6 ± 0.6 (n = 12), skull 0.6 ± 0.4 (n = 12)	Ratio of lead in tibia and skull/iliac/rib/vertebrae <1 from age 0 to 35 yrs then >1 from 36 to 75 yrs and greater than 75 yrs. Evidence of differential distribution amongst bones with age; the earliest difference is apparent during adolescence when trabecular bone of the vertebral body accumulates significantly more lead than that of the other 4 sites.
Saltzman et al. (1990) Cincinnati, OH 1970-71	29 tissues from 55 cadavers, mean age 50 yrs. Muffle furnace ashing. Pb concentrations by dithazone method.	Higher concentrations of Pb in tibia compared with rib and vertebrae and higher values for males compared with females. Males (n = 46): Ribs 6.70 ± 3.96 (μg/g, wet weight), tibia 12.55 ± 10.65, vertebrae 4.12 ± 2.49. Females (n = 8): Ribs 3.17 ± 0.91 (μg/g, wet weight), tibia 4.54 ± 2.04, vertebrae 2.01 ± 0.72.	Bone Pb increased with age. Results were similar to those of Barry (1978) and Wittmers et al. (1988).
Canada			
Samuels et al. (1989) Canada 1965-69	Ashed vertebral bones from male and female cadavers from three Canadian cities. AAS method.	Changes for different age ranges in Pb concentration for the period 1965 - 1969: 0-11 months: 3.98 μg/g (n = 28) 1-4 yrs: 10.02 μg/g (n = 32) 5-11 yrs: 12.91 μg/g (n = 26) 12-19 yrs 7.11 μg/g (n = 26) ≥20 yrs: 14.77 μg/g (n = 25)	For period 1965 to 1969 levels vary over age groups (p = 0.0001) but there was little gender difference. For the period 1980 to 1998 for Winnipeg, values were approximately half to one third those prevailing earlier.

Table AX6-2.3 (cont'd). Bone Lead Measurements in Cadavers

<table>
<tr><th>Reference, Study Location, and Period</th><th>Study Description</th><th>Lead Measurement</th><th>Findings, Interpretation</th></tr>
<tr><td>Europe</td><td></td><td></td><td></td></tr>
<tr><td>Drasch et al. (1987)
Germany
1983-85</td><td>Bone Pb in temporal bone, cortical part of the mid-femur, and pelvic bone from 120 female and 120 male adult cadavers. AAS.</td><td>Geometric means:
Males: Pelvic 1.95 ± 1.00 (µg/g, wet weight), mid-femur 4.75 ± 2.53, temporal 6.24 ± 3.17.
Females: Pelvic 1.41 ± 0.74 (µg/g), mid-femur 3.14 ± 1.89, temporal 5.00 ± 2.66.</td><td>Found cortical lead > trabecular lead. Limited difference in Pb for younger males and females; much higher Pb in bones of men >50 yr old compared with women</td></tr>
<tr><td>Drasch and Ott (1988)
Germany
1984</td><td>Bone Pb in temporal bone, cortical part of the mid femur, and pelvic bone from 82 child cadavers. Nitric acid digestion, AAS.</td><td><table>
<tr><th>Age</th><th colspan="2">0-1 yrs</th><th colspan="2">1-6 yrs</th><th colspan="2">10-20 yrs</th><th colspan="2">0-20 yrs</th></tr>
<tr><td>Sex</td><td>Male</td><td>Female</td><td>Male</td><td>Female</td><td>Male</td><td>Female</td><td>Male</td><td>Female</td></tr>
<tr><td>n</td><td>9</td><td>16</td><td>9</td><td>9</td><td>18</td><td>16</td><td>39</td><td>42</td></tr>
<tr><td>Temporal</td><td>0.331</td><td>0.334</td><td>0.530</td><td>0.732</td><td>1.770</td><td>1.740</td><td>0.858</td><td>0.749</td></tr>
<tr><td>Pelvic bone</td><td>0.230</td><td>0.278</td><td>0.461</td><td>0.522</td><td>0.748</td><td>0.511</td><td>0.455</td><td>0.404</td></tr>
<tr><td>Mid-femur</td><td>0.333</td><td>0.327</td><td>0.642</td><td>0.858</td><td>1.342</td><td>1.010</td><td>0.768</td><td>0.632</td></tr>
</table>(values in µg/g wet weight)</td><td>Negligible difference for 0 to 1 yr olds, for pre-school children (1-6 yrs) and for 10 to 20 yr olds; mean values for cortical bones showed higher Pb concentrations than trabecular bone; mean Pb in the mid femur and temporal bone was not statistically different for each of three age groups.</td></tr>
<tr><td>Hac et al. (1997)
Poland</td><td>Pb in rib bone and hair from 59 cadavers, aged 1-87 yrs. Perchloric acid digestion, AAS.</td><td>Bone Pb 3.0 (±1.5) µg/g (n = 54).</td><td>Small increases to age 50 yrs in rib bone. Number of samples for each age group not stated.</td></tr>
<tr><td>Asia</td><td></td><td></td><td></td></tr>
<tr><td>Noda et al. (1993)
Japan
1976, 1981, and 1986</td><td>76 cadavers, age range 0 to 83 yrs.</td><td>Age 0 yrs (1.25 µg/g wet weight) to 59 yrs (4.5 µg/g) after which there was a decrease (approximately 2.5 µg/g). For the age range 10-49 yrs, there was no significant difference in mean values of 2.8 to 3.1 µg/g.</td><td>Found no significant gender difference but levels in 1986 were significantly lower than in 1976.</td></tr>
</table>

Table AX6-2.4. Bone Lead Measurements in Environmentally-Exposed Subjects

Reference, Study Location, and Period	Study Description	Lead Measurement (SD or range) PbB in µg/dL, Bone Pb in µg/g Bone Mineral	Findings, Interpretation
United States			
Kim et al. (1996) Boston, MA 1989-90	Examination of the relationship between tooth Pb in children and bone Pb levels in young adults. Members of a cohort of young adults (n = 63, ~20 yr of age) were reassessed 13 yr after initial examination. Dentine Pb by anodic stripping voltammetry. Bone K-shell XRF. LOWESS smoothing, multiple linear regression.	No PbB. Tibia Pb 1.3 (± 4.4), patella Pb 5.4 (±8.4). Dentine Pb 13.4 (±10.7). Approximately one-third of tibia and one-fourth of patella estimates were negative values.	A 10 µg/g increase in dentine Pb levels in childhood was predictive of a 1 µg/g increase in tibia Pb levels and a 5 µg/g increase in patella PbB levels, and a 3 µg/g increase in mean bone Pb levels among the young adults. They concluded that Pb exposure in early life may be used to predict elevated body burden up to 13 yr later.
Hu et al. (1990) Boston, MA Unknown	To evaluate if K-shell XRF can be used to assess low-level Pb burdens in 34 employees (26 males, 8 females) ranging in age from 21 to 58 yr of a biomedical company with no known history of excessive Pb exposure. Medical environmental history questionnaire. Multiple linear regression.	18 (53%) of subjects had bone Pb levels included 0 or less within the estimate of uncertainty. Highest bone Pb 21 ± 4 µg/g bone mineral. For 16 young adults, age and year of home construction had a positive but statistically insignificant effect ($p > 0.05$) on bone Pb.	K-shell XRF may be useful for assessing low-level Pb burdens in epidemiological studies.
Hu et al. (1996) Boston, MA 1991+	Normative Aging Study. Subjects were middle-aged and elderly men who had community (nonoccupational) exposures to lead. Cross-sectional. Backwards elimination multivariate regression models that considered age, race, education, retirement status, measures of both current and cumulative smoking, and alcohol consumption.	47-59 yrs (n = 116): PbB 5.8 (±3.7), tibia 14.6 (±8.3), patella 23.6 (±12.4) 60-69 yrs (n = 360): PbB 6.3 (±4.2), tibia 21.1 (±11.4), patella 30.5 (±16.9) >70 yrs (n = 243): PbB 6.5 (±4.5), tibia 27 (±15.6), patella 38.8 (±23.5)	Factors that remained significantly related to higher levels of both tibia and patella Pb were higher age and measures of cumulative smoking, and lower levels of education. An increase in patella Pb from the median of the lowest to the median of the highest quintiles (13-56 µg/g) corresponded to a rise in PbB of 4.3 µg/dL. Bone Pb levels comprised the major source of circulating lead in these men.
Campbell et al. (2004) New York Unknown	Investigated the relationship between bone mineral density and environmental Pb exposure in 35 African American children.	High Pb exposure: PbB levels (mean 23.6 µg/dL; n = 19); low Pb exposure (mean 6.5 µg/dL; n = 16).	Unexpectedly, they found that children with high Pb exposure had a significantly higher bone mineral density than children with low Pb exposure. They hypothesized that this arises from the effect of Pb on accelerating bone maturation by inhibition of parathyroid hormone-related peptide.

Table AX6-2.4 (cont'd). Bone Lead Measurements in Environmentally-Exposed Subjects

Reference, Study Location, and Period	Study Description	Lead Measurement (SD or range) PbB in µg/dL, Bone Pb in µg/g Bone Mineral	Findings, Interpretation
United States (cont'd)			
Rosen et al. (1989) Bronx, NY Unknown	Comparison of L-shell XRF measures and EDTA provocation test in lead-toxic children 1-6 yr old. Eligible if PbB 25-55 µg/dL and erythrocyte protoporphyrin >35 µg/dL.	Negative EDTA test results (n = 30): PbB 30 ± 5 µg/dL, tibia Pb 12 ± 2 µg/g (range 7-52). Positive EDTA test results (n = 29): PbB 39 ± 8 µg/dL, tibia Pb 37 ± 3 µg/g (range 7-200).	From PbB and LXRF alone, 90% of Pb-toxic children were correctly classified as being EDTA-positive or -negative. LXRF may be capable of replacing EDTA testing.
Kosnett et al. (1994) Dickson City, PA 1991	Aim to determine the influence of demographic, exposure and medical factors on the bone Pb concentration of subjects with environmental Pb exposure. 101 subjects (49 males, 52 females; aged 11 to 78 yrs) recruited from 49 of 123 households geographically located in a suburban residential neighborhood. Tibia. Multiple regression.	Mean (SD) bone Pb12.7 (14.6). Log-transformed bone Pb highly correlated with age ($r = 0.71$; $p \leq 0.0001$). Gender differences in log-transformed bone Pb values were insignificant up until the 6th decade.	Bone Pb showed no significant change up to age 20 yr, increased with the same slope in men and women between ages 20 and 55 yrs, and then increased at a faster rate in men older than 55 yrs.
Rosen et al. (1993) Moosic and Throop, PA 1989-91	Suburban population (Throop, n = 269) exposed to unusually high emissions during 1963-81 from nearby battery recycling/secondary smelter. Moosic served as control community. ~9% children aged 5-12 yr, 15% 13-17 yr, 40% ≥ 18 yr. Soil and PbB, L-shell XRF.	No significant differences in tibia Pb found among three age groups in Moosic or Throop. Mooaic: means 5-12 yr, 6 µg/g; 13-17 yr, 8 µg/g; ≥18 yr, 7 µg/g Throop: means 5-12 yr, 12 ± 1 µg/g; 13-17 yr, 15 ± 2 µg/g; ≥18 yr, 12 ± 1 µg/g.	No change in bone Pb with age. Baseline values for bone Pb in the environmentally exposed population of Moosic can serve as a reference baseline for contemporary bone Pb levels in similar communities in the USA.
Stokes et al. (1998) Bunker Hill, ID; Spokane, WA 1994	Examined whether environmental exposure to Pb during childhood was associated with current adverse neurobehavioral effects. K-shell XRF. Formerly exposed as children 19-30 yr (n= 238, age 19-30 yr). Referents (n = 258)	Exposed group: PbB 2.9 µg/dL; tibia Pb 4.6 µg/g. Referent group: PbB 1.6 µg/dL; tibia Pb 0.6 µg/g.	

Table AX6-2.4 (cont'd). Bone Lead Measurements in Environmentally-Exposed Subjects

Reference, Study Location, and Period	Study Description	Lead Measurement (SD or range) PbB in µg/dL, Bone Pb in µg/g Bone Mineral	Findings, Interpretation
United States (cont'd)			
McNeill et al. (2000) Idaho and Washington 1994	To determine if high Pb exposure in childhood persisted until adulthood. 262 exposed subjects and 268 age and sex matched controls aged 19 – 29 yr. Tibia bone Pb, cumulative PbB index. Inverse weighted group mean data, linear regressions.	Group inverse weighted mean (SEM). Males: Exposed 4.54 (0.31); controls 0.03 (0.31) µg Pb/g bone mineral. Females: Exposed 5.61 (0.43); controls 1.67 (0.43) µg Pb/g bone mineral.	Lead from exposure in early childhood had persisted in the bone matrix until adulthood. Bone Pb significantly correlated with age for exposed groups. No significant correlation in regressions for control groups with age. Exposed subjects had group bone Pb levels significantly higher ($p < 0.005$) than control subjects in 7 of 11 age groups. Exposed subjects had increased current PbB concentrations that correlated significantly with bone Pb values. Incorporation rate of Pb into bone 0.039 (0.003) (μg Pb/g bone mineral)/ µg/dL yr).
Mexico			
Farias et al. (1998) Mexico City and suburbs 1995-96	Examined the relation of blood and tibia bone Pb levels to Pb determinants in 100 adolescents aged 11 to 21 yr. LOWESS smoothing, multivariate regressions.	Females (n = 62): PbB 6.4 (±3.2), tibia 5.5(±8.6). Males (n = 36): PbB 9.1 (±5.5), tibia 3.8 (±5.5). 25 subjects had bone Pb < 0. Bone Pb accounted for 4.1% of variation in PbB. Increase in bone Pb of 21.6 µg/g was associated with an increase in PbB of 1.2 µg/dL.	Predictors of bone Pb included higher traffic density near the home, mother's smoking history, and time spent outdoors. Predictors of log-transformed PbB included bone Pb levels, male sex, use of Pb-glazed ceramics, and living in Mexico City. Bone Pb accumulated over time constitutes a moderate source of circulating Pb during adolescence

Table AX6-2.5. Bone Lead Measurements in Occupationally-Exposed Subjects

Reference, Study Location, and Period	Study Description	Lead Measurement (SD or range) PbB in µg/dL, Bone Pb in µg/g Bone Mineral	Findings, Interpretation
United States			
Hu et al. (1994) U.S. 1991	Construction workers aged 23 to 67 yr (n = 19). Examination of Bone Pb and PbB as predictors of blood pressure in construction workers. Multivariate linear regression, LOWESS smoothing.	PbB 8.3 (±4.0), tibia Pb 9.8 (±9.5), patella Pb 13.9 (±13.6).	
Schwartz et al. (2000) U.S. 1995	Retired organolead employees (n = 543). Aim to determine influence of PbB, chelatable Pb, and tibial Pb on systolic and diastolic blood pressure.	PbB 4.6 (±2.6), tibia Pb14.4 (±9.3).	Tibia Pb was not associated with any blood pressure measures.
Popovich et al. (2005) Idaho	108 former female smelter employees and 99 referents to assess the PbB versus bone Pb relationship.	Exposed: PbB 2.73 (±2.39), tibia 14.4 (±0.5) Referents: PbB 1.25 (±2.10), tibia 3.22 (±0.50) Pb concentrations in tibia and blood significantly higher in the exposed group. Endogenous release rate (µg Pb per dL blood/µg Pb/g bone) in postmenopausal women was double the rate found in premenopausal women (0.132 ± 0.019 vs. 0.067 ± 0.014).	Higher tibia bone Pb (and PbB) was associated with use of estrogen (present or former) in both the whole referent group and postmenopausal women in the referent group.
Canada			
Fleming et al. (1997) Canada 1994	Primary smelter workers, 367 active and 14 retired. PbB in 204 workers returning after a 10-mo strike ended in 1991. Cumulative PbB index, K-shell measures with ^{109}Cd source.	Active (1975-81) median PbB 16.0, (1987-92) median PbB 8.0, tibia range 0-150, calcaneus 0-250. Retired tibia range 20-120, calcaneus 40-220. Bone Pb-cumulative PbB index slopes larger for retired compared with active workers, but not significant.	Nonlinearities in cumulative PbB index and tibia and calcaneus Pb suggest differences in Pb transfer from whole blood to bone among smelter employees. Contribution to PbB from bone stores at any instant in time is similar for all occupationally exposed populations, active or retired. Age-related variations in bone turnover are not a dominant factor in endogenous exposure of male lead workers. More rapid absorption of Pb in calcaneus than tibia.

Table AX6-2.5 (cont'd). Bone Lead Measurements in Occupationally-Exposed Subjects

Reference, Study Location, and Period	Study Description	Lead Measurement (SD or range) PbB in µg/dL, Bone Pb in µg/g Bone Mineral	Findings, Interpretation
Canada (cont'd)			
Fleming et al. (1998) Canada 1994	Primary smelter. ALAD 1-1 (n = 303) and ALAD1-2, 2-2 (n = 65). PbB, serum Pb, cumulative PbB index, ALAD genotype, K-shell measures with ^{109}Cd source.	1-1: PbB 22.9, tibia 41.2, calcaneus 71.6 1-2, 2-2: PbB 25.2, tibia 42.7, calcaneus 72.3. Slopes of linear relations of bone Pb to cumulative PbB index were greater for workers homoallelic for ALAD 1, indicating more efficient uptake of lead from blood into bone; effect most significant in calcaneus bone and for workers hired since improved safety measures enacted in 1977 [ALAD1-1: 0.0528 ± 0.0028 and ALAD1-2 or 2-2: 0.0355 ± 0.0031 ($p < 0.001$)].	Decreased transfer of blood lead into bone in individuals expressing the ALAD2 allele contrasted with increased blood lead. ALAD genotype affected lead metabolism and potentially modified lead delivery to target organs including the brain but ALAD genotype did not significantly affect the net accumulation of lead in bone.
Brito et al. (2000) Canada 1993-98	Aims were to: (i) investigate the long term human Pb metabolism by measuring the change of Pb concentration in the tibia and calcaneus between 1993 and 1998; and (ii) assess whether improved industrial hygiene was resulting in a slow accumulation of Pb in an exposed workforce. 101 workers in a secondary lead smelter, 51 subjects had similar bone Pb measurements in 1993. Most other subjects had been hired since 1993. Cumulative PbB index. Linear regressions.	Repeats (n = 51) 1993: Tibia 39 (±19), calcaneus 64 (±36). 1998: Tibia 33 (±18), calcaneus 65 (±38). Non-repeats (n = 50) 1998: Tibia 15 (±16), calcaneus 13 (±18). Tibia Pb decreased significantly ($p < 0.001$) in the 51 subjects with repeated bone Pb measurements. Tibia Pb in 1993 and changes in cumulative PbB index were significant predictors of changes in tibia Pb. An overall half-life of 15 yr (95% CI: 9, 55 yr) was estimated. Adding continuing lead exposure and recirculation of bone lead stores to the regression models produced half-life estimates of 12 and 9 yr, respectively, for release of lead from the tibia. Repeat subjects showed no net change in calcaneus Pb after 5 yr.	The decrease in new exposure coupled to release of previously stored bone Pb resulted in a significant decrease in tibia Pb in the repeat subjects. The rate of clearance of Pb from the tibia of 9 to 15 yr is towards the more rapid end of previous estimates. The lack of a significant change in the calcaneus Pb was surprising and if confirmed would have implications for models of Pb metabolism.

Table AX6-2.5 (cont'd). Bone Lead Measurements in Occupationally-Exposed Subjects

Reference, Study Location, and Period	Study Description	Lead Measurement (SD or range) PbB in µg/dL, Bone Pb in µg/g Bone Mineral	Findings, Interpretation
Canada (cont'd)			
Brito et al. (2002) Canada 1994, 1999	Evaluated endogenous release of Pb from bone to blood in 204 exposed subjects resuming their duties after a 10-mo strike in a primary lead smelter in 1991. Bone Pb (^{109}Cd source) measured in the tibia and calcaneus in 1994 (Fleming et al., 1997) and 1999. A linear model used to predict the current PbB upon the level of lead in bone. 327 subjects available on both occasions. Group H higher PbB and Group L lower PbB.	Group H: PbB 22.0, tibia 19.2 (n = 120) Group L: PbB 20.6, tibia 82.8 (n = 45) Group H: PbB 24.2, calcaneus 41.4 (n = 90) Group L: PbB 20.2,calcaneus 138.2 (n = 45) Structural analysis of data gave slopes for tibia (2.0, 95% CI: 1.66-2.54) and calcaneus (0.19, 95% CI: 0.16-0.23) that were significantly higher than those predicted by the commonly used simple linear regression method, for tibia (0.73, 95%, CI: 0.58-0.88) and calcaneus (0.08, 95% CI: 0.06-0.09).	Suggested that more Pb than previously predicted by regression analysis is released from bone to blood.
Europe			
Somervaille et al. (1988) England	K-shell measures with ^{109}Cd source on diverse Pb workers and controls Crystal glass (n = 87); Battery plant (n = 88); Precious metals (n = 15); Laboratory (n = 20). Cumulative PbB index.	Crystal glass: PbB 48.1, tibia 31.0 Battery plant: PbB 32.3, tibia 32.3 Precious metals: PbB 51.4, tibia 54.8 Laboratory: PbB 13.1, tibia 16.7	Correlation coefficients between tibia lead and duration of employment were consistently higher at all three factories respectively ($r = 0.86$, $p < 0.0001$; $r = 0.61$, $p < 0.0001$; $r = 0.80$, $p < 0.0001$). Strong relation between tibia Pb and cumulative PbB index among workers in factories from which PbB histories were available.
Christoffersson et al. (1984) Sweden Unknown	Lead smelter employees Active (n = 75); Former plant (n = 32) Finger bone measurement with ^{57}Co source.	Active: median PbB 53.8 (15.5), mean tibia 43 (<20, 122) Former: median PbB 24.9 (7.0), mean tibia 59.0 (<20, 135)	Increase of bone Pb with time of employment, no association between bone Pb and current PbB in active workers, in retired workers PbB rose with increasing bone Pb.
Christoffersson et al. (1986) Sweden 1978-84	Retired lead workers. Group 1: 7 smelter, 1 storage battery monitored for 2-5 yr directly after end of exposure. Group 2: 6 battery, bone Pb measured 7-13 yr after end of exposure. Finger bone measurement with ^{57}Co source from 4 to 9 times.	Group 1: mean initial bone Pb 97 (61, 131), decreasing bone Pb with time half-life 6.7 yr (3.4, 15) Group 2: mean initial bone Pb 72 (37, 96), mean half-life 8.2 yr (2.4,∞)	Decrease of lead in bone after the end of exposure considerably faster than estimated earlier from various data on lead metabolism.

Table AX6-2.5 (cont'd). Bone Lead Measurements in Occupationally-Exposed Subjects

Reference, Study Location, and Period	Study Description	Lead Measurement (SD or range) PbB in µg/dL, Bone Pb in µg/g Bone Mineral	Findings, Interpretation
Europe (cont'd)			
Hanninen et al. (1998) Finland Unknown	Storage battery workers Grouped into those whose PbB exceeded 50 µg/dL [High PbB (n = 28; 21 males)], and never [Low PbB (n = 26; 22 males)]. Evaluation of neuropsychological dysfunction.	High PbB: average PbB 39.3 (±8.3), tibia 35.3 (±16.6), calcaneus 100.4 (±43.1) Low PbB: average PbB 29.0 (±6.2), tibia 19.8 (±13.7), calcaneus 78.6(±62.4)	No relation was found between the neuropsychological test battery and tibial Pb.
Erkkilä et al. (1992) Finland Unknown	K-shell measures with ^{109}Cd source on acid battery employees and controls Active (n = 91); Former plant (n = 16); Office (n = 38); Laboratory (n = 26). K-shell XRF.	Active: PbB 30.0 (9.5), tibia 21.1 (17), calcaneus 76.6 (55.3) Former plant: PbB 12.2 (6.2), tibia 32.4 (34.9), calcaneus 73.5 (57.7) Office: PbB 6.4 (3.3), tibia 7.7 (11.3), calcaneus 14.2 (15.6) Laboratory: PbB 3.7 (1.7), tibia 3.5 (10.8), calcaneus 1.2 (10.6)	Tibia Pb concentration increased consistently both as a function of intensity of exposure and duration of exposure. Calcaneal Pb concentration strongly dependent on the intensity rather than duration of exposure. Biological half life of Pb in calcaneus <7-8 yr periods into which the duration of exposure was split. Retired workers: endogenous exposure to Pb arising from skeletal burdens accumulated over a working lifetime can easily produce the dominant contribution to systemic Pb concentrations once occupational exposure has ceased.
Nilsson et al. (1991) Sweden 1980s	Group A: 7 retired smelter workers and 1 battery worker monitored for ~10 yr with 11-17 finger bone measurements with ^{57}Co. Group B: 6 retired battery workers monitored for up to 18.5 yr with 7-13 finger bone measurements.	Bone Pb values decreased over time. A mono-exponential retention model was used. Group A: estimated half-life for bone Pb was 6.2-27 yr. Group B: half-life was 11-470 yr.	The "shared" half-life for bone Pb was 16 (CI: 12, 23) yr. These values are longer than ones of Christoffersson et al. (1986) for the same two groups; no "background" values were subtracted in the latter case.

Table AX6-2.5 (cont'd). Bone Lead Measurements in Occupationally-Exposed Subjects

Reference, Study Location, and Period	Study Description	Lead Measurement (SD or range) PbB in µg/dL, Bone Pb in µg/g Bone Mineral	Findings, Interpretation
Europe (cont'd)			
Gerhardsson et al. (1993) Sweden Unknown	Pb smelter and truck assembly (referent) workers; Active smelter (n = 70); Retired smelter (n = 30); Truck assembly (n = 31); Retired truck assembly (n = 10). K-shell measures with ^{109}Cd source.	Median values presented. Active smelter: PbB 31.9 (5.0, 47.4), tibia 13.0 (−4.1, 72.8), calcaneus 48.6 (0.4, 217.8) Retired smelter: PbB 9.9 (3.3, 21), tibia 39.3 (2.9, 73.4), calcaneus 100.2 (34.8, 188.9) Truck: PbB 4.1 (1.7, 12.4), tibia 3.4 (−9.4, 13.3), calcaneus 12.2 (−12.7, 43.0) Retired truck: PbB 3.5 (2.2, 12.2), tibia 12.0 (−6.7, 23.7), calcaneus 30.2 (−7.1, 56.7)	Higher calcaneus Pb than tibia Pb in active lead workers suggested more rapid absorption over time in this mainly trabecular bone. Estimated biological half-times were 16 yr in calcaneus (95% CI: 11, 29 yr) and 27 yr in tibia (95% CI: 16, 98 yr). Strong positive correlation between bone Pb and cumulative PbB index.
Börjesson et al. (1997) Sweden 1992	Pb smelter and referent male metal workers Active smelter (n = 71); Retired smelter (n = 18); Referent active (n = 27); Referent retired (n = 8). Similar cohort to Gerhardsson et al. (1993). Finger bone measurement with ^{57}Co source. Cumulative PbB index.	Median values presented. Active smelter: PbB 33.1 (8.3, 93), bone Pb 23.0 (−13, 99) Retired smelter: PbB 17.2 (8.9, 33.1), bone Pb 55 (3, 88) Active referent: PbB 3.7 (0.8, 7.0), bone Pb 3 (−21, 16) Retired referent: PbB 3.9 (3.1, 6.2), bone Pb 1.5 (−3, 12)	Multiple regression analyses showed bone Pb was best described by the cumulative PbB index, which explained 29% of the observed variance (multiple r^2) in bone Pb in active workers and about 39% in retired workers. Estimated biological half-life of bone Pb among active lead workers was 5.2 yr (95% CI: 3.3-13.0 yr).
Bergdahl et al. (1998) Sweden 1986	Secondary Pb smelter Exposed (n = 77); Referents (n = 24). K-shell measures with ^{109}Cd source. Cumulative PbB index and (calculated) plasma Pb.	Exposed: PbB 35.0 (14, 57), tibia 25 (5, 193), calcaneus 52 (−20, 458) Referents: PbB 5.0 (2.9, 16). tibia 10 (−6, 36), calcaneus 11(−12, 61)	Strong relationships between the tibia Pb (r^2 = 0.78) and calcaneus (r^2 = 0.80) and cumulative PbB index. Half-lives of Pb in tibia 13-24 yr and calcaneus 12-19.
Erfurth et al. (2001) Sweden	Secondary smelter Active (n = 62); Retired (n = 15); Referents (n = 26). Evaluation of effects of Pb on the endocrine system. Finger bone measures with ^{57}Co source.	Median values presented. Active: PbB 33.2 (8.3, 93.2), tibia 21 (−13, 99) Retired: PbB 18.6 (10.4, 49.7), tibia 55 (3, 88) Referents: PbB 4.1 (0.8, 6.2), tibia 2 (−21, 14)	No significant associations between bone Pb and pituitary and thyroid hormones, serum testosterone, gonadotropin-releasing hormone and thyroid releasing hormone.

Table AX6-2.5 (cont'd). Bone Lead Measurements in Occupationally-Exposed Subjects

Reference, Study Location, and Period	Study Description	Lead Measurement (SD or range) PbB in µg/dL, Bone Pb in µg/g Bone Mineral	Findings, Interpretation
Europe (cont'd)			
Roels et al. (1995) Belgium	Pb smelter and others. Active production (n = 73); Other departments (n = 50). K-shell measures with ^{109}Cd source. Cumulative PbB index.	Active: PbB 42.0, tibia 66.5 Others: PbB 14.5, tibia 31.4	Strong relationship between bone Pb and cumulative PbB index in smelter populations ($r = 0.80$, $p < 0.0001$; age explained ≤9.5% of variance). Slope of regression equation of log bone Pb versus log cumulative PbB index showed that doubling of cumulative PbB index corresponds to doubling of bone Pb.
Mexico			
Juarez-Perez et al. (2004) Mexico City 1996-7	Lithographic print shop workers; Males, n = 59, 10 females; mean age 47 yrs Plasma Pb by ultraclean ICP-MS methods. K-shell measures with ^{109}Cd source.	PbB 11.9 (±5.8), tibia 27.6 (±18.1; ND-73.8), patella 46.8 (±29.3; ND-139)	Statistically significant associations between: plasma Pb and PbB, patella Pb, tibia Pb, age, education, use of Pb-glazed ceramics but not air Pb, hand Pb or hygiene index at work. Multiple linear regression models with patella and tibia Pb as main predictors and adjusting for PbB and hygiene index explained 57% of variability in plasma Pb. Negative association between plasma Pb and hygiene index suggest oral exposure and gastrointestinal uptake of Pb predominant source of Pb exposure in these subjects.
Asia			
Schwartz et al. (2001) Korea 1997-99	Korean Pb workers (798, 639 male, 164 female) and controls (135, 124 male, 1 female). Evaluation of associations between PbB, tibia Pb, chelatable Pb, and neurobehavioral functions. K-shell measures with ^{109}Cd source.	Active: PbB 32 (±15), tibia 37.2 (±40.4) Controls: PbB 5.3 (±1.8), tibia 5.8 (±7.0).	After adjustment for covariates, tibia Pb was not associated with neurobehavioral test scores.

Table AX6-2.5 (cont'd). Bone Lead Measurements in Occupationally-Exposed Subjects

Reference, Study Location, and Period	Study Description	Lead Measurement (SD or range) PbB in µg/dL, Bone Pb in µg/g Bone Mineral	Findings, Interpretation
Asia (cont'd)			
Todd et al. (2001) Korea	Korean Pb workers active (n = 723), retired (n = 79), controls (n = 135). Evaluation of associations between PbB, tibia Pb, chelatable Pb. K-shell measures with ^{109}Cd source.	Active: median PbB 31.7, tibia 24.4 (−7.4, 337.6) Retired: median PbB 13.5, tibia 26.4 (−6.7, 196.7) Controls: median PbB 5.1, tibia 5.0 (−10.9, 26.6)	Control women higher bone Pb than men. Job duration, body mass index, and age were positive predictors of tibial Pb. Rate of increase in tibia Pb with age itself increased with increasing age. Tibial Pb stores in older subjects are less bioavailable and may contribute less to PbB than tibial stores in younger subjects.

Table AX6-2.6. Bone Lead Contribution to Blood Lead

Reference, Study Location, and Period	Study Description	Lead Measurement (SD or range) PbB in µg/dL, Bone Pb in µg/g Bone Mineral	Findings, Interpretation
United States			
Korrick et al. (2002) Boston, MA 1990-95	Nurses' Health Study. Cross-sectional study of 264 elderly women; 46-54 yr (n = 80) 55-64 yr (n = 102), 65-74 yr (n = 82). Tibia and patella Pb. Multivariate linear regression models.	46-54 yr: PbB 2.7 (SE ±0.3), tibia 10.5 (±1.0), patella 14.9 (±1.2) 55-64 yr: PbB 3.4 (±0.2), tibia 12.7 (±0.9), patella 17.0 (±1.1) 65-74 yr: PbB 3.3 (±0.3), tibia 16.4 (±0.9), patella 19.8 (±1.2). An increase from the first to the fifth quintile of tibia Pb level (19 µg/g) was associated with a 1.7 µg/dL increase in PbB (p 0.0001).	Tibia and patella Pb values were significantly and positively associated with PbB but only among postmenopausal women who were not using estrogens. Older age and lower parity were associated with higher tibia Pb; only age was associated with patella Pb. They suggested the observed interaction of bone Pb with estrogen status in determining PbB supports the hypothesis that increased bone resorption, as occurs postmenopausally because of decreased estrogen production, results in heightened release of bone Pb stores into blood.
Popovich et al. (2005) Bunker Hill, ID 1994	108 former female smelter employees and 99 referents to assess the PbB versus bone Pb relationship	Exposed: PbB 2.73 (±2.39), tibia 14.4 (±0.5) Referents: PbB 1.25 (±2.10), tibia 3.22 (±0.50) Pb concentrations in tibia and blood significantly higher in the exposed group. Endogenous release rate (µg Pb per dL blood/µ Pb/g bone) in postmenopausal women was double the rate found in premenopausal women (0.132 ± 0.019 vs. 0.067 ± 0.014).	Higher tibia bone Pb (and PbB) was associated with use of estrogen (present or former) in both the whole referent group and postmenopausal women in the referent group.

Table AX6-2.6 (cont'd). Bone Lead Contribution to Blood Lead

Reference, Study Location, and Period	Study Description	Lead Measurement (SD or range) PbB in µg/dL, Bone Pb in µg/g Bone Mineral	Findings, Interpretation
Canada			
Brito et al. (2000) Canada 1993-98	Aims were to: (i) investigate the long term human Pb metabolism by measuring the change of Pb concentration in the tibia and calcaneus between 1993 and 1998; and (ii) assess whether improved industrial hygiene was resulting in a slow accumulation of Pb in an exposed workforce. 101 workers in a secondary lead smelter, 51 subjects had similar bone Pb measurements in 1993. Most other subjects had been hired since 1993. Cumulative PbB index. Linear regressions.	Repeats (n = 51) 1993: Tibia 39 (±19), calcaneus 64 (±36). 1998: Tibia 33 (±18), calcaneus 65 (±38). Non-repeats (n = 50) 1998: Tibia 15 (±16), calcaneus 13 (±18). Tibia Pb decreased significantly ($p < 0.001$) in the 51 subjects with repeated bone Pb measurements. Tibia Pb in 1993 and changes in cumulative PbB index were significant predictors of changes in tibia Pb. An overall half-life of 15 yr (95% CI: 9, 55 yr) was estimated. Adding continuing lead exposure and recirculation of bone lead stores to the regression models produced half-life estimates of 12 and 9 yr, respectively, for release of lead from the tibia. Repeat subjects showed no net change in calcaneus Pb after 5 yr.	The decrease in new exposure coupled to release of previously stored bone Pb resulted in a significant decrease in tibia Pb in the repeat subjects. The rate of clearance of Pb from the tibia of 9 to 15 yr is towards the more rapid end of previous estimates. The lack of a significant change in the calcaneus Pb was surprising and if confirmed would have implications for models of Pb metabolism.
Brito et al. (2002) Canada 1994, 1999	Evaluated endogenous release of Pb from bone to blood in 204 exposed subjects resuming their duties after a 10-mo strike in a primary lead smelter in 1991. Bone Pb (^{109}Cd source) measured in the tibia and calcaneus in 1994 (Fleming et al., 1997) and 1999. A linear model used to predict the current PbB upon the level of lead in bone. 327 subjects available on both occasions. Group H higher PbB and Group L lower PbB.	Group H: PbB 22.0, tibia 19.2 (n = 120) Group L: PbB 20.6, tibia 82.8 (n = 45) Group H: PbB 24.2, calcaneus 41.4 (n = 90) Group L: PbB 20.2,calcaneus 138.2 (n = 45) Structural analysis of data gave slopes for tibia (2.0, 95% CI: 1.66, 2.54) and calcaneus (0.19, 95% CI: 0.16, 0.23) that were significantly higher than those predicted by the commonly used simple linear regression method, for tibia (0.73, 95% CI: 0.58, 0.88) and calcaneus (0.08, 95% CI: 0.06, 0.09).	Suggested that more Pb than previously predicted by regression analysis is released from bone to blood.
Mexico			
Brown et al. (2000) Mexico City 1994-5	Investigated determinants of bone Pb and PbB of 430 lactating Mexican women during the early postpartum period and contribution of bone Pb to PbB. Linear regression analyses.	PbB 9.5 (±4.5), tibia 10.2 (±10.1), patella 15.2 (±15.1).	Older age, use of Pb glazed pottery, and higher proportion of life spent in Mexico City were main predictors of higher tibia and patella Pb. Women in the 90th percentile for patella Pb had an untransformed predicted mean PbB 3.6 µg/dL higher than those in the 10th percentile.

Table AX6-2.6 (cont'd). Bone Lead Contribution to Blood Lead

Reference, Study Location, and Period	Study Description	Lead Measurement (SD or range) PbB in µg/dL, Bone Pb in µg/g Bone Mineral	Findings, Interpretation
Mexico (cont'd)			
Téllez-Rojo et al. (2002) Mexico City 1994-95	Evaluated the hypothesis that lactation stimulates Pb release from bone to blood. Cross–sectional examination of breastfeeding patterns and bone Pb as determinants of PbB among 425 lactating women (mean age 24.8 ± 5.3 yr) for 7 mo after delivery. Bone Pb at 1 mo postpartum. Maternal blood samples and questionnaire information collected at delivery and at 1, 4, and 7 mo postpartum. Generalized estimating equations.	Mean PbB decreased with time postpartum: 1 mo 9.4 (±4.4), 4 mo 8.9 (±4.0), 7 mo 7.9 (±3.3). Tibia 10.6 (11.6 after correction for negative values), patella 15.3 (16.9 after correction). After adjustment for bone Pb and environmental exposure, women who exclusively breastfed their infants had PbB levels that were increased by 1.4 µg/dL and women who practiced mixed feeding had levels increased by 1.0 µg/dL, in relation to those who had stopped lactation. A 10 µg Pb/g increment in patella and tibia bone Pb increased PbB by 6.1% (95% CI: 4.2, 8.1) and 8.1% (95% CI: 5.2, 11.1), respectively.	They concluded that their results support the hypothesis that lactation is directly related to the amount of Pb released from bone.
Garrido-Latorre et al. (2003) Mexico City 1995	Aim was to examine the relationship of blood lead levels to menopause and bone lead levels in 232 perimenopausal and postmenopausal women from Mexico City. Measured bone mineral density in addition to bone Pb. Information regarding reproductive characteristics and known risk factors for PbB was obtained using a standard questionnaire by direct interview. Mean age of the population was 54.7 yrs (±9.8). Linear regression analyses.	PbB 9.2 (±4.7), tibia 14.85 (±10.1), patella 22.73 (±14.9). A change of 10 µg Pb/g bone mineral in postmenopausal subjects was associated with an increase in blood lead of 1.4 µg/dL, whereas a similar change in bone lead among premenopausal women was associated with an increase in blood lead of 0.8 µg/dL.	Found that postmenopausal women using hormone replacement therapy had lower PbB levels and higher tibia and patella bone Pb levels than non-users; patella Pb explained the greatest part of variations in PbB. Found no association with PbB levels and did not describe any relationships between bone lead and bone density

Table AX6-2.7. Bone Lead Studies in Pregnant and Lactating Subjects

Reference, Study Location, and Period	Study Description	Lead Measurement (SD or range) PbB in µg/dL, Bone Pb in µg/g Bone Mineral	Findings, Interpretation
United States			
Hu et al. (1996) Boston, MA 1990	Cord blood PbB measured in 223 women, 41 bone Pb measured at 1-4 postpartum. ANOVA.	Values omitted if measurement uncertainty was >10 µg/g for tibia and 15 µg/g for patella. Cord PbB 1.19 (±1.32), maternal PbB 2.9 (±2.6), tibia 4.5 (±4.0) patella 5.8 (±4.5). Maternal age was the only factor marginally associated with combined bone Pb ($p = 0.08$) but not individually with tibia or patella Pb.	Umbilical cord blood Pb among women served by this Boston hospital declined dramatically from 1980 to 1990.
Rothenberg et al (2000) Los Angeles, CA 1995-98	Examined bone Pb contribution to PbB in a group of 311 immigrant women (mean age 27.8 ±7.5 yr), 99% from Latin America, during the 3rd trimester of pregnancy, and 1 to 2 mo after delivery. Multiple regression, variance-weighted least squares regression, structural equation modeling.	Prenatal PbB 2.2 (+4.8/−1.0, geometric mean), postnatal PbB 2.8 (+4.9/−1.2) ($p < 0.0001$), tibia 6.7(±12.5), calcaneus 8.4 (±13.2). Variance-weighted multiple regression and structural equation models showed that both calcaneus and tibia Pb were directly associated with prenatal PbB but only calcaneus Pb was associated with postnatal PbB. Increasing natural log yrs in the United States independently predicted decreasing calcaneus and 3rd trimester PbB.	Suggest that while some exogenous Pb sources and modulators of PbB, such as use of Pb-glazed pottery and calcium in the diet, control Pb exposure during and after pregnancy, endogenous Pb sources from past exposure before immigration continue to influence PbB levels in this cohort.
Rothenberg et al. (2002) Los Angeles, CA 1995-2001	Examined the effects of blood and bone PbB on hypertension and elevated blood pressure in the 3rd trimester and postpartum among 1,006 mostly Latina and Afro-American women. Multiple and logistic regression.	Returned and eligible: 3rd trimester PbB (n = 720) 1.9 (+3.6/−1.0), postpartum PbB (n = 704) 2.3 (+4.3/−1.2), tibia (n = 700) 8.0 (±11.4), calcaneus (n = 700) 10.7 (±11.9). Returned but ineligible: 3rd trimester PbB (n = 279) 1.9 (+4.2/−0.8), postpartum PbB (n = 274) 2.3 (+4.7/−1.1), tibia (n = 263) 8.7 (±13.9), calcaneus (n = 262) 11.2 (±15.1). For each 10 µg/g increase in calcaneus Pb level, the odds ratio for 3rd trimester hypertension (systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg) was 1.86 (95% CI: 1.04, 3.32). In normotensive subjects, each 10 µg/g increase in calcaneus Pb level was associated with a 0.70 mmHg (95% CI: 0.04, 1.36) increase in 3rd trimester systolic blood pressure and a 0.54 mmHg (95% CI: 0.01, 1.08) increase in diastolic blood pressure after adjusting for postpartum hypertension, education, immigration status, current smoking, current alcohol use, parity, age, and body mass index. Tibia bone Pb was not related to hypertension or elevated blood pressure either in the 3rd trimester or postpartum, nor was calcaneus Pb related to postpartum hypertension or elevated blood pressure.	The authors concluded that past Pb exposure influences hypertension and elevated blood pressure during pregnancy and controlling blood pressure may require reduction of Pb exposure long before pregnancy.

Table AX6-2.7 (cont'd). Bone Lead Studies in Pregnant and Lactating Subjects

Reference, Study Location, and Period	Study Description	Lead Measurement (SD or range) PbB in µg/dL, Bone Pb in µg/g Bone Mineral	Findings, Interpretation
Mexico			
Hernandez-Avila et al. (1996) Mexico City	Cross-sectional investigation of the interrelationships between environmental, dietary, and lifestyle histories, blood and bone Pb levels, among 98 recently postpartum women. Multivariate linear regression. Age 25.6 (±6.8) yr.	14-20 yr (n = 24): PbB 10.4 (±4.1), tibia 11.8 (±14.9), patella 14.1 (±13.3). 21-29 yr (n = 44): PbB 10.3 (±4.8), tibia 10.7 (± 10.9), patella 17.1 (±13.4) 30-43 yr (n = 27): PbB 7.8 (± 3.7), tibia 16.3 (±8.4), patella 18.1 (±12.7). A 34 µg/g increase in patella Pb (from the medians of the lowest to the highest quartiles) was associated with an increase in PbB of 2.4 µg/dL. Significant predictors of bone Pb included years living in Mexico City, lower consumption of high calcium content foods, and nonuse of calcium supplements for the patella and years living in Mexico City, older age, and lower calcium intake for tibia bone. Low consumption of milk and cheese, as compared to the highest consumption category (every day), was associated with an increase in tibia Pb of 9.7 µg/g.	Suggest that patella bone is a significant contributor to PbB during lactation and that consumption of high calcium content foods may protect against the accumulation of Pb in one.
Gonzales-Cossio et al. (1997) Mexico City Unknown	Examined relationship of Pb levels in cord blood and maternal bone to birth weight. Umbilical cord and maternal venous blood samples and anthropometric and sociodemographic data were obtained at delivery and 1 mo postpartum. Bone Pb at 1 mo postpartum. Multiple regression, LOWESS. Background information for calcium supplementation study Hernandez-Avila et al. (2003). Mother-infant pairs (n = 272).	Maternal PbB 8.9 (±4.1), cord PbB 7.1 (±3.5), tibia 9.8 (±8.9), patella 14.2 (±13.2). After adjustment for other determinants of birth weight, tibia Pb was the only Pb biomarker clearly related to birth weight. The decline in birth weight associated with increments in tibia Pb was nonlinear and accelerated at the highest tibia Pb quartile. In the upper quartile, neonates were on average, 156 g lighter than those in the lowest quartile.	Bone-lead burden is inversely related to birth weight.

Table AX6-2.7 (cont'd). Bone Lead Studies in Pregnant and Lactating Subjects

Reference, Study Location, and Period	Study Description	Lead Measurement (SD or range) PbB in µg/dL, Bone Pb in µg/g Bone Mineral	Findings, Interpretation
Mexico (cont'd)			
Brown et al. (2000) Mexico City 1994-5	Investigated determinants of bone Pb and PbB of 430 lactating Mexican women during the early postpartum period and contribution of bone Pb to PbB. Linear regression analyses.	PbB 9.5 (±4.5), tibia 10.2 (±10.1), patella 15.2 (±15.1).	Older age, use of Pb glazed pottery, and higher proportion of life spent in Mexico City were main predictors of higher tibia and patella Pb. Women in the 90th percentile for patella Pb had an untransformed predicted mean PbB 3.6 µg/dL higher than those in the 10th percentile.
Chuang et al. (2001) Mexico City 1994-95	Aim to estimate the contribution of maternal whole PbB and bone Pb, and environmental Pb to umbilical cord PbB (as a measure of fetal Pb exposure). Maternal and umbilical cord blood samples within 12 hr of each infant's delivery. Structural equation modeling.	Bone Pb measured within 1 mo after delivery. PbB 8.45 (±3.94, n = 608), tibia 9.67 (±9.21, n = 603), patella 14.24 (±14.19, n = 575). Tibia and patella Pb, use of Pb glazed ceramics, and mean air Pb level contributed significantly to plasma Pb. An increase in patella Pb and tibia Pb was associated with increases in cord PbB of 0.65 and 0.25 µg/dL, respectively.	Suggested that maternal plasma Pb varies independently from maternal whole PbB. Contributions from endogenous (bone) and exogenous (environmental) sources were approximately the same. (Plasma Pb not measured).
Ettinger et al. (2004) Mexico City 1994-95	Aim to quantify the relation between maternal blood and bone Pb and breast-feeding status among 310 lactating women in Mexico City, Mexico, at 1 mo postpartum. Breast milk measured. Multiple linear regression, LOWESS smoothing.	Breastfeeding: PbB 9.3 (±4.4, n = 310), tibia 9.6 (±10.1, n = 303), patella 14.5 (±14.9, n = 294). Non breastfeeding: PbB 9.3 (±4.9, n = 319), tibia 10.5 (±10.2, n = 306), patella 15.2 (±16.1, n = 289). Breast milk geometric mean 1.1 (range 0.21-8.02) µg/L. Breast milk Pb significantly correlated with umbilical cord Pb and maternal PbB at delivery and with maternal PbB and patella Pb at 1 mo postpartum. An interquartile range increase in patella Pb (20 µg/g) was associated with a 14% increase in breast milk lead (95% CI: 5, 25%). An IQR increase in tibia Pb (12.0 µg/g) was associated with a 5% increase in breast milk lead (95% CI: −3, 14).	Suggest that even among a population of women with relatively high lifetime Pb exposure, breast milk Pb levels are low, influenced both by current Pb exposure and by redistribution of bone Pb accumulated from past environmental exposures.

Table AX6-2.7 (cont'd). Bone Lead Studies in Pregnant and Lactating Subjects

Reference, Study Location, and Period	Study Description	Lead Measurement (SD or range) PbB in µg/dL, Bone Pb in µg/g Bone Mineral	Findings, Interpretation
Mexico (cont'd)			
Sanin et al. (2001) Mexico City 1994-95	Examined early postnatal growth in a cohort of healthy breastfed newborns in relation to maternal bone Pb burden. 329 mother-infant pairs sampled for umbilical cord blood at birth and maternal and infant venous blood at 1 mo postpartum. Maternal evaluations at 1 mo postpartum included Pb measures in blood and bone. Primary endpoints were attained weight 1 mo of age, and weight gain from birth to 1 mo of age. Linear regression.	Included in analyses (n = 329): Infant: cord PbB 6.8 (±3.9), PbB 1 mo 5.7 (±3.0) Maternal: PbB 9.7 (±5.2), tibia Pb 10.1 (±10.3), patella Pb 15.2 (±15.2) Excluded from analyses (n = 276): Infant: cord PbB 6.3 (±3.0), PbB 1 mo 5.5 (±3.3) Maternal: PbB 8.8 (±3.9), tibia Pb 9.75 (±10.3), patella Pb 14.2 (±17.3). Infant PbB were inversely associated with weight gain, with an estimated decline of 15.1 g/µg/dL of PbB. Children who were exclusively breastfed had significantly higher weight gains; however, this gain decreased significantly with increasing levels of patella Pb. Multivariate regression analysis predicted a 3.6 g decrease in weight at 1 mo of age/µg Pb/g bone mineral increase in maternal patella Pb levels.	The authors concluded that maternal Pb burden is negatively associated with infant attained weight at 1 mo of age and to postnatal weight gain from birth to 1 mo of age.
Gomaa et al. (2002) Mexico City Unknown	Aim to compare umbilical cord PbB and maternal bone Pb as independent predictors of infant mental development (n = 197). Prospective design. At 24 mo of age, each infant was assessed using the Bayley Scales of Infant Development-II (Spanish Version). Multiple linear regression.	Cord PbB 6.7 (±3.4), tibia 11.5 (±11.0), patella 17.9 (±15.2). After adjustment for confounders, Pb levels in umbilical cord blood and patella bone were significantly, independently, and inversely associated with the Mental Development Index (MDI) scores of the Bailey Scale. In relation to the lowest quartile of patella Pb, the 2nd, 3rd, and 4th quartiles were associated with 5.4-, 7.2-, and 6.5-point decrements in adjusted MDI scores. A 2-fold increase in cord PbB (e.g., from 5-10 µg/dL) was associated with a 3.1-point decrement in MDI score.	Suggest that higher maternal patella bone Pb levels constitute an independent risk factor for impaired mental development in infants at 24 mo of age. This effect is probably attributable to mobilization of maternal bone Pb stores.

Table AX6-2.7 (cont'd). Bone Lead Studies in Pregnant and Lactating Subjects

Reference, Study Location, and Period	Study Description	Lead Measurement (SD or range) PbB in µg/dL, Bone Pb in µg/g Bone Mineral	Findings, Interpretation
Mexico (cont'd)			
Hernandez-Avila et al. (2002) Mexico City 1994	Aim to evaluate the effects of maternal bone Pb stores on anthropometry at birth in 223 mother-infant pairs. Anthropometric data were collected within the first 12 hr following delivery. Maternal information was obtained 1 mo after delivery (mean age 24.4 ± 5.4 yr). Transformed anthropometric measurements to an ordinal 5-category scale, and association of measurements with other factors evaluated with ordinal logistic-regression models. Cumulative Odds Model.	Cord blood 7.01 (±3.5), maternal PbB 8.82 (± 4.0), tibia 10.70 (±7.58, adjusted for negative values), patella 15.39 (±11.18, adjusted for negative values). Maternal PbB increased linearly by 0.096/µg of tibia Pb and 0.078/µg patella Pb. Umbilical cord PbB increased by 0.111/µg tibia Pb and 0.061/µg patella Pb. Birth length of newborns decreased as tibia Pb levels increased (odds ratio of 1.03/µg/g bone mineral [95% CI: 1.01, 1.06]).	Compared with women in the lower quintiles of the distribution of tibia Pb, those in the upper quintile had a 79% increase in risk of having a lower birth length newborn (OR ratio 1.79; 95% CI: 1.10, 3.22). Patella Pb was positively related to the risk of a low head circumference score; this score remained unaffected by inclusion of birth weight. The increased risk was 1.02/ μg Pb/g bone mineral (95% CI: 1.01, 1.04). Odds ratios did not vary substantially after the authors adjusted for birth weight and other important determinants of head circumference.
Téllez-Rojo et al. (2002) Mexico City 1994-95	Evaluated the hypothesis that lactation stimulates Pb release from bone to blood. Cross-sectional examination of breastfeeding patterns and bone Pb as determinants of PbB among 425 lactating women (mean age 24.8 ±5.3 yr) for 7 mo after delivery. Bone Pb at 1 mo postpartum. Maternal blood samples and questionnaire information collected at delivery and at 1, 4, and 7 mo postpartum. Generalized estimating equations.	Mean PbB decreased with time postpartum: 1 mo 9.4 (±4.4), 4 mo 8.9 (±4.0), 7 mo 7.9 (±3.3). Tibia 10.6 (11.6 after correction for negative values), patella 15.3 (16.9 after correction). After adjustment for bone Pb and environmental exposure, women who exclusively breastfed their infants had PbB levels that were increased by 1.4 µg/dL and women who practiced mixed feeding had levels increased by 1.0 µg/dL, in relation to those who had stopped lactation. A 10 µg Pb/g increment in patella and tibia bone Pb increased PbB by 6.1% (95% CI: 4.2, 8.1) and 8.1% (95% CI: 5.2, 11.1), respectively.	They concluded that their results support the hypothesis that lactation is directly related to the amount of Pb released from bone.

Table AX6-2.7 (cont'd). Bone Lead Studies in Pregnant and Lactating Subjects

Reference, Study Location, and Period	Study Description	Lead Measurement (SD or range) PbB in µg/dL, Bone Pb in µg/g Bone Mineral	Findings, Interpretation
Mexico (cont'd)			
Hernandez-Avila et al. (2002) Mexico City 1994	Evaluated the effects that maternal bone Pb has on anthropometry at birth in 223 mother-infant pairs. Anthropometric data (birth length, head circumference) collected within the first 12 hr following delivery. Maternal information was obtained 1 mo postpartum. Transformed anthropometric measurements to an ordinal 5-category scale, ordinal logistic-regression models.	Participants (n = 223) Cord blood 7.01 (±3.5), maternal PbB 8.82 (±4.0), tibia 9.83 (±8.9), patella 14.14 (±13.0). Nonparticipants (n = 494): Cord blood 6.75 (±3.50), PbB 8.47 (±4.19). Birth length of newborns decreased as tibia Pb levels increased. Compared with women in the lower quintiles of the distribution of tibia Pb, those in the upper quintile had a 79% increase in risk of having a lower birth length newborn (odds ratio 1.79; 95% CI: 1.10, 3.22). The effect was attenuated– but nonetheless significant- even after adjustment for birth weight. Patella Pb was positively and significantly related to the risk of a low head circumference score; this score remained unaffected by inclusion of birth weight.	The authors estimated the increased risk of having a low head-circumference score to be 1.02/ µg Pb/g bone mineral (95% CI: 1.01, 1.04). Odds ratios did not vary substantially after the authors adjusted for birth weight and other important determinants of head circumference.
Hernandez-Avila et al. (2003) Mexico City 1994-95	Tested the hypothesis that in a randomized trial of lactating women a dietary calcium supplement will lower blood lead levels. Lactating women (mean age 24 yr) were randomly assigned to receive either calcium carbonate (1200 mg of elemental calcium daily) or placebo in a double-blind trial. Blood samples were obtained at baseline, and 3 and 6 mo after the trial began. Primary endpoint was change in maternal PbB in relation to supplement use and other covariates with multivariate generalized linear models for longitudinal observations.	Lactating calcium group (n = 296): PbB 9.2 (±4.2), tibia 10.7 (±9.8), patella 16.2 (±15.7) Lactating placebo (n = 321): PbB 9.4 (± 5.0), tibia 9.6 (±10.3), patella 13.5 (± 15.1) Women randomized to the calcium supplements experienced a small decline in PbB of 0.29 µg/dL (95% CI: −0.85, −0.26). The effect was more apparent among women who were compliant with supplement use and had high patella Pb of ≥5 µg/g. Among this subgroup, supplement use was associated with an estimated reduction in mean PbB of 1.16 µg/dL (95% CI: −2.08, −0.23), an overall reduction of 16.4%.	Among lactating women with relatively high Pb burden, calcium supplementation was associated with a modest reduction in PbB levels.

Table AX6-2.7 (cont'd). Bone Lead Studies in Pregnant and Lactating Subjects

Reference, Study Location, and Period	Study Description	Lead Measurement (SD or range) PbB in µg/dL, Bone Pb in µg/g Bone Mineral	Findings, Interpretation
Mexico (cont'd)			
Téllez-Rojo et al. (2004) Mexico City 1997-99	Tested the hypotheses that maternal bone Pb burden is associated with increasing maternal whole PbB and plasma Pb over the 3 trimesters of pregnancy and that this association is modified by rates of maternal bone resorption. Urine was analyzed for cross-linked N-telopeptides (NTx) of type I collagen, a biomarker of bone resorption. Patella and tibia Pb at 1 mo postpartum. Mixed models.	Participants (n = 193): PbB (µg/dL): initial 7.10 (±1.72), 1st trimester 6.47 (± 0.17), 2nd trimester5.80 (± 0.17), 3rd trimester 6.05 (± 0.17). Plasma (µg/L): 1st trimester 0.13 (±1.88), 2nd trimester 0.12 (± 1.95), 3rd trimester 0.12 (± 1.88) (geometric means and SD) Bone Pb during pregnancy: Tibia 11.35 (±8.82, adjusted for negative values), patella 13.82 (±10.97, adjusted for negative values). Nonparticipants (n = 134): PbB 6.82 (±1.75), tibia 13.71 (±9.17, adjusted for negative values), patella 11.79 (±9.75, adjusted for negative values). Found an increasing trend for plasma Pb among women with the highest bone Pb (≥median level of 12.1 µg/g) but a decreasing trend among less-exposed women(below the median level). The observed increase reached its maximum among women with both the highest bone Pb and the highest bone resorption. In comparison with women with a low bone Pb and a high NTx level, those with a high bone Pb and a high NTx level had, on average, an 80% higher mean plasma Pb. In the cross-sectional analyses for each trimester of pregnancy, there was an increasingly stronger association between bone Pb and plasma Pb (log-transformed) as pregnancy progressed. An increase in patella lead of 10 µg/g would be associated with 9% ($p = 0.07$), 24% ($p < 0.01$), and 25% ($p < 0.01$) increases in plasma Pb in the 1st, 2nd, and 3rd trimesters of pregnancy, respectively. The corresponding values for tibia lead were 8% ($p = 0.16$), 19% ($p < 0.01$), and 13% ($p = 0.01$), respectively. Dietary calcium intake was inversely associated with plasma lead.	They concluded that the results support the hypothesis of a biologic interaction between bone Pb burden and bone resorption. They also suggest that as pregnancy progresses, bone Pb may be mobilized increasingly into plasma.

Table AX6-2.7 (cont'd). Bone Lead Studies in Pregnant and Lactating Subjects

Reference, Study Location, and Period	Study Description	Lead Measurement (SD or range) PbB in µg/dL, Bone Pb in µg/g Bone Mineral	Findings, Interpretation
Mexico (cont'd)			
Moline et al. (2000) Morelos, Mexico 1999	Pilot study to assess the body burden of lead in 24 Mexican women (age 21-34 yr) who were lactating. Demographic and reproductive characteristics of women and potential sources of lead exposure were gathered by a direct interview. Multiple regression. Average time of lactation 22 (±17) months.	PbB 4.6 (± 2.0, geometric mean), tibia 9.2 (±4.2), patella 14.8 (±8.0), calcaneus 11.7 (±11.2). An inverse relationship was noted between months of lactation and age-adjusted calcaneus lead level ($p = 0.001$). No association was observed between age-adjusted patella or tibia lead level and months of lactation ($p = 0.15$).	This pilot study provides further limited evidence for the hypothesis that Pb mobilization occurs during lactation.

Table AX6-2.8. Bone Lead Studies of Menopausal and Middle-aged to Elderly Subjects

Reference, Study Location, and Period	Study Description	Lead Measurement (SD or range) PbB in µg/dL, Bone Pb in µg/g Bone Mineral	Findings, Interpretation
United States			
Hu et al. (1996) Boston, MA 1991+	Normative Aging Study. Subjects were middle-aged and elderly men who had community (nonoccupational) exposures to lead. Cross-sectional. Backwards elimination multivariate regression models that considered age, race, education, retirement status, measures of both current and cumulative smoking, and alcohol consumption.	47-59 yr: (n = 116): PbB 5.8 (±3.7), tibia 14.6 (±8.3), patella 23.6 (±12.4) 60-69 yr: (n = 360): PbB 6.3 (±4.2), tibia 21.1 (±11.4), patella 30.5 (±16.9) >70 yr: (n = 243): PbB 6.5 (±4.5), tibia 27 (±15.6), patella 38.8 (±23.5)	Factors that remained significantly related to higher levels of both tibia and patella Pb were higher age and measures of cumulative smoking, and lower levels of education. An increase in patella Pb from the median of the lowest to the median of the highest quintiles (13-56 µg/g) corresponded to a rise in PbB of 4.3 µg/dL. Bone Pb levels comprised the major source of circulating lead in these men.
Kim et al. (1997) Boston, MA 1991-95	Normative Aging Study (n = 70). Aim to examine age and secular trends in bone and blood lead levels of community-exposed men aged 52-83 yr. Bone and blood lead levels measured twice, with a 3-yr interval.	PbB 6.7 (±1.8), tibia 17.5 (±2.0), patella 29.1 (±1.8) 3 yr later: PbB 5.1 (±1.4), tibia 17.9 (±1.7), patella 22.2 (±1.8)	Cross-sectional analysis of each set of measurements indicated that, on average, a 1-year-older individual would have 2.7% and 2.4-3.2% higher levels of Pb in patella and tibia, respectively. Secular trend over time was decreasing for patella Pb levels and stable for tibia Pb levels.
Cheng et al. (1998) Boston, MA 1991-95	Normative Aging Study (n = 747). Aim to examine relationships of nutritional factors to body Pb burden. Cross-sectional. Multiple regression models adjusting for age, education level, smoking, and alcohol consumption.	PbB 6.2 (± 4.1), tibia 21.9 (± 13.3), patella 32.0 (±19.5). Multiple regression models men in the lowest quintile of total dietary intake levels of vitamin D (including vitamin supplements) (<179 i.u./day) had mean tibia and patella Pb levels 5.6 µg/g and 6.0 µg/g/ higher than men with intake in the highest quintile (≥589 i.u./day). Higher calcium intake was associated with lower bone Pb levels, but this relation became insignificant when adjustment was made for vitamin D. Subjects in the lowest vitamin C intake quintile (<109 mg/day) had a mean PbB level 1.7 µg/dL higher than men in the highest quintile (≥339 mg/day), while men in the lowest iron intake quintile (<10.9 mg/day) had a mean blood lead level 1.1 µg/dL higher than men in the highest quintile (≥23.5 mg/day).	Also observed inverse associations of blood lead levels with total dietary intake of vitamin C and iron. Suggested that low dietary intake of vitamin D may increase Pb accumulation in bones, while lower dietary intake of vitamin C and iron may increase PbB.

Table AX6-2.8 (cont'd). Bone Lead Studies of Menopausal and Middle-aged to Elderly Subjects

Reference, Study Location, and Period	Study Description	Lead Measurement (SD or range) PbB in µg/dL, Bone Pb in µg/g Bone Mineral	Findings, Interpretation
United States (cont'd)			
Hu et al. (2001) Boston, MA 1991+	Normative Aging Study. Aim to determine if ALAD polymorphism is associated with altered levels of lead in bone and blood. Multivariate linear regression models controlling for age, education, smoking, alcohol ingestion, and vitamin D intake.	ALAD 1-1 (n = 608): PbB 6.3(±4.1), tibia 22.2 (±13.9), patella 32.2 (±19.9) ALAD 1-2/2-2 (n = 118): PbB 5.7 (±4.2), tibia 21.2 (±10.9), patella 30.4 (±17.2) ALAD 1- 1 genotype was associated with cortical bone lead levels that were 2.55 µg/g (95% CI: 0.05, 5.05) higher than those of the variant allele carriers.	No significant differences by genotype with respect to Pb levels in trabecular bone or blood. In stratified analyses and a multivariate regression model that tested for interaction, the relationship of trabecular bone Pb to PbB appeared to be significantly modified by ALAD genotype, with variant allele carriers having higher PbB levels, but only when trabecular bone Pb levels >60 µg/g. The authors suggest that the variant ALAD-2 allele modifies lead kinetics possibly by decreasing lead uptake into cortical bone and increasing the mobilization of lead from trabecular bone.
Oliveira et al. (2002) Boston, MA 1991-98	Normative Aging Study. To determine if seasonal fluctuations in PbB levels are related to increased mobilization of bone Pb stores during the winter months. Measurements of blood and bone Pb during the high sun exposure months of May-August (summer; n = 290); the intermediate sun exposure months of March, April, September, and October (spring/fall; n = 283); and the low sun exposure months of November-February (winter; n = 191).	Mean PbB levels were slightly lower in summer (5.8 ± 3.4 µg/dL) compared with winter (6.6 ± 4.7 µg/dL). Mean bone Pb levels were higher during the summer than the winter months: 23.9 (±15.2) and 20.3 (±11.3) µg/g respectively for the tibia and 34.3 (±22.8) and 29.0 (±16.2) µg/g respectively for patella.	Found a significant interaction between season and bone Pb with bone Pb during the winter months exerting an almost 2-fold greater influence on PbB levels than during the summer months. The authors attributed this to increased mobilization of endogenous bone Pb stores arising potentially from decreased exposure to sunlight, lower levels of activated vitamin D and enhanced bone resorption.

Table AX6-2.8 (cont'd). Bone Lead Studies of Menopausal and Middle-aged to Elderly Subjects

Reference, Study Location, and Period	Study Description	Lead Measurement (SD or range) PbB in µg/dL, Bone Pb in µg/g Bone Mineral	Findings, Interpretation
United States (cont'd)			
Korrick et al. (2002) Boston, MA 1990-95	Nurses' Health Study. Cross-sectional study of 264 elderly women; 46-54 yr (80) 55-64 yr (102), 65-74 yr (82). Tibia and patella Pb. Multivariate linear regression models.	46-54 yr: PbB 2.7 (SE ±0.3), tibia 10.5 (±1.0), patella 14.9 (±1.2) 55-64 yr: PbB 3.4 (±0.2), tibia 12.7 (±0.9), patella 17.0 (±1.1) 65-74 yr: PbB 3.3 (±0.3), tibia 16.4 (±0.9), patella 19.8 (±1.2). An increase from the first to the fifth quintile of tibia Pb level (19 µg/g) was associated with a 1.7 µg/dL increase in PbB ($p = 0.0001$).	Tibia and patella Pb values were significantly and positively associated with PbB but only among postmenopausal women who were not using estrogens. Older age and lower parity were associated with higher tibia Pb; only age was associated with patella Pb. They suggested the observed interaction of bone Pb with estrogen status in determining PbB supports the hypothesis that increased bone resorption, as occurs postmenopausally because of decreased estrogen production, results in heightened release of bone Pb stores into blood.
Tsaih et al. (1999) Boston, MA 1991-97	Normative Aging Study. Aim to evaluate hypothesis that bone and erythrocyte Pb make independent contributions to urine Pb excreted over 24 hour. Urine used as a proxy for plasma Pb. Age range 53-82 yr (n = 71). Generalized additive model.	PbB: 5.94 (±3.0), tibia 21.7 (±10.9), patella 31.1 (±15.1), urinary Pb 5.69 (±1.9) μg/day. Both erythrocyte Pb and bone Pb variables remained independently and significantly associated with urinary Pb.	Finding suggests that bone influences plasma Pb in a manner that is independent of the influence of erythrocytic lead on plasma Pb. Reinforces superiority of bone Pb over PbB in predicting some chronic forms of toxicity may be mediated through bone's influence on plasma Pb. Urinary lead might be useful as a proxy for plasma Pb.
Wright et al. (2004) Boston, MA 1991-97	Normative Aging Study. Aim to evaluate if hemochromatosis gene (HFE) was associated with body lead burden. Tibia and patella bone Pb. DNA samples genotyped. Multivariate linear regression analyses.	Of 730 subjects, 94 (13%) carried the C282Y variant and 183 (25%) carried the H63D variant. In multivariate analyses that adjusted for age, smoking, and education, having an HFE variant allele was an independent predictor of significantly lower patella Pb levels ($p < 0.05$).	Suggested that HFE variants have altered kinetics of Pb accumulation after exposure and these effects may be mediated by alterations in Pb toxicokinetics via iron metabolic pathways regulated by the HFE gene product and body iron stores.

Table AX6-2.8 (cont'd). Bone Lead Studies of Menopausal and Middle-aged to Elderly Subjects

Reference, Study Location, and Period	Study Description	Lead Measurement (SD or range) PbB in µg/dL, Bone Pb in µg/g Bone Mineral	Findings, Interpretation
United States (cont'd)			
Lin et al. (2004) Boston, MA 1999-2000	Community Lead Study. Measured PbB and bone Pb levels among minority individuals from Boston. Compared with earlier studies of predominantly white subjects, the 84 volunteers in this study (33:67 male to female ratio; 31-72 yrs of age) had similar educational, occupational, and smoking profiles and mean blood, tibia, and patella Pb levels. LOWESS smoothing curves. Multiple linear regression analyses to predict blood, tibia and patella Pb.	<45 yr (n = 28): PbB 2.0 (±1.2), tibia 8.3 (±8.4), patella 8.9 (±14.3) 46-60 yr (n = 41): PbB 2.8 (±1.7), tibia 10.8 (±11.5), patella 11.8 (±11.4) 61-75 yr (n = 15): PbB 5.3 (±3.2),tibia 21.7 (±8.6), patella 30.9 (±15.7) Slopes of the univariate regressions of blood, tibia, and patella lead versus age were 0.10 µg/dL/yr ($p < 0.001$), 0.45 µg/g/yr ($p < 0.001$), and 0.73 µg/g/yr ($p < 0.001$), respectively.	Analyses of smoothing curves and regression lines for tibia and patella Pb suggested an inflection point at 55 yr of age, with slopes for subjects ≥55 yr of age that were not only steeper than those of younger subjects but also substantially steeper than those observed for individuals >55 yr of age in studies of predominantly white participants.
Berkowitz et al. (2004) New York 1994-99	Longitudinal study of 91 premenopausal and perimenopausal women aged ≥ 30 yrs of age from New York who were undergoing surgical menopause (baseline; n 84) to determine if bone Pb values decrease and PbB values increase during menopause. Tibia Pb concentrations measured at baseline, 6 mo (70) and 18 mo (62) postsurgery.	Baseline: Median PbB 2.5 (0.3, 11.7), tibia 6.1 (−22.2, 36.4) 6 mo: PbB 3.2 (0.4, 12.0), tibia 6.8 (−14.2, 29.0) 18 mo: PbB 3.1 (0.5, 9.1), tibia 5.8 (−15.4, 24.2)	Marginal decline in tibia Pb values between 6 and 18 mo post surgery for women who took estrogen replacement therapy (ERT) but not for those who did not take ERT. They concluded that there was no substantial mobilization of Pb (from the tibia) during menopause but common ERT use may have masked this effect, the amounts of Pb released were too low to detect in blood, or the numbers of subjects was too small to detect an effect.
Schafer et al. (2005) Baltimore, MD	Evaluated the relations among PbB, tibia Pb, and homocysteine levels by cross-sectional analysis among subjects in the Baltimore Memory Study, a longitudinal study of 1, 140 randomly selected residents in Baltimore, MD, aged 50-70 yr and 66.0% female, 53.9% white, and 41.4% black or African American. Multiple linear regression analyses.	PbB 3.5 (±2.4) µg/dL, tibia 18.9 (±12.5) µg/g, homocysteine 10.0 (±4.1) μmol/L. Tibia lead was modestly correlated with PbB (Pearson $r = 0.12$, $p < 0.01$) but was not associated with homocysteine levels.	Suggested that homocysteine could be a mechanism that underlies the effects of lead on the cardiovascular and central nervous systems, possibly offering new targets for intervention to prevent the long-term consequences of lead exposure.

Table AX6-2.8 (cont'd). Bone Lead Studies of Menopausal and Middle-aged to Elderly Subjects

Reference, Study Location, and Period	Study Description	Lead Measurement (SD or range) PbB in µg/dL, Bone Pb in µg/g Bone Mineral	Findings, Interpretation
United States (cont'd)			
Kosnett et al. (1994) Dickson City, PA	Aim to determine the influence of demographic, exposure and medical factors on the bone Pb concentration of subjects with environmental Pb exposure. 101 subjects (49 males, 52 females; aged 11 to 78 yrs) recruited from 49 of 123 households geographically located in a suburban residential neighborhood.	Log-transformed bone Pb highly correlated with age ($r = 0.71$; $p \leq 0.0001$).	Bone Pb showed no significant change up to age 20 yr, increased with the same slope in men and women between ages 20 and 55 yr, and then increased at a faster rate in men older than 55 yr.
Popovich et al. (2005) Bunker Hill, ID 1994	108 former female smelter employees and 99 referents to assess the PbB versus bone Pb relationship.	Exposed: PbB 2.73 (±2.39), tibia 14.4 (±0.5) Referents: PbB 1.25 (±2.10), tibia 3.22 (±0.50) Pb concentrations in tibia and blood significantly higher in the exposed group. Endogenous release rate (µg Pb per dL blood/ µg Pb/g bone) in postmenopausal women was double the rate found in premenopausal women (0.132 ± 0.019 versus 0.067 ± 0.014).	Higher tibia bone Pb (and PbB) was associated with use of estrogen (present or former) in both the whole referent group and postmenopausal women in the referent group.
Canada			
Webber et al. (1995) Canada Unknown	Tested hypothesis that women on hormone replacement therapy should have higher bone Pb content and lower plasma Pb as hormone replacement therapy would suppress the transfer of endogenous Pb to the circulation. 56 women, some using hormone replacement therapy over approximately 4 yrs.	Low dose hormone replacement therapy (n = 15): PbB 4.08 (±1.60), tibia 19.37 (±8.62), calcaneus 24.02 (±10.88) Moderate dose hormone replacement therapy (n = 11): PbB 5.22 (±3.36), tibia 16.80 (±11.68), calcaneus 23.83 (±14.18) Calcium only (n = 22): PbB 4.6 (±1.59), tibia 11.13 (±6.22), calcaneus 21.12 (±13.55)	Women not taking hormones had significantly lower Pb values in cortical bone compared to all women on hormone replacement therapy ($p = 0.007$). Showed higher tibia Pb levels but no increase in calcaneus Pb level or decrease in PbB.

Table AX6-2.8 (cont'd). Bone Lead Studies of Menopausal and Middle-aged to Elderly Subjects

Reference, Study Location, and Period	Study Description	Lead Measurement (SD or range) PbB in µg/dL, Bone Pb in µg/g Bone Mineral	Findings, Interpretation
Mexico			
Garrido-Latorre et al. (2003) Mexico City 1995	Aim was to examine the relationship of blood lead levels to menopause and bone lead levels in 232 perimenopausal and postmenopausal women from Mexico City. Measured bone mineral density in addition to bone Pb. Information regarding reproductive characteristics and known risk factors for PbB was obtained using a standard questionnaire by direct interview. Mean age of the population was 54.7 yrs (±9.8). Linear regression analyses.	PbB 9.2 (±4.7), tibia 14.85 (±10.1), patella 22.73 (±14.9). A change of 10 µg Pb/g bone mineral in postmenopausal subjects was associated with an increase in blood lead of 1.4 µg/dL, whereas a similar change in bone lead among premenopausal women was associated with an increase in blood lead of 0.8 µg/dL.	Found that postmenopausal women using hormone replacement therapy had lower PbB levels and higher tibia and patella bone Pb levels than non-users; patella Pb explained the greatest part of variations in PbB. Found no association with PbB levels and did not describe any relationships between bone lead and bone density.
Australia			
Gulson et al. (2002) Sydney, Australia 2000	Environmentally exposed females (n = 7) and males (n = 3) aged 44-70 yr. Treated for 6 mo with the bisphosphonate alendronate. PbB and isotopic ratios measured by TIMS for 6 mo prior to treatment and 12 mo post-treatment. Bone mineral density and bone markers including NT_x measured.	Found a decrease in PbB concentrations and changing blood lead isotopic composition in the direction predicted during treatment. Upon cessation of treatment, PbB increased and the isotopic compositions changed.	Results consistent with changes in bone remodeling associated with bisphosphonate use.

Table AX6-2.9. Lead in Deciduous Teeth from Urban and Remote Environments

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Canada			
Tsuji et al. (2001) Ontario, Canada	Dentine chips from schoolchildren living in a remote area.	Mean value of 9.2 µg/g dry weight (n = 61)	Attributed the high values to consumption of lead contaminated game meat.
Europe			
Tvinnereim et al. (1997) Norway 1990-94	2,746 deciduous whole teeth.	Mean 1.27 ± 1.87 µg/g of dry tooth substance	Observed an ~50% reduction in lead concentrations since the 1970s.
Lyngbye et al. (1991) Denmark	In 2,033 teeth from 1, 848 children.	Geometric mean for the largest group from Arhus to be 8.4 µg/g (wet weight) with similar values from Copenhagen suburbs with a secondary lead smelter (9.6 µg/g) and a lead battery factory (9.9 µg/g).	Concluded that automobile exhausts and indirect occupational exposure were important sources for the lead in dentine.
Gil et al. (1996) Coruna, Spain	220 whole deciduous and permanent teeth (one per subject).	Permanent teeth showed higher mean values (13.1 ±1.1 µg/g) than deciduous teeth (4.0 ± 1.1 µg/g)	Found no gender differences.
Nowak and Chmielnicka (2000) Poland	Compared permanent teeth from two cohorts, one from the highly polluted Katowice district and a control town of Beskid.	In the control teeth they observed decreases in lead for incisors (41.8 µg/g) to canines (37.5 µg/g) to molars (35.3 µg/g) to premolars (32.0 µg/g). However, there was no difference in the mean values for the two centers: Katowice 36.5 ± 16.3 µg/g and Beskid 36.3 ± 11.5 µg/g.	These values are very high compared with most other studies.
Mexico			
Hernandez-Guerrero et al. (2004) Mexico City	100 healthy deciduous teeth collected from 2 to 13 yr old children.	Higher mean concentrations of lead in the 10-13 yr old group (7.7 µg/g) than in other age groups and the mean concentrations were higher in girls (7.3 µg/g) than boys (6.3 µg/g).	No association between pollution intensity and tooth lead.

Table AX6-2.9 (cont'd). Lead in Deciduous Teeth from Urban and Remote Environments

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Mexico (cont'd)			
Frank et al. (1990) Alsace, Mexico	Circular biopsies 500 μm in diameter punched in the vertical sections of the crown and cervical third of each root. The age of the European subjects ranged from 10 to 80 yrs in Europe and 12 to 29 yrs in Mexico City. Energy-dispersive X-ray fluorescence method to compare lead in enamel and dentine of premolars and permanent molars.	Compared with the European values, there were approximately 6 times higher inner coronal dentine and 7 to 9 times higher pulpal root dentine concentrations for samples from Mexico City.	The authors found no significant difference in the relationship between traffic and mean lead values for enamel and dentine in the European communities but a significantly higher lead concentration in relation to age. The differences were attributed to traffic exposure.
Asia			
Karakaya et al. (1996) Ankara, Turkey	103 whole deciduous teeth from primary school aged children aged 7 to 12 yrs.	Significant differences in lead for urban (4.99 ± 0.46 µg/g dry weight) compared with suburban children (1.69 ± 0.25 µg/g).	

Table AX6-2.10. Lead In Deciduous Teeth from Polluted Environments

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe			
Begerow et al. (1994) Germany 1991	790 children aged 6 yrs old living in urban and rural areas in western and eastern Germany. Incisors sampled.	Lead levels of 1.50 to 1.74 µg/g from the western sector and from 1.51 to 2.72 µg/g in the eastern sector.	Major decrease (40-50%) since 1976.
Cikrt et al. (1997) Czech Republic	Compared tooth (n = 162) and blood lead levels in children living at various distances from a lead smelter.	Significant difference in the mean tooth lead for children from the most contaminated zone less than 0.5 km from the smelter (6.44 µg/g; n = 13) and those >5 km from the smelter (1.45 µg/g; n = 36). Blood lead levels varied from 15.42 µg Pb/100 ml (n = 6; 95% CI: 7.17, 33.17) close to the smelter to 4.66 µg/100 ml (n = 165, 95% CI: 4.30, 5.04) at larger distances.	No descriptions of the teeth type were available.
Australia			
Gulson (1996) Broken Hill, Australia	36 exposed and nonexposed children from Broken Hill lead-zinc mining community. Sectioned teeth into mainly enamel (incisal section) and mainly dentine (cervical section). Lead isotope ratios and lead concentrations by TIMS with isotope dilution.	For subjects with low exposure (n = 13), lead concentrations in the incisal section ranged from 0.4 to 3.5 µg/g with a mean and standard deviation of 1.2 ± 0.8 µg/g (n = 13). For the cervical sections in low exposure children the values ranged from 0.8 to 8.3 and mean 3.7 ± 2.4 µg/g. For subjects with high exposure (n = 23), lead concentrations in the incisal section ranged from 1.0 to 8.9 µg/g with a mean and standard deviation of 2.6 ± 1.8 µg/g. For the cervical sections in high exposure children the values ranged from 1.5 to 31.5 µg/g and mean 13.7 ± 8.0 µg/g.	The isotopic results in dentine were interpreted to reflect an increased lead exposure from the lead-zinc-silver orebody during early childhood, probably associated with hand-to-mouth activity.
Gulson et al. (2004) Lake Macquarie, Australia	10 children from six houses in a primary zinc-lead smelter community at North Lake Macquarie, New South Wales, Australia. Sectioned deciduous teeth compared with environmental samples. Lead isotope ratios and lead concentrations by TIMS with isotope dilution.	Blood lead levels in the children ranged from 10 to 42 µg/dL and remained elevated for a number of years. Median lead level in the enamel section of the teeth was 2.3 µg/g with a range from 0.6 to 7.4 µg/g; in dentine the median value was 5.3 µg/g with a range from 1.4 to 19.9 µg/g.	Approximately 55 to 100% of lead could be derived from the smelter.

Table AX6-2.11. Summary of Selected Measurements of Urine Lead Levels in Humans

Reference, Study Location, and Period	Study Description	Urine Lead Measurement			Comment
United States					
CDC (2005) U.S. 1999-2002	Design: national survey (NHANES IV) stratified, multistage probability cluster design Subjects: children and adults (≥6 yrs, n = 5140) in general population Biomarker measured: urine lead Analysis: ICP-MS	Units: µg/g creatinine Geometric mean (95% CI)			Geometric mean blood lead concentrations in age strata ranged from 0.94 to 1.51 µg/dL.
		Age (yr)	1999-2000	2001-2002	
		≥6:	0.72 (0.70, 0.74)	0.64 (0.60, 0.68)	
		n:	2465	2689	
		6-11:	1.17 (0.98, 1.41)	0.92 (0.84, 1.00)	
		n:	340	368	
		12-19:	0.50 (0.46, 0.54)	0.40 (0.38, 0.43)	
		n:	719	762	
		≥20:	0.72 (0.68, 0.76)	0.66 (0.621, 0.70)	
		n:	1406	1559	
		Males:	0.72 (0.68, 0.76)	0.64 (0.61, 0.67)	
		n:	1227	1334	
		Females:	0.72 (0.68, 0.76)	0.64 (0.59, 0.69)	
		n:	1238	1355	
Schwartz et al. (1999, 2000b) U.S. 1993-1997	Design: prospective Subjects: adult male (n = 543) former TEL manufacture workers (age range: 42-74 yrs) Biomarker measured: DMSA (10 mg/kg)-provoked urine lead Analysis: GFAAS	Units: µg/4 hr Arithmetic mean (SD): ≥2 yr exposure: 17.1 (15.7) <2 yr exposure: 20.4 (17.9)			Arithmetic mean (SD) blood lead (µg/dL) was 5.0 (2.8) for workers exposed ≥2 yr and 2.8 (1.9) for workers exposed <2yr. Blood lead was strongest predictor or DMSA-provoked urine lead. Arithmetic mean (SD) tibia lead (µg/g, XRF) was 15.6 (9.8) for workers exposed ≥2 yr and 12.1 (7.7) for workers exposed <2yr.
Rabinowitz et al. (1976) New York NR	Design: experimental study Subjects: adult (n:5) males, age range 25-53 yrs, ingested 300 µg Pb/day (approximately 50% as ^{204}Pb) for 10-210 days Biomarker measured: urine lead Analysis: MS	Units: µg/day Arithmetic mean (range): 36 (36-41)			Arithmetic mean (range) blood lead (µg/dL) was 19.4 (16.7-25.1). Blood-to urine clearance estimate was 0.19 (range 0.15-0.23) L/day (from Diamond, 1992).

Table AX6-2.11 (cont'd). Summary of Selected Measurements of Urine Lead Levels in Humans

Reference, Study Location, and Period	Study Description	Urine Lead Measurement	Comment
United States (cont'd)			
Berger et al. (1990) Ohio 1983-1986	Design: cross-sectional, convenience sample Subjects: children (n = 39), age range not reported. Biomarker measured: timed urine lead Analysis: AAS	Units: µg/day range: 5-70	Blood lead range was 22-55 µg/dL. Blood-to urine clearance estimate was 0.07 L/day (from Diamond, 1992).
Europe			
Chamberlain et al. (1978) United Kingdom 1975-1976	Design: experimental Subjects: adult males (n = 6), intravenous injection of ^{203}Pb tracer Biomarker: urinary lead clearance Analysis: gamma spectrometer (^{203}Pb)	Units: L/day Arithmetic mean (range) Blood-to-urine: 0.09 (0.08-0.10) Pplasma-to-urine: 20	
Brockhaus et al. (1988) Germany 1982-1986	Design: cross-sectional Subjects: children (n = 184), age range 4-11 yrs residing in 2 areas impacted by smelting operations Biomarker measured: urine lead Analysis: GFAAS	Units: µg/g creatinine Geometric mean (GSD, range) Stolberg (n = 106): 9.6 (2.3, 0.2-43.0) Dortmund (n = 78): 6.7 (2.0, 1.6-41.0)	Geometric mean blood lead levels were approximately 7 µg/dL.
Koster et al. (1989) Germany NR	Design: cross-sectional Subjects: adult (n = 46, 40 males) hospital workers, age range 20-78 yr. Biomarker measured: urine lead Analysis: GFAAS	Units: µg/24 hr-1.73 m^2 (adult body surface area) Arithmetic mean (range): 6.8 (2.3-18.9)	Arithmetic mean (range) blood lead (µg/dL) was 7.6 (2.6-18.7). Blood-to urine clearance estimate was 0.15 L/day (from Diamond, 1992).
Australia			
Gulson et al. (2000) Australia	Design: longitudinal Subjects: women (n = 58) during pregnancy, age range 18-35 yrs Biomarker measured: blood-to-urine clearance Analysis: TIMS	Units: µg/h Arithmetic mean (SD, range): 3.2 (0.8-10.2) Geometric mean: 2.7	Reported blood-to-urine clearance corresponds to approximately 0.08 L/day.

Table AX6-2.11 (cont'd). Summary of Selected Measurements of Urine Lead Levels in Humans

Reference, Study Location, and Period	Study Description	Urine Lead Measurement	Comment
Asia			
Araki et al. (1986, 1990) Japan NR	Design: cross-sectional Subjects: adult (n = 19) male, gun metal foundry workers, age range 34-59 yr. Biomarker measured: urine lead Analysis: AAS	Units: µg/24 hr Arithmetic mean (range): 94 (37-171)	Arithmetic mean plasma concentration was 0.67 µg/dL (range 0.37-0.92). Plasma-to urine clearance estimate was 22 L/day. Blood-to urine clearance estimate was 0.33 L/day (from Diamond, 1992).
Lee et al. (1990) Korea NR	Design: cross-sectional Subjects: adults (n = 95) male workers in lead smelting, battery manufacture, PVC-stabilizer manufacture facilities, age range: 19-64 yrs; reference subjects (n = 13), age range 22-54 yr. Biomarker measured: DMSA (10 mg/kg)-provoked urine lead Analysis: GFAAS	Units: µg/4 hr Arithmetic mean (SD, range) Lead workers: 288.7 (167.7, 32.4-789) Reference: 23.7 (11.5, 10.5-43.5)	Arithmetic mean (SD, range) blood lead concentration (µg/dL) was 44.6 (12.6, 21.4-78.4) in lead workers and 5.9 (1.2, 4.0-7.2) in reference subjects. Blood lead was strongest predictor or DMSA-provoked urine lead.
Schwartz et al. (2000a), Lee et al. (2001) Korea 1997-1999	Design: cross-sectional Subjects: adult lead (inorganic) workers (n = 798, 634 males), age range 18-65 yrs. Biomarker measured: DMSA (10 mg/kg)-provoked urine lead Analysis: GFAAS	Units: µg/4 hr Arithmetic mean (SD, range) 186 (208, 4.8-2100)	Arithmetic mean (SD, range) blood lead (µg/dL) was 32.0 (15, 4-86). Blood lead was strongest predictor or DMSA-provoked urine lead Arithmetic mean (SD, range) tibia lead (µg/g, XRF) was 37.1 (40.4, -7 to 338).

AAS - atomic absorption spectroscopy; ET-AAS - electro-thermal atomic absorption spectrometry; GFAAS - graphite furnace atomic absorption spectroscopy; ICP-AES - inductively coupled plasma/atomic emission spectroscopy; ICP-MS - inductively coupled plasma-mass spectrometry; MS - mass spectrometry; NR - not reported; Pct - percentile; TEL -tetraethyl lead; TIMS - thermal ionization mass spectrometry.

Table AX6-2.12. Summary of Selected Measurements of Hair Lead Levels in Humans

Reference, Study Location, and Period	Study Description	Hair Lead Measurement	Comment
United States			
DePietro et al. (1989) GA, SC, TX, VA 1976-1980	Design: cross-sectional (random sample from NHANES II, HHANES Stands) Subjects: adults (n = 270, 200 males; age range: 20-73 yrs) from general population Biomarker measured: hair lead Analysis: ICP-AES	Units: µg/g Geometric mean (10-90th Pct range) 2.42 (<1.0-10.8)	Hair lead level varied with hair treatment (e.g., shampoo, coloring).
Tuthill (1996) MA NR	Design: cross-sectional Subjects: children (n = 277, 141 males, age range 6.5-7.5 yrs) Biomarker measured: hair lead Analysis: ICP-AES	Units: µg/g <0.1-0.9 : 13.5% 1-1.9: 40.8% 2-2.9: 25.6% 3-3.9: 9.0% ≥4: 11.1%	Study examined associations between hair lead levels and attention-deficit behaviors.
Europe			
Annesi-Maesano et al. (2003) France 1985, 1991-1992	Design: cross-sectional Subjects: mother (mean age 29 yr)-infant pairs (n:374) Biomarker measured: hair lead Analysis: ICP-AES	Units: µg/g Arithmetic mean (SD): Infant: 1.38 (1.26) Mother: 5.16 (6.08)	Mean blood lead concentrations were 96 µg/dL (SD 58) in mothers and 67 (SD 48) in infant cord blood. Infant hair-cord blood lead correlation (Spearman, r) was 0.21 ($p < 0.01$).
Drasch et al. (1997) Germany 1993-1994	Design: cross-sectional Subjects: adults (n = 150, 75 males; age range: 16-93 yrs) from general population with no known occupational exposure Biomarker measured: hair lead (post-mortem) Analysis: ET-AAS	Units: µg/g Median (range): 0.76 (0.026-20.6) 25-75th Pct range: 0.45-1.48	Median blood lead (µg/dL) was 2.8 (range <0.9-16.1). Median temporal bone lead was 2.84 µg/g (range 0.25-22.3), Hair lead correlation (Spearman r) was 0.35 ($p < 0.001$) for blood, 0.10 ($p > 0.05$) for temporal bone, and 0.16 ($p > 0.05$) for body burden.0.512 for liver ($p = 0.003$) and 0.57 ($p = 0.001$) for kidney.
Gerhardsson et al. (1995) Sweden NR	Design: cross-sectional Subjects: adult male smelter workers (n = 32) and referents (n = 10) Biomarker measured: hair lead (post-mortem) Analysis: XRF	Units: µg/g Median (range): Active workers: 8.0 (1.5-29,000) Retired workers: 2.6 (0.6-9.3) Reference: 2.05 (0.3-96)	Based on reported a cumulative annual blood lead index of 1,374 µg/dL and average duration of employment of 31.4 yrs, average blood lead may have been approximately 44 µg/dL in workers. Hair lead correlation (Spearman, r).

Table AX6-2.12 (cont'd). Summary of Selected Measurements of Hair Lead Levels in Humans

Reference, Study Location, and Period	Study Description	Hair Lead Measurement	Comment
Europe (cont'd)			
Esteban et al. (1999) Russia 1996	Design: cross-sectional Subjects: children (n = 189, 110 females; age rage 1.9-10.6 yr) living in the vicinity of lead battery and leaded glass manufacture facilities. Biomarker measured: hair lead Analysis: ICP-AES	Units: ng/g Geometric mean (range): 5.4 (1-39.2) 90^{th} Pct: ~15	Geometric mean blood lead was 8.5 µg/dL (range 3.1-35.7) log blood lead = 1.44 + 0.35 (log hair) + 0.24 (gender), $r^2 = 0.20$.

AAS - atomic absorption spectroscopy; ET-AAS - electro-thermal atomic absorption spectrometry; GFAAS - graphite furnace atomic absorption spectroscopy; HHANES - Hispanic Health and Nutrition Examination Survey; ICP-AES - inductively coupled plasma/atomic emission spectroscopy; ICP-MS - inductively coupled plasma-mass spectrometry; NR - not reported; Pct - percentile.

CHAPTER 6 ANNEX

ANNEX TABLES AX6-3

Table AX6-3.1. Prospective Longitudinal Cohort Studies of Neurocognitive Ability in Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States			
Bellinger et al. (1992) U.S.	148 subjects from the Boston Prospective Study were re-evaluated at 10 years of age. The WISCR was used to index intellectual status. Extensive assessment of medical and sociodemographic covariates.	Cord and serial postnatal blood lead assessments. Cord blood lead grouping <3, 6-7, >10 µg/dL. Blood lead at 2 years 6.5 (SD 4.9) µg/dL	Increase of 10 µg/dL in blood lead level at age two was associated with a decrement of approximately 6 IQ points. Relationship was stronger for verbal compared to performance IQ. Prenatal exposure to lead as indexed by cord blood lead levels was unrelated to psychometric intelligence.
Dietrich et al. (1991, 1992, 1993); Ris et al. (2004) U.S.	253-260 children followed since birth in the Cincinnati Lead Study were re-evaluated at 4, 5, and 6.5 years of age. At 4 and 5 years the KABC, was used to index intellectual status. At 6.5 years, the WISCR was administered. At 15-17 years of age, 195 Cincinnati Lead Study subjects were re-evaluated with a comprehensive neuropsychological battery that yielded a "Learning/IQ" factor in a principal components analysis. Extensive assessment of medical and sociodemographic covariates.	Prenatal (maternal) and serial postnatal blood lead assessments. Prenatal blood lead 8.3 (SD 3.7) µg/dL Blood lead at 2 years 17.4 (SD 8.8) µg/dL	Few statistically significant relationships between blood lead indices and covariate-adjusted KABC scales at 4 and 5 years of age. One KABC subscale that assesses visual-spatial skills was associated with late postnatal blood lead levels following covariate adjustment. After covariate adjustment, average postnatal blood lead level was significantly associated with WISCR performance IQ at 6.5 years. Blood lead concentrations in excess of 20 µg/dL were associated with deficits in performance IQ on the order of 7 points compared with children with mean blood lead concentrations of less than 10 µg/dL. At 15-17 years, late childhood blood lead levels were significantly associated with lower covariate-adjusted Learning/IQ factor scores.
Canfield et al. (2003) U.S.	172 predominantly African-American, lower socioeconomic status children in Rochester, NY followed since they were 5 to 7 months were evaluated at 3 and 5 years. An abbreviated form of the Stanford-Binet Intelligence Scale-4 (SBIS-4) was used to index intellectual status. Extensive assessment of medical and sociodemographic covariates.	Serial postnatal blood lead Blood lead at 2 years 9.7 µg/dL	Following covariate adjustment, there was a significant inverse relationship between blood lead indices and IQ at all ages. Overall estimate indicated that an increase in average lifetime blood lead concentration of 1 µg/dL was associated with a loss of ½ IQ point. Effects were stronger for subjects whose blood lead levels never exceeded 10 µg/dL. Semiparametric analysis indicated a decline in IQ of 7.4 points for a lifetime average blood lead concentration up to 10 µg/dL while for levels between 10 and 30 µg/dL a more gradual decrease in IQ was estimated. Authors concluded that the most important aspect of their findings was that effects below 10 µg/dL that have been observed in previous cross-sectional studies have been confirmed in a rigorous prospective investigation.

Table AX6-3.1 (cont'd). Prospective Longitudinal Cohort Studies of Neurocognitive Ability in Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Bellinger and Needleman (2003) U.S.	Reanalysis of data from the Boston Prospective Study focusing on 48 subjects at 10 years of age whose blood lead levels never exceeded 10 µg/dL. WISCR was used to index intellectual status. (see Bellinger, et al. (1992)	Serial postnatal blood lead Blood lead at 2 years 6.5 (SD 4.9) µg/dL	IQ was inversely related to two-year blood lead levels following covariate adjustment. Blood lead coefficient (-1.56) was greater than that derived from analyses including children with concentrations above 10 µg/dL (-0.58). Authors conclude that children's IQ scores are reduced at lead levels still prevalent in US
Chen et al. (2005) U.S.	Repeat measure psychometric data on 780 children enrolled in the Treatment of Lead-Exposed Children (TLC) clinical trial for were analyzed to determine if blood lead concentrations at 2 years of age constitute a critical period of exposure for the expression of later neurodevelopmental deficits. Data for placebo and active drug groups were combined in these analyses, which spanned the ages of approximately 2 to 7 years of age. Measures of intellectual status included the Bayley Mental Development Index (MDI), and full scale IQ derived from age-appropriate Wechsler scales.	Blood lead Range 20-44 µg/dL Baseline blood lead 26 (SD 26.5) µg/dL in both drug and placebo groups. Blood lead at 7 years 8.0 (SD 4.0) µg/dL	Association between blood lead and psychometric intelligence increased in strength as children became older, whereas the relation between baseline (2 year) blood lead and IQ attenuated. Peak blood lead concentration thus does not fully account for the observed association in older children between their lower blood lead concentrations and IQ. The effect of concurrent blood lead on IQ may therefore be greater than currently believed. Authors conclude that these data support the idea that lead exposure continues to be toxic to children as they reach school age, and does not lend support to the interpretation that majority of the damage is done by the time the child reaches 2 to 3 years of age.

Table AX6-3.1 (cont'd). Prospective Longitudinal Cohort Studies of Neurocognitive Ability in Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe			
Wasserman et al. (1992, 1994, 2003); Factor-Litvak et al. (1999) Yugoslavia	Birth cohort of approximately 300-400 infants followed since birth residing in two towns in Kosovo, Yugoslavia, one group near a longstanding lead smelter and battery manufacturing facility and another in a relatively unexposed location 25 miles away. Intellectual status was monitored from 2 to 10-12 years of age with the Bayley Scales of Infant Development, McCarthy Scales of Children's Abilities, and WISCIII. Extensive assessment of medical and sociodemographic covariates.	Maternal prenatal, umbilical cord and serial postnatal blood lead Maternal blood lead in: exposed area 19.9 (SD 7.7) µg/dL, unexposed area 5.6 (SD 2.0) µg/dL Umbilical cord blood lead in: exposed area 22.2 (SD 8.1) µg/dL, unexposed area 5.5 (SD 3.3) µg/dL Blood lead at 2 years in: exposed area 35.4 µg/dL, unexposed area 8.5 µg/dL	Rise in postnatal blood lead from 10 to 30 µg/dL at two years of age associated with a covariate-adjusted decline of 2.5 points in Bayley MDI. Maternal and cord blood lead not consistently associated with Bayley outcomes. Higher prenatal and cord blood lead concentrations associated with lower McCarthy General Cognitive Index (GCI) scores at 4 years. Scores on the Perceptual-Performance subscale particularly affected. After covariate-adjustment, children of mothers with prenatal blood lead levels >20 µg/dL scored a full standard deviation below children in the lowest exposure group (<5 µg/dL prenatal blood lead). Postnatal blood lead also associated with poorer performance. Increase in blood lead level from 10-25 µg/dL was associated with a reduction of 3.8 points in GCI after covariate-adjustment. Effects even more pronounced on the Perceptual-Performance subscale. At 7 years, significant inverse associations between lifetime average blood lead and WISCIII IQ were observed, with consistently stronger associations with Performance IQ and later blood lead measures. Adjusted intellectual loss associated with an increase in lifetime average blood lead from 10-30 µg/dL was over 4 points in WISCIII Full-Scale and Performance IQ. At 10-12 years, subjects were again assessed with the WISCIII. Following covariate-adjustment, average lifetime blood lead was associated with all components of the WISCIII with effect sizes similar to those observed at 7 years. In most instances, bone lead-IQ relationships were stronger than those for blood lead among subjects residing near the lead smelter.

Table AX6-3.1 (cont'd). Prospective Longitudinal Cohort Studies of Neurocognitive Ability in Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Latin America			
Schnaas et al. (2000) Mexico	112 children followed since birth with complete psychometric data from the Mexico City Prospective Study were examined. Intellectual status was indexed with the General Cognitive Index (GCI) from the McCarthy Scales of Children's Abilities (MSCA). Purpose of the study was to determine if the magnitude of the effect of postnatal blood lead levels on cognition varies with the time between blood lead and cognitive assessments.	Serial postnatal blood lead Average blood lead 24-36 months 9.7 (range 3-48) µg/dL.	A number of significant interactions observed between blood lead levels and age of assessment. Greatest effect observed at 48 months where a 5.8 deficit in adjusted GCI scores was observed for each natural log increment in blood lead. Authors concluded that four to five years of age appears to be a critical period for the manifestation of earlier postnatal blood lead level effects on cognition.
Gomaa et al. (2002) Mexico	197 two year-olds residing in Mexico City followed since birth. The Bayley Scales of Infant Development Mental Development Index (MDI) was used to index intellectual status. Extensive assessment of medical and sociodemographic covariates.	Umbilical cord and serial postnatal blood lead Umbilical cord blood lead 6.7 (SD 3.4) µg/dL Blood lead at 2 years 8.4 (SD 4.6) µg/dL. Maternal tibial and patellar bone lead Patellar (trabecular) bone lead 17.9 (SD 15.2) µg/g	Umbilical cord blood lead and patellar (trabecular) bone lead were significantly associated with lower scores on the Bayley MDI. Maternal trabecular bone lead levels predicted poorer sensorimotor functioning at two years independent of the concentration of lead measured in cord blood. Increase in cord blood lead level from 5-10 µg/dL was associated with a 3.1 point decrement in adjusted MDI scores. In relation to lowest quartile of trabecular bone lead, the second, third, and fourth quartiles were associated with 5.4, 7.2, and 6.5 decrement in MDI following covariate adjustment. Authors concluded that higher maternal trabecular bone lead concentrations constitute an independent risk factor for impaired mental development in infancy, likely due to the mobilization of maternal bone lead stores over gestation.

Table AX6-3.1 (cont'd). Prospective Longitudinal Cohort Studies of Neurocognitive Ability in Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Australia			
Baghurst et al. (1992); McMichael et al. (1994); Tong et al. (1996) Australia	400-500 subjects residing in and near Port Pirie, Australia and followed since birth were re-evaluated at 7 to 8 and 11-13 years of age. WISCR was used to index intellectual status at both ages. Extensive assessment of medical and sociodemographic covariates.	Maternal prenatal, umbilical cord and serial postnatal blood lead Antenatal average blood lead 10.1 (SD 3.9) µg/dL Umbilical cord blood lead 9.4 (SD 3.9) µg/dL Blood lead at 2 years geometric mean 21.3 (SD 1.2) µg/dL Deciduous central incisor whole tooth lead Tooth lead geometric 8.8 (SD 1.9) µg/g	Significant decrements in covariate-adjusted full scale IQ were observed in relationship to postnatal blood lead levels at both ages. At seven to eight years a loss of 5.3 points was associated with an increase in blood lead from 10 to 30 µg/dL. At 11-13 years mean full scale IQ declined by 3.0 points for an increase in lifetime average blood lead concentrations from 10 to 20 µg/dL. Lead levels in central upper incisors were also associated with lower 7-8 year IQ following covariate adjustment. Adjusted estimated decline in IQ across the range of tooth lead from 3 to 22 ppm was 5.1 points.
Cooney et al. (1991) Australia	175 subjects from the Sydney, Australia Prospective Study were assessed at 7 years of age. The WISCR was used to index intellectual status. Extensive assessment of medical and sociodemographic characteristics.	Maternal and cord blood lead Cord blood lead 8.4 µg/dL (SD not given) Blood lead at 2 year 15.8 µg/dL (SD not given)	Blood indices of lead exposure were not associated with any measure of psychometric intelligence. Authors conclude that the evidence from their study indicates that if developmental deficits do occur at blood lead levels <25 µg/dL, the effect size is likely to be small (<5%). Sydney results are difficult to interpret from the statistical presentation in their report. It is not clear which covariates were entered into regression analyses nor is the empirical or substantive basis for their conclusion.
Asia			
Shen et al. (1998) China	Pregnant women and newborns in Shanghai, China recruited from health care facilities in the community on the basis of cord blood lead concentration percentiles (30^{th} and 70^{th}) yielding a total N of 173 subjects. The Bayley Scales of Infant Development Mental Development Index (MDI) and Psychomotor Development Index (PDI) were used to index sensorimotor/intellectual status at 3, 6, and 12 months. Extensive assessment of medical and sociodemographic characteristics.	Cord blood lead Cord blood lead "high group" 13.4 (SD 2.0) µg/dL "low group" 5.3 (SD 1.4) µg/dL Blood lead at 1 year "high group" 14.9 (SD 8.7) µg/dL "low group" 14.4 (SD 7.7) µg/dL	At all ages the Bayley MDI was associated with cord blood lead groupings following adjustment for covariates. Postnatal blood lead unrelated to any Bayley measures. Differences in MDI between prenatal blood lead exposure groupings generally in accord with similar investigations in Boston, Cincinnati, and Cleveland. Authors conclude that the adverse effects of prenatal lead exposure are readily discernible and stable over the first year of life.

Table AX6-3.2. Meta- and Pooled-Analyses of Neurocognitive Ability in Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States			
Lanphear et al. (2005) International	Pooled analysis of seven international prospective studies involving 1,333 school-age children. Primary outcome measure was full-scale IQ as assessed by age-appropriate Wechsler scale. Measures of exposure were concurrent, peak, average lifetime and "early" blood lead (i.e. mean blood lead from 6-24 months). Cord blood lead was also investigated for those studies that collected these samples at birth. Multivariate regression models were developed adjusting for site as well as 10 common covariates. Blood lead measure with the largest adjusted R^2 was nominated a priori as the preferred index related lead exposure to IQ in subsequent analyses. Results evaluated by applying a random-effects model.	Umbilical cord blood lead Serial postnatal blood lead Lifetime average blood lead 12.4 (range 4.1-34.8) µg/dL	Concurrent blood lead level exhibited the strongest relationship with IQ, although results of regression analyses for all blood lead variables were similar. Steepest declines in IQ were at blood lead concentrations below 10 µg/dL. For the entire pooled data set, a decline of 6.2 IQ points (95% CI: 3.8-8.6) was observed for an increase in blood lead from 1-10 µg/dL.
Needleman and Gatsonis (1990) International	Meta analysis of 12 studies chosen on the basis of quality—covariate assessment and application of multiple regression techniques. Studies weighted on basis of sample size. Studies divided according to tissue analyzed (blood or teeth). Joint p-values and average effect sizes calculated using two different methods.	Blood lead Tooth lead	Joint p-values for blood lead studies were <0.0001 for both methods while for teeth joint p-values of <0.0006 and <0.004 were obtained. Partial correlations ranged from –0.27 to –0.0003. No single study was responsible for the significance of the final findings. Authors concluded that the hypothesis that lead lowers children's IQ at relatively low dose is strongly supported by results of this quantitative review.
Schwartz (1994) International	Meta analysis of 7 recent studies relating blood lead to IQ were reviewed, three longitudinal and four cross-sectional. Measure of effect was estimated decrease in IQ for an increase in blood lead from 10-20 µg/dL. Studies were weighted by the inverse of the variances using random	Blood lead	Estimated decrease in IQ for increase in blood lead from 10-20 µg/dL was –2.6 points (SE 0.41). Results were not determined by any individual study. Effect estimates similar for longitudinal and cross-sectional studies. For studies with mean blood lead concentrations below 15 µg/dL estimated effect sizes were larger. When the study with the lowest exposures was examined alone using nonparametric smoothing (Boston), no evidence of a threshold was observed down to a blood lead level of 1 µg/dL. Author concludes that these data provide further evidence of lead effects on cognition at levels below 10 µg/dL.

Table AX6-3.2 (cont'd). Meta- and Pooled-Analyses of Neurocognitive Ability in Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Pocock et al. (1994) International	Meta-analysis of five prospective and fourteen cross-sectional studies (including tooth and blood tissues) were included. The fixed effect method of Thompson and Pocock (1992) was employed. Only blood lead at or near two years of age was considered for the prospective studies.	Blood lead Tooth lead	Overall conclusion was that a doubling of blood lead levels from 10-20 µg/dL, or tooth lead from 5-10 µg/g was associated with an average estimated deficit in IQ of around 1-2 points. Authors caution interpretation of these results and lead literature in general citing questions surrounding the representativeness of the samples, residual confounding, selection bias, and reverse causality.

Table AX6-3.3. Cross-sectional Studies of Neurocognitive Ability in Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States			
Lanphear et al. (2000) U.S.	4,853 US children ages six to 16 years enrolled in NHANES-III. Two subtests of the WISC-R (Block Design and Digit Span) used to assess intellectual status. Medical and sociodemographic covariates were assessed	Blood lead at time of testing Geometric blood Lead 1.9 (SE 0.1) µg/dL 2.1% with blood lead ≥10 µg/dL	Multivariate analyses revealed a significant association between blood lead levels and both WISC-R subtests. Associations remained statistically significant when analyses were restricted to children with blood lead levels below 10 µg/dL. Authors caution that lack of control for parental intelligence and variables like the HOME scale should temper any conclusions regarding observed effects.
Emory et al. (2003) U.S.	77 healthy, lower-risk African-American infants age 7 months. The Fagan Test of Infant Intelligence (FTII) was administered to assess intellectual status. Birth weight and gestational age examined as potential covariates/confounders.	Maternal blood lead Blood lead 0.72 (SD 0.86) µg/dL	Infants scoring in the upper 5th to 15th percentiles for the FTII had mother with significantly lower maternal blood lead levels when compared to those scoring in the lower 5th or 15th percentile. Findings of this study should be considered preliminary due to small sample size and lack of covariate assessment or control.
Chiodo et al. (2004) U.S.	237 African-American inner-city children assessed at 7.5 years of age. Cohort was derived from a larger study of the effects of prenatal ETOH exposure on child development. 83% of children in lead study had little or no gestational exposure to ETOH. WISC-III was administered to assess intellectual status. Medical and sociodemographic covariates were assessed.	Blood lead at time of testing Blood lead 5.4 (SD 3.3) µg/dL	Following covariate adjustment statistically significant relationships between blood lead and full-scale, verbal and performance IQ were observed. Significant effects of lead on full-scale and performance IQ was evident at blood lead concentrations below 7.5 µg/dL.
Europe			
Walkowiak et al. (1998) Germany	384 six-year-old children in three German cities. Two subtests of the WISC (Vocabulary and Block Design) used to estimate IQ. Both subscales were combined to form a "WISC Index." Medical and sociodemographic covariate covariates were assessed.	Blood lead at time of testing Blood lead 4.2 µg/dL 95th percentile 8.9 µg/dL	Following covariate-adjustment, WISC Vocabulary was significantly associated with blood lead but combined WISC index was borderline. Authors conclude that findings roughly correspond with those of other studies that find effects below 10 µg/dL but caution that potentially important covariates such as HOME scores were not controlled.
Prpic-Majic et al. (2000) Croatia	275 third and fourth grade students in Zagreb, Croatia. WISC-R was administered to assess intellectual status. Covariate factors limited to parents' educational status and gender of child.	Blood lead at time of testing Blood lead 7.1 (SD 1.8) µg/dL	Following covariate adjustment, no statistically significant associations were observed for lead or other indicators of toxicity (ALAD, EP) on WISC-R. Authors argue that study had sufficient power and that the "no-effect" threshold for lead must be in the upper part or above the study's range of exposures.

Table AX6-3.3 (cont'd). Cross-sectional Studies of Neurocognitive Ability in Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Latin America			
Kordas et al. (2004) Mexico	602 first grade children in public schools in a highly industrialized area of northern Mexico. Premise of study was that effects of lead could be explained by correlated nutritional factors such as iron status, anemia, and growth. Peabody Picture Vocabulary Test-Revised (PPVT-R), Cognitive Abilities Test (CAT), and an abbreviated form of the WISC-R were administered to assess intellectual status. Medical and sociodemographic covariates were assessed.	Blood lead at time of testing Blood lead 11.5 (SD 6.1) µg/dL	Following covariate adjustment blood lead levels were significantly associated with poorer performance on the PPVT-R, WISC-R Coding, and Number and Letter Sequencing. Authors concluded that lead's association with iron deficiency anemia or growth retardation could not explain relationship between lead and cognitive performance. Authors acknowledge study's limitations in that parental intelligence and quality of caretaking in home were not directly assessed as potentially confounding variables.
Counter et al. (1998) Ecuador	77 chronically lead-exposed children living in Ecuadorian villages where lead is used extensively in commercial ceramics production. Ravens Colored Progressive Matrices (RCPM) used to index intellectual status. Only half of the sample was assessed. No assessment of medical or sociodemographic covariates.	Blood lead at time of testing Blood lead 47.4 (SD 22) µg/dL	Simple regression analysis revealed a correlation between blood lead and RCMP of only borderline significance. Results difficult to interpret because there was no attempt to age-adjust. When analysis restricted to children 9-11 years of age, a highly significant negative correlation was obtained. Study has little relevance to the question of lead hazards in the US because of unusually high levels of exposure.
Asia			
Rabinowitz et al. (1991) Taiwan	443 children in grades one to three in Taipei City and three schools near lead smelters. Ravens Colored Progressive Matrices (RCMP) used to index intellectual status. Medical and sociodemographic covariate factors were assessed.	Dentin tooth lead Taipei City 4.3 (SD 3.7) µg/g Smelter areas 6.3 (SD 3.3) µg/g	Scores on the RCMP were negatively correlated with tooth lead concentrations. In multivariate analyses, parental education was the most important predictor of RCMP scores, but tooth lead concentrations still significantly predicted lower scores in females residing in low-income families.
Bellinger et al. (2005) India	74 four to fourteen year-old children residing in Chennai, India were enrolled in the study, 31 of which were assessed with the Binet-Kamath Intelligence test. Data were collected on sociodemographic features of subjects' families.	Blood lead at time of testing Blood lead 11.1 (SD 5.6) µg/dL	Covariate-adjusted blood lead coefficient was negative but nonsignificant, perhaps due to small sample size and highly variable performance of subjects with the least elevated blood lead concentrations.

Table AX6-3.4. Effects of Lead on Academic Achievement in Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States			
Lanphear et al. (2000) U.S.	Design: Cross-sectional. 4,853 US children ages six to 16 years enrolled in NHANES-III. Subjects were administered the Arithmetic and Reading subtests of the Wide Range Achievement Test-Revised (WRATR). A number of medical and sociodemographic covariates were assessed and entered into multivariable models.	Blood lead at time of testing Geometric blood lead 1.9 (SE 0.1) µg/dL. 2.1% with blood lead ≥10 µg/dL	Following covariate adjustment, a statistically significant relationship between blood lead and WRATR performance was found. A 0.70 point decrement in Arithmetic scores and a 1 point decrement in Reading scores for each 1 µg/dL increase in blood lead concentration was observed. Statistically significant inverse relationships between blood lead levels and performance for both Reading and Arithmetic subtests were found for children with blood lead concentrations < 5 µg/dL. Authors concluded that results of these analyses suggest that deficits in academic skills are associated with blood lead concentrations lower than 5 µg/dL. They cautioned, however, that two covariates that have been shown to be important in other lead studies (i.e., parental IQ and HOME scores) were not available. This may have over or under estimated deficits in academic skills related to lead. They further caution that, as with all cross-sectional studies utilizing blood lead as the index of dose it is not clear whether deficits in academic skills were due to lead exposure that occurred sometime during early childhood or due to concurrent exposure. Nevertheless, concurrent blood lead levels reflect both ongoing exposure and preexisting body burden.

Table AX6-3.4 (cont'd). Effects of Lead on Academic Achievement in Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Needleman et al. (1990) U.S.	Design: Prospective cohort. Re-examination of the Chelsea and Somerville cohort recruited in the 1970's (Needleman et al., 1979). 132 adolescents were recalled. Large battery of tests was administered to examine neurobehavioral deficits and academic achievement in high school and shortly following graduation. Extensive assessment of medical and sociodemographic covariates.	Tooth (dentin) lead Tooth lead median 8.2 µg/g	Subjects with dentin lead levels >20 ppm were at higher risk of dropping out of high school (adjusted OR = 5.8, 95% CI: 1.4-40.7) and of having a reading disability (adjusted OR: 5.8, 95% CI: 1.7-19.7). Higher dentin lead levels were also significantly associated with lower class standing, increased absenteeism, and lower vocabulary and grammatical reasoning scores on the Neurobehavioral Evaluation System (NES). Authors conclude that undue exposure to lead has enduring and important effects on objective parameters of success in life.
Bellinger et al. (1992) U.S.	Design: Prospective longitudinal. 148 children in the Boston Lead Study cohort were examined at 10 years of age. The short-form of the Kaufman Test of Educational Achievement (KTEA) was used to assess academic achievement. Primary outcome was the Battery Composite Score. Extensive assessment of medical and sociodemographic covariates.	Cord and serial postnatal blood lead assessments. Cord blood lead grouping <3, 6-7, >10 µg/dL. Blood lead at 2 years 6.5 (SD 4.9) µg/dL	After covariate-adjustment, blood lead levels at 24 months were significantly predictive of lower academic achievement (β = -0.51, SE 0.20). Battery Composite Scores declined by 8.9 points for each 10 µg/dL increase in blood lead. This association was significant after adjustment for IQ. Authors conclude that lead-sensitive neuropsychological processing and learning factors not reflected in measures of global intelligence may contribute to deficits in academic achievement.
Leviton et al. (1993) U.S.	Design: Prospective cohort. Teachers of approximately 2000 eight year-old children born in 1 hospital in Boston between 1979 and 1980 filled out the Boston Teachers Questionnaire (BTQ) to assess academic performance and behavior. Limited information is provided on the assessment of covariate factors but a number were considered and controlled for in multivariable analyses.	Cord blood lead Cord blood lead 6.8 µg/dL Tooth (dentin) lead Tooth lead 2.8 µg/g	Following adjustment for potential confounding variables, elevated dentin lead concentrations were associated with statistically significant reading and spelling difficulties as assessed by the BTQ among girls in the sample. Authors conclude that their findings support the case for lead-associated learning problems at levels that were prevalent at that time in the general population. However, authors add that the inability to assess child-rearing quality in this study conducted by mail limits the inferences that can be drawn.

Table AX6-3.4 (cont'd). Effects of Lead on Academic Achievement in Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Australia			
Fergusson et al. (1993, 1997); Fergusson and Horwood (1993) New Zealand	Design: Prospective cohort. Academic performance was examined in a birth cohort of 1200 New Zealand children enrolled in the Christchurch Health and Development Study. Measures of academic performance at 12-13 years included the Brut Reading Test, Progressive Achievement Test, Test of Scholastic Abilities, and teacher ratings of classroom performance in the areas of reading, writing, and mathematics. The growth of word recognition skills from 8 to 12 years was also examined using growth curve modeling methods. Academic achievement in relationship to lead was re-examined in this cohort at 18 years. Measures of academic achievement included the Burt Reading Test, number of years of secondary education, number of certificates passed (based on national examinations), and leaving school without formal qualifications (failing to graduate). Extensive assessment of medical and social covariates.	Tooth (dentin) lead Tooth lead 6.2 (SD 6.2) μg/g	Following covariate adjustment, dentin lead levels were significantly associated with virtually every formal index of academic skills and teacher ratings of classroom performance in 12-13 year-olds. After adjustment for covariates, tooth lead levels greater than 8 μg/g were associated with significantly slow growth in word recognition abilities with no evidence of catch up. At 18 years, tooth lead levels were significantly associated with lower reading test scores, having a reading level of less than 12 years, failing to complete three years of high school, leaving school without qualifications, and mean number of School Certificates passed. Authors conclude that early exposure to lead is independently associated with detectable and enduring deficits in children's academic abilities. They further conclude that their findings are particularly significant in that they confirm the findings of Needleman (1990), albeit in a cohort with lower levels of exposure to environmental lead.

Table AX6-3.4 (cont'd). Effects of Lead on Academic Achievement in Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia			
Wang et al. (2002) Taiwan	Design: Cross-sectional. 934 third graders living in an urban industrial area of Taiwan. Outcome variables were grades for Chinese (reading, writing), mathematics, history, and natural science. Grades were converted into individual class rankings to avoid teacher bias. Limited data on medical and sociodemographic covariates.	Blood lead at time of evaluation Blood lead 5.5 (SD 1.9) µg/dL	Following covariate adjustment, blood lead was significantly associated with lower class ranking in all academic subjects. Major shortcoming of this study is lack of control for potentially important covariates such as parental IQ. However, the relatively low levels of exposure in this sample and strength and consistency of the reported relationships suggest that lead may be playing some role in lowering academic performance.
Rabinowitz et al. (1992) Taiwan	Design: Cross-sectional. Teachers of 493 children in grades 1-3 filled out the Boston Teachers Questionnaire (BTQ) to assess academic performance and behavior. Sociodemographic and medical covariate factors were assessed.	Tooth (dentin) lead Tooth lead 4.6 (SD 3.5) µg/g	Prior to adjustment for covariates, girls with higher exposures to lead evinced a borderline significant trend for reading difficulties while byes displayed significantly increased difficulties with respect to activity levels and task attentiveness. In logistic regression models that include significant covariate factors, the tooth lead terms failed to achieve statistical significance. Authors conclude that lead levels found in the teeth of children in this Taiwanese sample are not associated with learning problems as assessed by the BTQ.

Table AX6-3.5. Effects of Lead on Specific Cognitive Abilities in Children — Attention/Executive Functions, Learning, and Visual-Spatial Skills

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States			
Bellinger et al. (1994) U.S.	Design: Prospective cohort. 79 subjects from the original Chelsea and Somerville, MA lead study were re-evaluated at 19-20 years of age with the Mirsky battery of attentional measures. Extensive measures of medical and sociodemographic covariates.	Tooth (dentin) lead Tooth lead 13.7 (SD 11.2 µg/g) KXRF Bone lead Tibial bone lead (range <1 - >10 µg/g) Patellar bone lead (range <1 - > 15 µg/g)	Higher tooth lead concentrations were significantly associated with poorer scores on the Focus-Execute and Shift factors of the Mirsky battery. Few significant associations were observed between bone lead levels and performance. Authors conclude that early lead exposure may be associated with poorer performance on executive/regulatory functions, which are thought to depend on the frontal or prefrontal regions of the brain.
Stiles and Bellinger (1993) U.S.	Design: Prospective longitudinal. 148 subjects from the Boston Lead Study were re-evaluated at 10 years of age with an extensive neuropsychological battery. Tests included the California Verbal Learning Test, Wisconsin Card Sorting Test, Test of Visual-Motor Integration, Rey-Osterieth Complex Figure, Story Recall, Finger Tapping, and Grooved Pegboard. Extensive measures of medical and sociodemographic covariates.	Cord and serial postnatal blood lead assessments. Cord blood lead grouping <3, 6-7, >10 µg/dL. Blood lead at 2 years 6.5 (SD 4.9) µg/dL	Authors point out that the number of significant associations was about equal to those that would be expected by chance. However, tasks that assess attentional behaviors and executive functions tended to among those for which lead was a significant predictor of performance. Following covariate adjustment, higher blood lead concentrations at two year were significantly associated with lower scores on Freedom from Distractibility factor of the Wechsler scales, increase in percentage of perseverative errors on the Wisconsin Card Sorting Test and the California Verbal Learning Test.
Canfield et al. (2003, 2004) U.S.	Design: Prospective longitudinal. 170-174 children from the Rochester Lead Study were administered a number of learning and neuropsychological functioning at 48, 54, and 66 months of age. At 48 and 54 months the Espy Shape School Task was administered while at 66 months the Working Memory and Planning assessment protocols of the Cambridge Neuropsychological Test Automated Battery (CANTAB) was given. Extensive measures on medical and sociodemographic covariates.	Serial postnatal blood lead Blood lead at 2 years 9.7 µg/dL Lifetime average blood lead 7.2 µg/dL (range 0-20 µg/dL)	Following covariate adjustment, blood lead level at 48 months was negatively associated with children's focused attention while performing the Shape School Tasks, efficiency at naming colors, and inhibition of automatic responding. Children with higher blood lead concentrations also completed fewer phases of the Espy tasks and knew fewer color and shape names. On the CANTAB battery, children with higher lifetime average blood lead levels showed impaired performance on spatial working memory, spatial memory span, and cognitive flexibility and planning. Authors conclude that the effects of pediatric lead exposure are not restricted to global measures of intellectual functioning and executive processes may be at particular risk.

Table AX6-3.5 (cont'd). Effects of Lead on Specific Cognitive Abilities in Children — Attention/Executive Functions, Learning, and Visual-Spatial Skills

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Ris et al. (2005) U.S.	Design: Prospective longitudinal. 195 subjects from the Cincinnati Lead Study were administered an extensive and comprehensive neuropsychological battery at 16-17 years of age. Domains assessed included Executive Functions, Attention, Memory, Achievement, Verbal Skills, Visuoconstructional, and Fine Motor. Factor scores transformed to ranks derived from a principal components factor analysis of the neuropsychological test scores were the primary outcome variables. Extensive measures on medical and sociodemographic covariates.	Prenatal (maternal) and serial postnatal blood lead assessments. Prenatal blood lead 8.3 (SD 3.7) µg/dL Blood lead at 2 years 17.4 (SD 8.8) µg/dL	Following covariate adjustment, strongest associations between lead exposure and performance were observed for factor scores derived from the Attention component, which included high loadings on variables from the Conners Continuous Performance Test. This relationship was strongest in males. Authors speculate that since the incidence of Attention Deficit/Hyperactivity Disorder is greater in males in general, early exposure to lead may exacerbate a latent potential for such problems.

Table AX6-3.6. Effects of Lead on Disturbances in Behavior, Mood, and Social Conduct in Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States			
Sciarillo et al. (1992) U.S.	Design: Cross-sectional. 150 2-5 year-old children in Baltimore separated into "high" (2 consecutive blood lead levels >15 µg/dL) and "low" groups. Mothers filled out the Achenbach Child Behavior Checklist (CBCL). The Center for Epidemiologic Studies Depression Scale (CESD) was administered to mothers as a control measure.	Screening Blood leads at various times before assessment. Blood lead high group 28.6 (SD 9.3) µg/dL, blood lead low group 11.3 (SD 4.3) µg/dL	When compared to lower exposed group, children in the high group had a significantly higher CBCL Total Behavior Problems Score (TBPS) and Internalizing and Externalizing scores. After adjustment for maternal depression, blood lead concentrations were still significantly associated with an increase in the TBPS. Children in high group were nearly 3 times more likely to have a TBPS in the clinical range. A significantly higher percentage of children in the high group scored in the clinical range for CBCL subscales measuring aggressive and destructive behavioral tendencies.
Bellinger et al. (1994) U.S.	Design: Prospective cohort: 1782 children born within a 1-year period at a single Boston hospital were examined at 8 years of age. Teachers filled out the Achenbach Child Behavior Profile (ACBP). Medical and sociodemographic characteristics assessed by questionnaire and chart review.	Umbilical cord blood lead Cord blood lead 6.8 (SD 3.1) µg/dL Tooth (dentin) lead 3.4 (SD 2.4) µg/g	Cord blood lead levels were not associated with the prevalence or nature of behavioral problems reported by teachers. Tooth lead levels were significantly associated with ACBP Total Problem Behavior Scores (TPBS). Statistically significant tooth lead-associated increases in both Externalizing and Internalizing scores were observed. Each log unit increase in tooth lead was associated with a 1.5-point increase in T scores for these scales. Authors caution that residual confounding cannot be ruled out because of the lack of information on parental psychopathology or observations of the family environment. However, these results are in accord with other studies that social and emotional dysfunction may be an important expression of elevated lead levels during early childhood.
Denno (1990) U.S.	Design: Prospective cohort. Survey of 987 Philadelphia African-American youths enrolled in the Collaborative Perinatal Project. Data available from birth through 22 years of age. Analysis considered 100 predictors of violent and chronic delinquent behavior.	Blood lead Values not provided	Repeat offenders presented consistent features such as low maternal education, prolonged male-provider unemployment, frequent moves, and higher lead intoxication. In male subjects, a history of lead poisoning was among the most significant predictors of delinquency and adult criminality.

Table AX6-3.6 (cont'd). Effects of Lead on Disturbances in Behavior, Mood, and Social Conduct in Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Needleman et al. (1996) U.S.	Design: Prospective cohort. 850 boys enrolled in the Pittsburgh Youth Study were prescreened to assess delinquent behavioral tendencies. Subjects who scored in the 30th percentile on the risk score and an equal number randomly selected from the remainder form the sample of 530 subjects. Measures of antisocial behavior were administered at 7 and 11 years of age including the Self Reported Antisocial Behavior scale (SRA), Self Report of Delinquent Behavior (SRD), and parents' and teachers' versions of the Achenbach Child Behavior Profile (CBCL). Extensive assessment of medical and sociodemographic covariates.	Bone lead by K-XRF Bone lead (exact concentrations not reported) Negative values treated categorically as 1 and positive values grouped into quintiles.	Following covariate-adjustment, parents of subjects with higher lead levels in bone reported significantly more somatic complaints, more delinquent and aggressive behavior, and higher Internalizing and Externalizing scores. Teachers reported significant increase in scores on somatic complaints, anxious/depressed, social problems, attention problems, delinquent behavior, aggressive behavior, internalizing and externalizing problems in the higher bone lead subjects. At 11 years, subject's SRD scores were also significantly related to bone lead levels. More high lead subjects had CBCL T scores in the clinical range for attention, aggression, and delinquency. Authors conclude that lead exposure is associated with increased risk for antisocial and delinquent behavior.
Dietrich et al. (2001) U.S.	Design: Prospective longitudinal. 195 subjects from the Cincinnati Lead Study were examined at 16-17 years of age. Parents were administered a questionnaire developed specifically for the study while CLS subjects were given the Self Report of Delinquent Behavior. Extensive assessment of medical and sociodemographic covariates.	Prenatal (maternal) and serial postnatal blood lead assessments. Prenatal blood lead 8.3 (SD 3.7) µg/dL Blood lead at 2 years 17.4 (SD 8.8) µg/dL	Prenatal (maternal) blood lead was significantly associated with a covariate-adjusted increase in the frequency of parent-reported delinquent and antisocial acts. Prenatal and measures of postnatal lead exposure were significantly associated with self-reported delinquent and antisocial behaviors. Authors concluded that lead might play a measurable role in the development of behavioral problems in inner-city children independent of other important social and biomedical cofactors.
Needleman et al. (2000) U.S.	Design: Case-control. 194 adjudicated delinquents and 146 non-delinquent controls recruited from high schools in the City of Pittsburgh and Allegheny County, PA. Covariate assessments were not extensive but did include race, parental sociodemographic factors, and neighborhood crime rates.	Bone lead by KXRF Bone lead Cases 11.0 (SD 32.7 µg/g), Controls 1.5 (SD 32.1 µg/g)	Cases had significantly higher average concentrations of lead in tibia than controls. Following covariate adjustment, adjudicated delinquents were 4 times more likely to have bone lead concentration >25 µg/g then controls. Bone lead level was the second strongest factor in the logistic regression models, exceeded only by race. In models stratified by race, bone lead was exceeded as a risk factor only by single parent status. Authors conclude that elevated body lead burdens are associated with increased risk for adjudicated delinquency.

Table AX6-3.6 (cont'd). Effects of Lead on Disturbances in Behavior, Mood, and Social Conduct in Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe			
Wasserman et al. (1993) Yugoslavia	Design: Prospective longitudinal. Birth cohort of approximately 300-400 infants followed since birth residing in two towns in Kosovo, Yugoslavia, one group near a longstanding lead smelter and battery manufacturing facility and another in a relatively unexposed location 25 miles away. 379 children at 3 years of age were examined. Parents were interviewed with the Achenbach Child Behavior Checklist (CBCL). Extensive assessment of medical and sociodemographic covariates.	Maternal prenatal, umbilical cord and serial postnatal blood lead Maternal blood lead in: exposed area 19.9 (SD 7.7) µg/dL, unexposed area 5.6 (SD 2.0) µg/dL Umbilical cord blood lead in: exposed area 22.2 (SD 8.1) µg/dL, unexposed area 5.5 (SD 3.3) µg/dL. Blood lead at 2 years in: exposed area 35.4 µg/dL, unexposed area 8.5 µg/dL.	Following covariate adjustment, concurrent blood lead levels were associated with increased Destructive Behaviors on the CBCL subscale, although the variance accounted for by lead was small compared to sociodemographic factors. As blood lead increased from 10 to 20 µg/dL, subscale scores increased by 0.5 points. The authors conclude that while statistically significant, the contribution of lead to social behavioral problems in this cohort was small compared to the effects of correlated social factors.
Australia			
Burns et al. (1999) Australia	Design: Prospective longitudinal. 322 subjects residing in and near Port Pirie, Australia and followed since birth were re-evaluated at 11-13 years of age. Parents completed the Achenbach Child Behavior Checklist. Extensive assessment of medical and sociodemographic characteristics.	Maternal prenatal, umbilical cord and serial postnatal blood lead Antenatal average blood lead 10.1 (SD 3.9) µg/dL Umbilical cord blood lead 9.4 (SD 3.9) µg/dL Blood lead at 2 years geometric mean 21.3 (SD 1.2) µg/dL	After adjustment for covariates, regression models revealed that for an increase in average lifetime blood lead concentrations from 10 to 30 µg/dL, the Externalizing behavior problem T score increased by 3.5 points in boys (95% CI: 1.6, 5.4), but only 1.8 points (95% CI: −0.1, 11.1) in girls. Internalizing behavior problems were predicted to rise by 2.1 points (95% CI: 0.0, 4.2) in girls by only 0.8 (95% CI: −0.9, 2.4) in boys. Authors concluded that lead exposure is associated with an increase in externalizing (undercontrolled) behaviors in boys.
Fergusson et al. (1993) New Zealand	Design: Prospective cohort. 690-891 children ages 12 and 13 years from the Christchurch Child and Health Study, New Zealand were examined. Mothers and teachers were asked to respond to a series of items derived from the Rutter and Conners parental and teacher questionnaires. Extensive assessment of sociodemographic and medical covariates.	Tooth (dentine) lead Tooth lead (range 3–12 µg/g)	Statistically significant dose-effect relationships were observed between tooth lead levels and the inattention/restlessness variable at each age. Authors conclude that this evidence is consistent with the view that mildly elevated lead levels are associated with small but long term deficits in attentional behaviors.

Table AX6-3.7. Effects of Lead on Sensory Acuities in Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States			
Schwartz and Otto (1991) U.S.	Design: Cross-sectional. 3545 subjects 6-19 years old who participated in the Hispanic Health and Nutrition Examination Survey. Pure tone audiometric evaluations were performed at 500 Hz, 2000 Hz, and 4000 Hz. Extensive measures on medical and sociodemographic covariates.	Blood lead at the time of testing. Blood lead 50th percentile 8 µg/dL	Following covariate adjustment, higher blood lead concentrations were associated with an increased risk of hearing thresholds that were elevated above the standard reference level at all four frequencies. Blood lead was also associated higher hearing threshold when treated as a continuous outcome. These relationships extended to blood lead levels below 10 µg/dL. An increase in blood lead from 6 to 18 µg/dL was associated with a 2-dB loss at all frequencies. Authors conclude that HHANES results those reported earlier for NHANES-II.
Dietrich et al. (1992) U.S.	Design: Prospective/longitudinal. 215 subjects drawn from the Cincinnati Lead Study at the age of 5 years. Children were administered the SCAN-a standardized test of central auditory processing. Extensive measurement of medical and sociodemographic covariates	Prenatal (maternal) and serial postnatal blood lead assessments. Prenatal blood lead 8.3 (SD 3.7) µg/dL Blood lead at 2 years 17.4 (SD 8.8) µg/dL	Higher prenatal (maternal), neonatal and postnatal blood lead concentrations were associated with more incorrect identification of common monosyllabic words presented under conditions of muffling. Following covariate adjustment, average childhood blood lead level remained significantly associated with impaired performance on the SCAN subtest. Authors conclude that lead-related deficits in hearing and auditory processing may be one plausible mechanism by which an increased lead burden might impede a child's learning.
Europe			
Osman et al. (1999) Poland	Design: Cross-sectional. 155 children 4-14 year-old living in an industrial region of Poland. Pure tone audiometric evaluations were performed at 500 Hz, 1000 Hz, 2000 Hz, 4000 Hz, 6000Hz, and 8000 Hz. Basic data on medical history, limited information on sociodemographic covariates such as family structure and income.	Blood lead at the time of testing Blood lead median 7.2 µg/dL (range 1.9-28 µg/dL)	Higher blood lead concentrations were significantly associated with increased hearing thresholds at all frequencies studied. This relationship remained significant when analyses were limited to subjects with blood lead levels below 10 µg/dL. Authors conclude that auditory function in children is impaired at blood lead concentrations below 10 µg/dL.

Table AX6-3.8. Effects of Lead on Neuromotor Function in Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States			
Dietrich et al. (1993); Bhattacharya et al. (1995); Ris et al. (2005) U.S.	Design: Prospective longitudinal. Relationship between lead exposure and neuromotor function has been examined in several studies on the Cincinnati Lead Study Cohort from 6 to 17 years of age. At 6 years of age 245 subjects were administered the Bruininks-Oseretsky Test of Motor Proficiency (BOTMP); at 6-10 years of age subjects were assessed for postural instability using a microprocessor-based strain gauge platform system and at 16-17 years of age the fine-motor skills of study subjects were assessed with the grooved pegboard and finger tapping tasks (part of a comprehensive neuropsychological battery). Extensive measurement of medical and sociodemographic factors.	Prenatal (maternal) and serial postnatal blood lead assessments. Prenatal blood lead 8.3 (SD 3.7) µg/dL Blood lead at 2 years 17.4 (SD 8.8) µg/dL	Following covariate adjustment, postnatal lead exposure was significantly associated with poorer scores on BOTMP measures of bilateral coordination, visual-motor control, upper-limb speed and dexterity and the Fine Motor Composite score. Low-level neonatal blood lead concentrations were also significantly associated with poorer scores on the aforementioned subtests, as well as measures of visual-motor control. Postnatal lead exposure was significantly associated with greater postural instability in 6-10 year-old subjects and poorer fine-motor coordination when examined at 16-17 years. Authors conclude that effects of early lead exposure extend into a number of dimensions of neuromotor development.
Europe			
Wasserman et al. (2000) Yugoslavia	Design: Prospective longitudinal. Birth cohort of approximately 300-400 infants followed since birth residing in two towns in Kosovo, Yugoslavia, one group near a longstanding lead smelter and battery manufacturing facility and another in a relatively unexposed location 25 miles away. 283 children at age 54 months were administered the Beery Developmental Test of Visual-Motor Integration (VMI) and the Bruininks-Oseretsky Test of Motor Proficiency (BOTMP). Extensive measurement of medical and sociodemographic factors.	Maternal prenatal, umbilical cord and serial postnatal blood lead Maternal blood lead in: exposed area 19.9 (SD 7.7) µg/dL, unexposed area 5.6 (SD 2.0) µg/dL Umbilical cord blood lead in: exposed area 22.2 (SD 8.1) µg/dL, unexposed area 5.5 (SD 3.3) µg/dL. Blood lead at 2 years in: exposed area 35.4 µg/dL, unexposed area 8.5 µg/dL.	Following covariate-adjustment, the log average of serial blood lead assessments to 54 months was associated with lower Fine Motor Composite and VMI scores. Lead exposure was unrelated to gross motor performance. With covariate adjustment, an increase in average blood lead from 10 to 20 µg/dL was associated with a loss of 0.62 and 0.42 points respectively, in Fine Motor Composite and VMI. Authors noted that other factors such as indicators of greater stimulation in the home make a larger contribution to motor development than lead.

Table AX6-3.9. Effects of Lead on Direct Measures of Brain Anatomical Development and Activity in Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States			
Trope and Lopez Villegas (1998) U.S.	Design: Case-control. One 10 year-old subject with history of lead poisoning and unexposed 9 year-old cousin. Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS) were used to assess differences in cortical structures and evidence of neuronal loss. This was the first study to attempt to determine in vivo structural and/or metabolic differences in the brain of a child exposed to lead compared with a healthy control.	Blood lead lead poisoned case 51 µg/dL at 38 mos. Unexposed control not reported.	Both children presented with normal volumetric MRI. MRS revealed a significant alteration in brain metabolites, with a reduction in N-acetylaspartate:creatine ratio for both gray and white matter compared to the subject's cousin. Authors conclude that results suggest neuronal loss related to earlier lead exposure.
Trope et al. (2001) U.S.	Design: Case-control. 16 subjects with a history of elevated blood lead levels before 5 years of age and 5 age-matched siblings or cousins were evaluated. Average age at time of evaluation was 8 years. Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS) were used to assess differences in cortical structures and evidence of neuronal loss.	Blood lead range in lead-exposed 23 to 65 µg/dL Controls <10 µg/dL	All children had normal MRI examinations, but lead-exposed subjects exhibited a significant reduction in N-acetylaspartate:creatine and pohosphocreatine ratios in frontal gray matter compared to controls. Authors conclude that lead has an effect on brain metabolites in cortical gray matter suggestive of neuronal loss.
Cecil et al. (2005) U.S.	Design: Prospective/longitudinal. 48 young adults ages 20 to 23 years were re-examined. Functional MRI (fMRI) was used to examine the influence of childhood lead exposure on language function. Subjects performed a verb generation/finger-tapping paradigm. Extensive measurement of medical and sociodemographic covariates	Blood lead Average childhood blood lead 13.9 (SD 6.6 µg/dL (range 4.8-31.1 µg/dL)	Higher average childhood blood lead levels was significantly associated with reduced activation in Broca's area in the left hemisphere and increased activation in the right temporal lobe, the homologue of Wernicke'a area in the left hemisphere. Authors conclude that elevated childhood lead exposure strongly influences neural substrates of semantic language function on normal language areas with concomitant recruitment of contra-lateral regions resulting in a striking dose-dependent atypical organization of language function.

Table AX6-3.9 (cont'd). Effects of Lead on Direct Measures of Brain Anatomical Development and Activity in Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Latin America			
Rothenberg et al. (2000) Mexico	Design: Prospective/longitudinal. 113 5-7 year-old children from the Mexico City Prospective Study were re-examined. Brain stem auditory evoked potentials were recorded to assess the impact of prenatal and postnatal lead exposure on development of auditory pathways. Results adjusted for gender and head circumference.	Blood lead Prenatal (20 wks) 8.1 (SD 4.1) µg/dL Cord 8.7 (SD 4.3) µg/dL Postnatal 18 mos. 10.8 (SD 5.2) µg/dL	Prenatal blood lead at 20 weeks was associated with decreased interpeak intervals. After fitting a nonlinear model to these data, I-V and III-V interpeak intervals decreased as blood lead rose from 1 to 8 µg/dL and increased as blood lead rose from 8 to 30 µg/dL. Increased blood lead at 12 and 48 months was related to decreased conduction intervals for I-V and II-V across the entire blood lead range suggesting pathway length effects.
Asia			
Meng et al. (2005) China	Design: Case-control. 6 subjects with blood lead concentrations ≥27 µg/dL and 6 controls with blood lead concentrations < 10 µg/dL were evaluated with Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy to evaluate structural abnormalities and differences in N-acetylaspartate, creatine, and choline in frontal lobes and hippocampus of cases and controls.	Blood lead Blood lead cases 37.7 (SD 5.7) µg/dL Blood lead controls 5.4 (SD 1.5) µg/dL	All children presented with normal MRI. Peak values of N-acetylaspartate, choline, and creatine in all four brain regions were reduced in lead exposed children relative to controls. Authors conclude that reduced brain N-acetylaspartate in cases may be related to decreased neuronal density or loss. Reduced choline signal may indicate decreased cell membrane turnover or myelin alterations while lower creatine may indicate reduced neuronal cell viability.

Table AX6-3.10. Effects of Lead on Reversibility of Lead-Related Deficits in Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States			
Ruff et al. (1993) U.S.	Design: Intervention study, non-randomized. 126 children with complete data age 13 to 87 months and with blood lead levels between 25 and 55 µg/dL were given chelation with ETDA and/or therapeutic iron where indicated. At baseline and follow-up, patients were evaluated with the Bayley Scales of Infant Development, Mental Development Index, or Stanford Binet Scales of Intelligence depending upon age.	Blood lead at time of treatment 31.2 (SD 6.5) µg/dL.	Without respect to treatment regimen, changes in performance on cognitive measures after 6 months were significantly related to changes in blood lead levels after control for confounding factors. Standardized scores on tests increased 1 point for every 3 µg/dL decrement in blood lead.
Rogan et al. (2001); Dietrich et al. (2004) U.S.	Design: Double blind, placebo-controlled randomized clinical trial. The Treatment of Lead-Exposed Children (TLC) clinical trial of 780 children in 4 centers was designed to determine if children with moderately elevated blood lead concentrations given succimer would have better neuropsychological outcomes than children given placebo. Children between 12 and 33 months of age were evaluated 3 years following treatments and again at 7 and 7.5 years of age. A wide range of neurological, neuropsychological, and behavioral tests was administered. Assessment of potentially confounding factors included sociodemographics and parental IQ.	Blood lead Range 20-44 µg/dL Baseline blood lead 26 (SD 26.5) µg/dL in both drug and placebo groups.	Succimer was effective in lowering blood lead levels in subjects on active drug during the first 6 months of the trial. However, after 1 year differences in the blood lead levels of succimer and placebo groups had virtually disappears. 3 years following treatment, no statistically significant differences between active drug and placebo groups were observed for IQ or other more focused neuropsychological and behavioral measures. When evaluated at 7 and 7.5 years of age, TLC could demonstrate no benefits of earlier treatment on an extensive battery of cognitive, neurological, behavioral and neuromotor endpoints. Authors conclude that the TLC regimen of chelation therapy is not associated with neurodevelopmental benefits in children with blood lead levels between 20 and 44 µg/dL and that these results emphasize the importance of taking environmental measures to prevent exposure to lead in light of the apparent irreversibility of lead-associated neurodevelopmental deficits.

Table AX6-3.10 (cont'd). Effects of Lead on Reversibility of Lead-Related Deficits in Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Liu et al. (2002) U.S.	Design: Prospective longitudinal clinical trial. Data from the Treatment of Lead-Exposed Children (TLC) used to examine prospective relationships between falling blood lead levels and changes in cognitive functioning. 741 children recruited between 13 and 33 months of age were assessed at baseline and 6 months later with the Bayley Mental Development Index (MDI) and 36 months post-randomization with the Wechsler Preschool and Primary Scales of Intelligence-Revised to obtain IQ.	Blood lead Baseline blood lead 26.2 (SD 5.1) µg/dL 36 months post-randomization blood lead 12.2 (SD 5.2) µg/dL	TLC found no overall effect of changing blood lead level on change in cognitive test scores from baseline to 6 months. Slope estimated to be 0.0 points per 10 µg/dL change in blood lead. From baseline to 36 months and 6 months to 36 months, falling blood lead levels were significantly associated with increased cognitive test scores, but only because of an association in the placebo group. Authors conclude that because improvements were not observed in all children, the data do not provide support that lead-induced cognitive impairments are reversible. Although the possible neurotoxicity of succimer cannot be ruled out.
Latin America			
Kordas et al. (2005); Rico et al. (2005) Torreon, Mexico	Design: Double-blind, placebo-controlled nutritional supplementation clinical trial conducted among 602 first grade children ages 6-8 years in Torreon, Mexico. Subjects received iron, zinc, both or placebo for 6 months. Parents and teachers filled out the Conners Rating Scales at baseline and follow up six months following the end of supplementation to index behavioral changes following therapy. In addition, 11 cognitive tests of memory, attention, visual-spatial abilities, and learning were administered, including WISCR-M at baseline and follow-up 6 months later.	Blood lead Baseline blood lead 11.5 (SD 6.1) µg/dL	No significant effects of treatment on behavior or cognition could be detected with any consistency. Authors conclude that this regimen of supplementation does not result in improvements in ratings of behavior or cognitive performance.
Australia			
Tong et al. (1998) Australia	Design: Prospective longitudinal. 375 children from the Port Pirie Prospective Study were followed from birth to the age of 11-13 years. Bayley Mental Development Index (MDI) at 2 years, the McCarthy Scales General Cognitive Index (GCI) and IQs from the Wechsler Intelligence Scale served as the primary indicators of intellectual status. The purpose of the study was to assess the reversibility of lead effects on cognition in relationship to declines in blood lead over time.	Postnatal Blood lead Average Blood lead at 2 years 21.2 µg/dL declining to 7.9 µg/dL at 11-13 years.	Although blood lead levels declined substantially, covariate adjusted scores on standardized measures of intellectual attainment administered at 2, 4, 7, and 11-13 years of age were unrelated to declining body burden. Authors conclude that effects of early exposure to lead during childhood are not reversed by a subsequent decline in blood lead concentration.

Table AX6-3.11. Neurobehavioral Effects Associated with Environmental Lead Exposure in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States			
Muldoon et al. (1996)	325 women from rural location (mean age 71) and 205 women from a city location (mean age 69) participants in the Study for Osteoporotic Fractures had the association of nonoccupational lead exposure and cognitive function examined. Logistic regression determined effect of blood lead on neuropsychological performance.	Rural group Blood lead 5 µg/dL Urban group Blood lead 5 µg/dL	Groups were significantly different with the urban group more educated and smoked and drank more. Performance in each group stratified by exposure into three groups (low <4 µg/dL, medium 4-7 µg/dL, high >7 µg/dL rural and >8 µg/dL) - no significant associations were present in the urban group but the rural group had significantly poorer performance with increasing blood lead for Trails B (OR = 2.6, 95% CI: 1.04, 6.49), Digit Symbol (OR 3.73, 95% CI: 1.57, 8.84),and Reaction Time in the lower (OR 2.84, 95% CI: 1.19, 6.74) and upper extremities (OR 2.43, 95% CI: 1.01, 5.83). The fact that marked differences exist between the low lead groups for rural and urban (the lowest 15th percentile) suggests the differences between the two groups are unrelated to lead. Response time for reaction time across lead groups increased for the rural group and decreased or remained the same for the urban group. As response time is sensitive to lead effect, this raises question whether factors not measured accounted for difference. Namely MMSE for the whole population was 25 (15-26) with poorer performance in the rural group. The clinical cutoff score for MMSE is 24 suggesting the presence of clinical cognitive disorders. Even though this is a simple neuropsychological battery up to 9 were unable to perform some of the tests including 3 on the MMSE.
Payton et al. (1998)	141healthy men in VA normal aging study evaluated every 3 to 5 years with cognitive battery and blood lead and once a measurement of patella and tibia bone lead. Statistics are confusing as it is not clear when ANCOVA is used and how the groups are created.	Mean blood lead 6 µg/dL, patella bone lead 32 and tibia bone lead 23 µg/g bone mineral	Regressions adjusted for age and education found significant relationship of blood lead with Pattern Comparison (perceptual speed), Vocabulary, Word List Memory, Constructional Praxis, Boston Naming Test, and Verbal Fluency Test. Only for Constructional Praxis were bone lead and blood lead significantly associated. Mechanism most sensitive to low levels lead exposure believed to be response speed. It is unusual that Vocabulary, a test resistant to neurotoxic insult is significantly associated with blood lead. This may be related to the significant negative correlation of bone lead with education, a similar trend is present for blood lead. It is not clear how multiple comparisons were handled.

Table AX6-3.11 (cont'd). Neurobehavioral Effects Associated with Environmental Lead Exposure in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Rhodes et al. (2003)	526 participants with mean age 67 years, 47% had education level of high school or less. Mood symptoms evaluated with Brief Symptom Inventory (BSI). Use of logistic regression adjusting for covariates examined association of BSI scales and blood lead and bone lead levels.	Mean blood lead 6 µg/dL Mean tibia Pb 22 µg/g Mean patella Pb 32 µg/g	BSI found mood symptoms for anxiety and depression were potentially associated with bone lead levels. However education was inversely related to bone lead and high school graduates had significantly higher odds of Global Severity Index and Positive Symptom Total. BSI appears to be detecting general stress related to socioeconomic status.
Wright et al. (2003)	736 healthy men (mean age 68) in Normative Aging Study examined every 3 to 5 years were administered the Mini-Mental State Exam (MMSE). Linear regression examined relationship of MMSE and blood lead, Patella and Tibia bone lead measurements after adjusting for covariates.	Mean blood lead 5 µg/dL, patella bone lead 30 and tibia bone lead 22 µg/g bone mineral	Mean MMSE score 27. Relation of MMSE scores <24 (n = 41) and blood lead by logistic regression found OR 1.21 (95% CI: 1.07, 1.36) and for patella lead OR 1.21 (95% CI: 1.00, 1.03) and tibia lead OR 1.02 (95% CI: 1.00, 1.04). Risk of MMSE <24 when comparing the lowest and highest quartiles of patella lead was 2.1 (95% CI: 1.1, 4.1), for tibia lead was 2.2 (95% CI: 1.1, 3.8) and blood lead was 3.4 (95% CI: 1.6, 7.2). Interaction between patella lead and age, and blood lead and age in predicting MMSE found steeper decrease in MMSE score relative to age in the higher quartiles of patella lead and blood lead. MMSE very sensitive to years of education below 8 years. In this study 213 subjects had less than high school education. If the community dwelling population had older individuals with less education living in areas with higher past pollution the confounding may be impossible to sort out. Initially at beginning of NAS subjects were eliminated with chronic medical problems or blood pressure >140/90. It is not addressed how the development of medical conditions during the duration of the study are handled.

Table AX6-3.11 (cont'd). Neurobehavioral Effects Associated with Environmental Lead Exposure in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Weisskopf et al. (2004)	466 men, mean age 70 years, in the VA Normative Aging Study had 2 MMSE tests 3.5 years apart.	Mean blood lead 4 μg/dL, patella bone lead 23 and tibia bone lead 19 μg/g bone mineral	Baseline mean MMSE score was 27 and mean change in MMSE score over 3.5 years was 0.3. Change in MMSE associated with one interquartile range increment for bone lead and blood lead found relationship between patella lead and change in MMSE was unstable when patella lead is ≥90 μg/g bone mineral. Examination of patella lead below this level found a greater inverse association with MMSE at lower lead concentrations (β = −0.25, 95% CI: −0.45, −0.05). A similar but weaker association existed for tibia lead when values ≥67 μg/g bone mineral were removed (β = −0.19, 95% CI: −0.39, 0.02). There was no association of MMSE change and blood lead (β = −0.01, 95% CI: −0.13, 0.11). There was no interaction of age and bone lead. These are very high bone lead levels for environmental exposure. The biological plausibility of change in the MMSE over 3.5 years would have been reinforced if the change by functional domain in the MMSE was provided.
Europe			
Nordberg et al. (2000) Sweden	762 participants, mean age 88 years, in a study of aging and dementia examined MMSE. Used blood lead as dependent and examined contribution of covariates and MMSE.	Mean blood lead 3.7 μg/dL	Mean MMSE 25 found no relation of blood lead and MMSE. In this population was fairly homogenous, all elderly Swedes, and the likelihood of prior exposure to elevated lead levels was low.

Table AX6-3.12. Symptoms Associated with Occupational Lead Exposure in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Canada			
Lindgren et al. (1999)	Smelter workers (n = 467) with a mean age of 40 years completed the Profile of Mood Scale. Factor structure of POMS validated in this occupational population. Regression analysis determined association with lead exposure.	Mean blood lead 28 (8.5, 4-62) µg/dL Mean IBL 711 (415.5, 1-1537) µg-yr/dL.	Factor analysis found one factor labeled "general distress" composed of POMS subscales anger, confusion, depression, fatigue and tension and a second factor labeled 'psychological adjustment'. IBL was significantly associated with 'general distress' after adjustment for the covariates ($\beta = 0.28$ [SE 1.51×10^{-4}] $p = 0.01$) while there was no relation with blood lead. The factor structure of POMS originally validated in a clinical population had six mood subscales however the factor structure in this occupational population was found to have only two subscales.
Holness et al. (1988)	47 demolition workers with acute lead intoxication - Phase 1-were followed with blood lead and symptoms during engineering modifications to control exposure -Phases 2-4. Workers stratified by blood lead and symptom frequency was analyzed.	Phase I- Mean blood lead 59 µg/dL SD N/A Phase 2-Mean blood lead 30 µg/dL SD N/A Phase 3-Mean blood lead 19 µg/dL SD N/A Phase 4-Mean blood lead 17 µg/dL SD N/A	Below blood lead <50 µg/dL percentage of workers reporting symptoms was fatigue-25, headache-14 dizzy-9, sleep-8, abdominal cramps-8, muscle ache-8, paresthesiae-8, appetite-7, constipation-6, and weakness-6. All symptoms were significantly lower except for paresthesiae when compared to group with blood lead >70 µg/dL. Of interest, at beginning of Phase 4 when mean blood lead was 13 µg/dL, no symptoms were reported. At the end of Phase 4, mean blood lead was 17 µg/dL and one worker complained of fatigue.
Europe			
Lucchini et al. (2000) Italy	66 workers in lead manufacturing, mean age 40 (8.7) years and 86 controls mean age 43 (8.8) years were administered a questionnaire with neuropsychological (14 items), sensory-motor (3 items), memory (4 items) and extrapyramidal (8 items), 10 Parkinson symptoms and the Mood Scale. Group comparisons and linear regression examined relationship of symptoms and lead exposure.	Mean blood lead 27 (11.0, 6-61) µg/dL Mean TWA 32 (14.1, 6-61) µg/dL Mean IBL 410 (360.8, 8-1315) µg-yr/dL. Controls-mean blood lead 8 (4.5, 2-21) µg/dL	Lead exposed worker reported confusion, somnolence, abnormal fatigue, irritability, and muscular pain more frequently ($p < 0.04$). There were no group differences for the parkinsonism symptoms or Mood Scale. Linear regression combing exposed and control group found neurological symptoms significantly associated with blood lead ($r = 0.22$, $p = 0.006$). Neuropsychological symptoms were significantly higher in the High-IBL compared to the Low-IBL group. The estimated threshold for a significant increase (prevalence of 5%) of a high score for neurological symptoms was at a blood lead of 12 µg/dL.

Table AX6-3.12 (cont'd). Symptoms Associated with Occupational Lead Exposure in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Latin America			
Maizlish et al. (1995) Venezuela	43 workers from a lead smelter, mean age 34 (9) years and 47 nonexposed workers, mean age 35 (11) years completed the profile of mood states (POMS) questionnaire and a questionnaire of symptoms of the central and peripheral nervous system, and gastrointestinal. Prevalence ratios used to examine symptoms and lead. ANCOVA and linear regression adjusting for potential confounders examined relationship of lead exposure and POMS.	Mean blood lead 43 (12.1) µg/dL Mean peak blood lead 60 (20.3) µg/dL Mean TWA 48 (12.1) µg/dL Controls mean blood lead 15 (6) µg/dL mean peak blood lead 15 (6) µg/dL mean TWA 15 (6) µg/dL	Significantly increased relative risks found for difficulty concentrating (RR 1.8 [95% CI: 1.0-3.1]), often being angry or upset without reason (RR 2.2 [95% CI: 1.2, 4.1]), feeling abnormally tired (RR 2.2 [95% CI: 0.9, 5.3]) and joint pain (RR 1.8, [95% CI: 1.0, 3.3]). The six subscales of the POMS were not significantly different between the exposed and control groups. However dose-related analysis found significantly poorer scores for tension-anxiety and blood lead ($p = 0.009$), hostility and blood lead ($p = 0.01$) and TWA ($p = 0.04$), and depression and blood lead ($p = 0.003$) and peak lead ($p = 0.003$) and TWA ($p = 0.004$).
Asia			
Schwartz et al. (2001) Korea	803 lead-exposed Korean workers, mean age 40 years completed the Center for Epidemiologic Studies Depression Scale. Linear regression examined for association of CES-D and lead biomarkers after adjusting for the covariates.	Mean blood lead 32 (15.0) µg/dL Mean tibia lead 37 (40.3) µg/g bone mineral	After adjustment for age, gender and education significant associations found for CES-D and tibia lead ($\beta = 0.0021$ [SE 0.0008]; $p < 0.01$) but not with blood lead. This occupational lead-exposed populations had higher past lead exposure compared to the current mean blood lead of 32 µg/dL.

Table AX6-3.12 (cont'd). Symptoms Associated with Occupational Lead Exposure in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Lee et al. (2000) Korea	95 Korean lead exposed workers, mean age 43 years, completed questionnaire of lead-related symptoms present over last three months. Relationship between symptom score and measures of lead exposure assessed by linear regression. Logistic regression use to model presence or absence of symptoms for gastrointestinal, neuromuscular, and general.	DMSA-chelatable lead Mean 289 (167.7) µg ZPP 108 (60.6) µg/dL Mean ALAU3 (2.8) mg/l Mean blood lead 45 (9.3) µg/dL	Workers with DMSA -chelatable lead above the median of 261 µg were 6.2 (95% CI: 2.4, 17.8) times more likely to have tingling or numbness in their extremities, 3.3 (95% CI: 1.2, 10.5) times more likely to experience muscle pain and 3.2 (95% CI: 1.3, 7.9) times more likely to feel irritable. The workers with higher chelatable lead were 7.8 (95% CI: 2.8, 24.5) times more likely to experience neuromuscular symptoms compared to workers with lower chelatable lead. In this study ZPP predicted weakness of ankle and wrist (OR 2.9 [95% CI: 1.1, 8.1]) and fatigue (OR 2.9 [95% CI: 1.1, 8.7]) while ALAU predicted inability to sleep (OR 5.4 [95% CI: 1.2, 33.2]) and blood lead was not significantly associated with any symptoms. A measure of lead in bioavailable storage pools was the strongest predictor of symptoms particularly neuromuscular.
Niu et al. (2000) China	44 lead-exposed workers (17 men, 27 women) from lead printing houses, mean age 35 (4.9) and education 9.3 (no SD) years and 34 controls (19 men and 15 women), mean age 33 (7.4) years and education 9.5 (no SD) years completed the profile of mood state as part of the NCTB. ANCOVA controlling for age, sex and education examined group differences and linear regression for dose-response relationship.	Mean blood lead 29 (26.5) µg/dL (8 workers blood lead exceeded 50 µg/dL) Controls Mean blood lead 13 (9.9) µg/dL (1 control blood lead exceeded 50 µg/dL)	POMS subscales for confusion ($F = 3.02$, $p < 0.01$), fatigue ($F = 3.61$, $p < 0.01$), and tension ($F = 2.82$, $p < 0.01$) were significantly elevated in the lead exposed group. Regression analyses found a dose response (data not shown).

Table AX6-3.13. Neurobehavioral Effects Associated with Occupational Lead Exposure in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States			
Fiedler et al. (2003) New Jersey	40 workers with lead exposure, mean age 48 (9.5) years completed a neurobehavioral battery and was compared to 45 lead/solvent workers, mean age 47 (10.2), 39 solvent exposed workers, mean age 43 (9.4), and 33 controls, mean age 44 (10.2). Group differences and dose-effect relationships were assessed after adjusting for potential confounding.	Mean blood lead µg/dL Mean bone lead ppm µg/g (dw) Lead workers 14 (11.7)/2.7 (0.7) Lead/Solvent workers 12 (11.6)/2.8 (0.6) Solvent workers 5 (4.1)/−1.8 (1.8) Controls 4 (1.4)/−1.1 (1.6)	Of nineteen outcomes, significant differences found on the California verbal learning test (CVLT) ($p = 0.05$) and positive symptom distress index on the Symptom checklist-90-R. On the CVLT the controls performed significantly better on trials 2 and 3 demonstrating efficiency of verbal learning. Symbol digit substitution (SDS) approached significance ($p = 0.09$) with lead and lead/solvent group slower on latency of response but not accuracy. Bone lead was a significant predictor of latency of response on SDS, total errors on paced auditory serial addition task and simple reaction time non-preferred hand. Bone lead and SRT, preferred hand approached significance. This is a confusing study design as bone lead is used as a predictor in workers both with and without occupational lead exposure.
Balbus-Kornfeld et al. (1995)	Reviewed 21 studies from 28 publications; number of subjects ranged from 9-708.	Mean blood lead in most exposed group 28-68 µg/dL. Only 5 studies used a measure of cumulative exposure or absorption of Pb, 2 studies used duration of exposure.	Dexterity (17/21 studies) and executive or psychomotor 11/21 studies were the functional domains most commonly associated with lead. Age not adequately controlled in most studies, usually matching means or medians. Intellectual abilities prior to exposure usually adjusted for with education however Vocabulary, a measure of overall intellectual ability still different between the groups. The conclusion reached that evidence of effects from cumulative exposure or absorption of lead was inadequate.

Table AX6-3.13 (cont'd). Neurobehavioral Effects Associated with Occupational Lead Exposure in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Canada			
Lindgren et al. (1996)	467 Canadian former and current, French and English speaking lead smelter workers, mean age 43 (11.0) years and education 10 (3.2) years were administered a neuropsychological battery in English or French. Data analyses used MANCOVA adjusting for age, education, measure of depressive symptoms and self reported alcohol use.	Mean years employment 18 (7.4) Mean blood lead 28 (8.4) µg/dL Mean TWA 40 (4-66) µg/dL Mean IBL 765 (1-1626) µg-yr/dL	Fourteen neuropsychological variables examined by MANCOVA with the grouping variable exposure (high, medium and low) and the covariates, age, education, CES-D, and alcohol use found no exposure term significant until years of employment, a suppressor term, was added as a covariate. IBL exposure groups differed significantly (df 2,417) on digit symbol ($F = 3.03$, $p = 0.05$), logical memory ($F = 3.29$, $P = 0.04$), Purdue dominant hand ($F = 4.89$, $p = 0.01$), and trails A ($F = 3.89$, $p = 0.02$) and B ($F = 3.2$, $p = 0.04$). This study showed a dose-effect relationship between cumulative lead exposure (IBL) and neuropsychological performance at a time when there was no association with current blood lead.
Bleecker et al. (2002)	256 smelter workers from the above population were currently employed and took the test battery in English. Their mean age was 41 (7.9) years, and education 10 (2.8) years. The goal was to determine if educational achievement as measured by WRAT-R Reading modified performance on MMSE. Linear regression assessed the contribution of age, WRAT-R, education, alcohol intake, cigarette use, IBL and IBLXWRAT-R on MMSE performance.	Mean blood lead 28 (8.8) µg/dL Mean IBL 725 (434) µg-yr/dL	MMSE had a median (range) score of 29 (19-30). The most common errors were recall of 3 items (38%), spell world backwards (31%), repetition of "no ifs ands or buts" (21%) and copy a design to two intersecting pentagons (16%). WRAT-R reading used as an additional measure of educational achievement because it was a stronger predictor of MMSE performance than years of education. The significant interaction ($\Delta R^2 = 2\%$, $p = 0.01$) explained by a dose-effect between IBL and MMSE only in the 78 workers with a WRAT-R reading grade level less than 6. The workers with higher reading grade levels and the same cumulative lead exposure were able to compensate for the effects of lead on the MMSE because of increased cognitive reserve.

Table AX6-3.13 (cont'd). Neurobehavioral Effects Associated with Occupational Lead Exposure in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Canada (cont'd)			
Bleecker et al. (2005)	256 smelter workers currently employed and took the test battery in English. Their mean age was 41 (7.9) years, and education 10 (2.8) years. The purpose was to determine whether components of verbal memory as measured on the Rey Auditory Verbal Learning Test (RAVLT) were differentially affected by lead exposure. Linear regression and ANCOVA assessed the relationship of lead and components of verbal learning and memory.	Mean blood lead 28 (8.8) µg/dL Mean TWA 39 (12.3) µg/dL Mean IBL 725 (434) µg-yr/dL	Outcome variables RAVLT a word list test included measures of immediate memory span and attention (Trial 1), best learning (Trial V), incremental learning across the five trials (Total Score), and storage (Recognition) and retrieval (Delayed Recall) of verbal material. TWA significantly contributed to the explanation of variance for Trial V ($\Delta R^2 = 1.4\%$, $p < 0.03$) and Delayed Recall ($\Delta R^2 = 1.4\%$, $p = 0.03$) after adjusting for age and WRAT-R while IBL did the same with Recognition ($\Delta R^2 = 2.0\%$, $p = <0.02$) and Delayed Recall ($\Delta R^2 = 1.1\%$, $p = 0.06$). Workers stratified into 3 group by increasing clinical memory difficulties-Group1 had normal encoding, storage and retrieval; Group2 could encode and store verbal information but had difficulty with retrieval and Group 3 had abnormal encoding, storage and retrieval but was still able to learn new verbal information. ANCOVA adjusting for age and WRAT-R compared lead exposure across the memory groups. Blood lead showed no difference but TWA and IBL were significantly higher in Group 3 compared to Group 1 ($p < 0.05$ for both). Internal strategies used on the RAVLT over the five trials found that Groups 1 and 2 remembered more words from the beginning of the list while group 3 remembered more from the end. At a time when blood lead was not associated with performance, cumulative lead exposure resulted in poorer storage and retrieval of previously learned material. Alterations in the ability to organize materials in long term memory interferes with retrieval efficiency.

Table AX6-3.13 (cont'd). Neurobehavioral Effects Associated with Occupational Lead Exposure in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Canada (cont'd)			
Bleecker et al. (1997a) New Brunswick 1992-1993	The performance of the 467 current and retired smelter workers as described in Lindgren et al. (1996) administered a screening neuropsychological battery by testers blinded to the degree of lead exposure of the worker had their performance compared to age matched norms. If performance on two or more tests in any functional domain was below 1.5 standard deviations the worker was invited for a complete clinical evaluation. Eighty current workers were identified by this criterion. Mean years- age 44 (8.4), education 8 (2.8) and duration employed 20 (5.3). Five neuropsychological tests commonly associated with lead exposure were examined for a differential association with blood lead, IBL,TWA and bone lead.	Mean blood lead 26 (7.07) µg/dL Mean TWA 42 (8.4) µg/dL Mean IBL 903 (305.9) µg-yr/dL, Mean tibial bone lead 41 µg/g bone mineral	Relationship of 5 neuropsychological tests with 4 measures of lead dose after adjusting for age age^2 and education, education^2 found RAVLT trial V and Verbal Paired Associates were associated with blood lead (ΔR^2 = 6.2%, p = 0.02; ΔR^2 = 5.5%, p = 0.07) and TWA (ΔR^2 = 3.2%, p = 0.09; ΔR^2 = 13.9%; P = 0.00) while Digit Symbol and Grooved Pegboard were associated with TWA (ΔR^2 = 6.1%, p = 0.00; ΔR^2 = 5.5%, p = 0.02) and IBL (ΔR^2 = 4.8%, p = 0.01; ΔR^2 = 5.7%, p = 0.02). Only grooved pegboard was associated with bone lead (ΔR^2 = 4.2%, p = 0.05). Block design was not associated with any measures of lead dose. Age was an effect modifier with grooved pegboard. There was enhanced slowing in older workers when compared to younger workers with the identical IBL.
Bleecker et al. (1997b) New Brunswick 1992-1993	Of the 80 current smelter workers described above 78 completed a simple visual reaction time (SRT) and had mean years age 44 (8.2) years, education 8 (7.2) and duration employed20 (5.6).	Mean blood lead, 26 (7.2) µg/dL Mean blood lead from bone 7 (4.2) µg/dL Mean blood lead from environment 19 (7.0) µg/dL Mean bone lead 40 (25.2) µg/g bone mineral	SRT consisted of 44 responses to a visual stimulus at interstimulus intervals (ISI) varying between 1 through 10 seconds with a mean SRT (median) of 262 (179 to 387) ms. Blood lead and median SRT had a curvilinear relationship R^2 = Pb + Pb^2, 13.7%, p < 0.01) after adjusting for age and education with slowing of SRT beginning at a blood lead of approximately 30 µg/dL. No relationship existed between bone lead and SRT. There was a stronger association between Pb and Pb^2 and SRT for the longer ISI = s, 6 to 10 seconds (R^2 = 13.9%, p < 0.01), as age was significantly related to the shorter ISI = s but not the longer ones. In this population the contribution of bone lead to blood lead had been previously where estimated where for a bone lead level of 100 µg Pb/g bone mineral, 17 µg Pb/dL of the blood lead was derived from internal bone stores with the remainder from the environment. Blood lead was fractionated to that from bone (blood lead-bn) versus blood lead from the environment (blood lead-en). Regression analysis to examine the relationship of blood lead-bn and blood lead-en and SRT after adjusting for the covariates found significant contribution to the variance of SRT only for blood lead-en (R^2 for blood lead-en + blood lead-en^2 = 14.4%, p < 0.01). The absence of a contribution by age and more stable responses with ISIs of 6 to 10 sec supports using this component of SRT.

Table AX6-3.13 (cont'd). Neurobehavioral Effects Associated with Occupational Lead Exposure in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Canada (cont'd)			
Lindgren et al. (2003) New Brunswick 1992-1993	In an attempt to separate the effects of past high lead exposure from a lower proximate exposure, examination of the pattern of lead levels of the 467 Canadian lead smelter workers found 40 workers who had high past exposure followed by years where 90% of blood lead were above 40 µg/dL (High-high = H-H) while another group of 40 workers had similar past high lead exposure followed by years where 90% of blood lead were below 40 µg/dL (High-low = H-L). The groups did not differ on age, education, years of employment or CES-D. Five outcomes examined-Purdue Pegboard assembly, Block Design, Digit Symbol, Rey Auditory Verbal Learning Test-total score, delayed Logical Memory.	Mean IBL for past exposure H-H 633 (202.2) µg-yr/dL H-L 557 (144.8) µg-yr/dL Mean IBL for the proximate exposure H-H 647 (58.7) µg-yr/dL H-L 409 (46.4) µg-yr/dL Mean blood lead H-H 37 (5.1) µg/dL H-L 24 (5.2) µg/dL	Of the five neuropsychological measures examined only RAVLT (total score) and Logical Memory (delayed) were significantly different after adjusting for the covariates in the two pattern groups. Use of regression analyses found pattern group contributed significantly ($R^2 = 4\%$, $p < 0.05$) to the explanation of variance in RAVLT after accounting for current blood lead ($R^2 = 3\%$, $p < 0.10$) and IBL measures ($R^2 = 7\%$, $p < 0.01$). For past IBL, H-H pattern correlated more strongly with RAVLT ($r = -0.21$) while H-L pattern had no relationship with past exposure ($r = 0.08$). For proximate IBL the difference was maintained between H-H ($r = -0.11$) and H-L pattern ($r = 0.00$). The authors suggested that the absence of an association between past high lead exposure and verbal memory in the H-L pattern group may reflect reversibility of function when blood lead is maintained below 40 µg/dL.
Braun and Daigneault (1991) Quebec	41 workers from a secondary lead smelter, mean age 35 (9.6) years and years of education 10 (2.1) were compared to a control group mean age 37 (10.1) years and years of education 11 (1.3) on tests of cognitive and motor function. MANCOVA and dose-effect relationships after adjusting for potential confounders were performed.	Mean TWA 53 (7.5) µg/dL Mean maximum blood lead 87 (22.4) µg/dL	None of the measures of cognitive executive function showed group differences. Partial correlation adjusting for age and education with dose related variables found no statistical significance. On motor function the exposed workers had significantly slower simple reaction time ($p = 0.05$). However partial correlations with measures of dose found dose-effect correlation in both negative and positive directions. Group of exposed workers was mixed for lead exposure with 11 currently working and the remainder with no exposure up to 84 months. Also two of the exposed workers had been treated with chelation.

Table AX6-3.13 (cont'd). Neurobehavioral Effects Associated with Occupational Lead Exposure in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe			
Hanninen et al. (1998) Finland	Fifty-four lead battery workers were stratified by those whose blood lead never exceeded 50 µg/dL (n = 26) (group 1) and those who had higher exposure in the past (n = 28) (group 2) to examine the neuropsychological effects of current low level blood lead from higher blood lead in the past. Mean age group 1was 42 (9.3) years, education 8 (1.7) years and years of exposure 12 (6.7). Mean age group 2 was 47 (6.2) years, education 8 (1.0) years and years of exposure 21 (6.9). Analysis included partial correlations within the groups and ANCOVA within group 1 divided at the median TWA3 of 29 µg/dL.	Markers of lead exposure for the group 1 were mean IBL 330 µg-yr/dL, Maximum blood lead 40 µg/dL, TWA 29 µg/dL Tibial lead 20 µg/g Calcaneal lead 79 µg/g Past high exposure, group 2 Mean IBL 823 µg-yr/dL, Maximum blood lead 69 µg/dL, TWA 40 µg/dL, Tibial lead 35 µg/g Calcaneal lead 100 µg/g IBL, TWA and maximum blood lead were also calculated for the previous 3 years with a median TWA3 of 29 µg/dL	Partial correlations controlling for age, sex and education in group 1 found block design, digit symbol, digit span, similarities, Santa Ana 1 and memory for design significantly associated with recent measures of exposure and embedded figures with maximum blood lead. In group 2 embedded figures, digit symbol, block design, and associative learning were associated with IBL and /or maximum blood lead. Calcaneal lead was weakly associated with digit symbol, digit symbol retention, and synonyms. There was no association with tibial lead in either group. Group 1 divided at the median TWA3 of 29 µg/dL found the high group had lower scores for visuospatial and visuoperceptive tasks (digit symbol, embedded figures and memory for design). Overall past high exposure, blood lead >50 µg/dL, had the greatest effect on tests requiring the encoding of complex visually presented stimuli. The authors conclude that the effect of lead on brain function is better reflected by history of blood lead than content of lead in bone.
Lucchini et al. (2000) Italy	66 workers in lead manufacturing, mean age 40 (8.6) years, mean education 8 (2.4) years and mean exposure time 11 (9) years and a control group of 86 with mean age 43 (8.8) years, mean years of education 9 (2.7) years. Group differences examined and dose-effect relationship with correlation and ANOVA.	Mean blood lead 28 (11) µg/dL Control-mean blood lead 8 (4.5) µg/dL Mean IBL 410 (360.8) µg-yr/dL, Mean TWA 32 (14.1) µg/dL, Mean years exposed 11(8.1)	No association with neuropsychological tests (addition, digit span, finger tapping symbol digit and motor test from Luria) and blood lead, TWA or IBL were found. Blood lead and visual contrast sensitivities at the high frequencies were significantly associated for the entire group. Blood lead and serum prolactin in the whole group was significantly associated. Increased prolactin secretion occurs with a variety of neurotoxins and reflects impaired dopamine function in the pituitary. The estimated threshold for a significant increase of high prolactin levels was at a blood lead of 10 µg/dL.

Table AX6-3.13 (cont'd). Neurobehavioral Effects Associated with Occupational Lead Exposure in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Osterberg et al. (1997) Sweden	38 workers, median age 42 (no range) years at a secondary smelter stratified by finger bone lead concentration and along with 19 controls matched triplets for age, education and job level. Median years employed 10 (2-35).	High bone lead Median bone 32 (17-101) µg/g Median blood lead 38 (19-50) µg/dL Median peak blood lead 63 (46-90) µg/dL Median IBL 408 (129-1659) µg-yr/dL Low bone lead Median bone 16 (−7-49) µg/g Median blood lead 34 (17-55) µg/dL Median peak blood lead 57 (34-78) µg/dL Median IBL 250 (47-835) µg-yr/dL Controls Median bone 4 (−19-18) µg/g Median blood lead 4 (1-7) µg/dL	A cognitive test battery (36 tests) covering learning and memory, visuomotor function, visuospatial function, concentration and sustained attention found no impairment or dose-response relationships with any of the markers of lead exposure. Deviating test scores (belong to 10% lowest reference norms) were less in high bone lead (1 vs. 4 vs. 4). None of the deviating parameters were significantly correlated with any of the lead indices. Even when age was taken into account the significant associations between outcome and lead exposure metrics did not exceed chance in light of the numerous analyses performed. These were the most heavily lead-exposed workers in Sweden. It was unusual that the 2 visuomotor tasks significantly different had better performance in the lead-exposed workers compared to the controls.

Table AX6-3.13 (cont'd). Neurobehavioral Effects Associated with Occupational Lead Exposure in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Stollery et al. (1991) England	Seventy lead-exposed workers, mean age 41(no SD) years, grouped by blood lead (<20 µg/dL, 21-40 µg/dL and 41-80 µg/dL) examined on three occasions each separated by four months. Tested on a computer for syntactic reasoning, delayed five choice reaction time, visual spatial memory, and category search task.	Low blood lead (no SD provided) Mean blood lead 14 µg/dL Mean ZPP 13 mg/dL Mean urinary ALA 2 mg/L Mean years exposed 7 Medium blood lead Mean blood lead 31 µg/dL Mean ZPP 33 mg/dL Mean urinary ALA 3 mg/L Mean years exposed 10 High blood lead Mean blood lead 52 µg/dL Mean ZPP 77 mg/dL Mean urinary ALA 6 mg/L Mean years exposed 11	Lead exposure was stable over the 8 months of testing. The low lead group drank significantly less alcohol and rated their work as less demanding. Performance and exposure stable except in the high lead group where decision time was slowed more than movement time along with concentration difficulties that remained stable across testing sessions. Movement and decision times were significantly correlated for each duration of waiting. On the memory test of recalled nouns, the memory deficit associated with lead ($r = -0.35$, $p = 0.003$) was restricted to recall of nouns unrelated to task (distracters) ($p = 0.04$) that did not improve with repetition suggestive of difficulties with incidental learning. Workers with blood lead >40 µg/dL had impairments that correlated best with average blood lead over the preceding 8 months. Workers with blood lead between 21 to 40 µg/dL had essentially no impairment.
Stollery et al. (1996) England	Same as above except this was a further analysis of the five choice reaction time.	Same as above	Movement and decision slowing was correlated with blood lead. Slowed movement time was constant across response-stimulus intervals in contrast to decision time that was increasingly affected by lead especially at the shortest response-stimulus intervals. This supported the finding that decision gaps, central in origin, as opposed to movement gaps are selectively affected by lead exposure in this population.
Barth et al. (2002) Austria	47 lead storage-battery workers, mean age 40 (9.7) years and 53 nonexposed controls, mean age 39 (8.4) years were matched for age and verbal intelligence. Group differences and dose-response relationship were explored.	Mean blood lead 31 (11.2) µg/dL IBL 384 (349.0) µg-yr/dL Years employed 12 (9.0) Controls Mean blood lead 4 (2.0) µg/dL	Significant differences were found for block design ($p \leq 0.01$), visual recognition ($p \leq 0.01$) and Wisconsin card sorting (categories $p = 0.0005$, total errors $p = 0.0025$, perseverations $p = 0.001$, loss of sorting principle $p = 0.003$) but not SRT or digit symbol. In the exposed group partial correlation adjusting for age found no significant associations with IBL ($n = 53$). In the entire group the full correlation was significant for blood lead and Wisconsin card sorting, block design and visual recognition ($n = 100$). Visuospatial abilities and executive function were better predicted by blood lead than cumulative lead exposure. It is unusual that a frontal lobe task is associated with blood lead when SRT and digit symbol sensitive to the affects of lead are not.

Table AX6-3.13 (cont'd). Neurobehavioral Effects Associated with Occupational Lead Exposure in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Latin America			
Maizlish et al. (1995) Venezuela	43 workers from a lead smelter, mean age 34 (9) years and 47 nonexposed workers, mean age 35 (11) years completed the WHO neurobehavioral core test battery. ANCOVA and linear regression adjusting for potential confounders examined relationship of lead exposure and NCTB.	Mean blood lead 43 (12.1) µg/dL Mean peak blood lead 60 (20.3) µg/dL Mean TWA 48 (12.1) µg/dL Controls Mean blood lead 15 (6) µg/dL Mean peak blood lead 15 (6) µg/dL Mean TWA 15 (6) µg/dL	Group comparison was significant for SRT ($p = 0.06$) but the lead exposed workers performed faster. Linear regression found SRT poorer performance with blood lead and TWA but not significant. With peak blood lead SRT improved with increasing lead exposure. In this study only symptoms were significantly different between the groups. (See above).
Asia			
Schwartz et al. (2001) South Korea	803 Korean lead exposed workers, 80% men and 20% women, mean age 40 (10.1) years from a variety of industries, and 135 controls, 92% men and 8% women, mean age 35 (9.1) years. Educational levels lead-exposed workers/controls ≤6 years = 23% / 7%, 7-9 years 23% / 11%, 10-12 years = 46% / 70%, and >12 years 8% / 12%. Group differences on neurobehavioral testing after controlling for covariates and linear regression controlling for covariates examined the presence of a dose-effect relationship.	Lead-exposed workers Mean blood lead 32 (15) µg/dL Tibia bone lead 37 (40.3) µg/g DMSA-chelatable lead level 186 (208.1) µg Controls Mean blood lead 5 (1.8) µg/dL Tibia bone lead 6 (7) µg/g	Nineteen outcomes examined. Compared to controls lead exposed workers performed significantly worse on SRT, Digit Span, Benton Visual Retention, Colored Progressive Matrices, Digit Symbol, and Purdue Pegboard after controlling for age, gender and education. The association of DMSA with test performance was lost by the addition of blood lead. Bone lead was not associated with neurobehavioral performance. blood lead was the best predictor for significant decrements in neurobehavioral performance on trails B ($\beta = -0.0025$ [SE 0.0009], $p < 0.01$), Purdue Pegboard (dom $\beta = -0.0159$ [SE 0.0042], $p < 0.01$; non-dom $\beta = 0.0169$ [SE 0.0042], $p < 0.01$; both $\beta = -0.0142$ [SE 0.0038], $p < 0.01$; assem $\beta = -0.0493$ [SE 0.0151], $p < 0.01$) and Pursuit Aiming (#corr $\beta = -0.1629$ [SE 0.0473], $p < 0.01$; #incorr $\beta = -0.0046$ [SE 0.0023], $p < 0.05$). The magnitude of the effect for these eight tests significantly associated with blood lead was an increase in blood lead of 5 µg/dL was equivalent to an increase of 1.05 years in age. Use of Lowess lines for Purdue Pegboard (assembly) and Trails B suggested a threshold at blood lead 18 µg/dL after which there is a decline of performance.

Table AX6-3.13 (cont'd). Neurobehavioral Effects Associated with Occupational Lead Exposure in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Schwartz et al. (2003) South Korea 1997-2001	Longitudinal decline in neurobehavioral performance examined in 576 of the above group of lead exposed workers who completed 3 visits at one year intervals. Mean age at baseline was 41 (9.5) years and job duration 9 (6.3) years and 76% were men. Compared to non-completers lead workers who completed 3 visits were 3.3 years older, baseline mean blood lead was 2.0µg/dL lower, on the job 1.6 years longer, 24% women vs. 10% of noncompleters, and usually had less than high school education. Models examined short-term versus long-term effects. Final model had current blood lead, tibia bone lead and longitudinal blood lead and covariates.	Baseline mean blood lead 31 (14.2) µg/dL Tibia lead 38 (43) µg/g	Blood lead from baseline correlated with those from visit 2 and 3 and baseline tibial lead correlated with that measured at visit 2. Cross-sectional associations of blood lead or short-term change occurred with Trails A (β = −0.0020 [95% CI: −0.0040, −0.0001]) and B (β = −0.0037 [95% CI: −0.0057, −0.0017]), Digit Symbol (β = −0.0697 [95% CI: −0.1375, −0.0019]) , Purdue Pegboard (dom β = −0.0131 [95% CI: −0.0231, −0.0031]; non-dom (β = −0.0161 [95% CI: −0.0267, −0.0055]); both (β = −0.0163, [95% CI: −0.0259, −0.0067]); assem (β = −0.0536 [95% CI: −0.0897, −0.0175]), and Pursuit Aiming #corr (β = 0.1526, [95% CI: −0.2631, −0.0421]) after covariates. However longitudinal blood lead was only associated with poorer performance on Purdue Pegboard non-dom (β = −0.0086 [95% CI: −0.0157, −0.0015](; both (β = −0.0063 [95% CI: −0.0122, 0.0004]); assem (β = −0.0289 [95% CI: −0.0532, −0.0046]). Historical tibial bone lead was associated with digit symbol (β = −0.0067 [95% CI: −0.0120, 0.0014]) and Purdue Pegboard dom (β = −0.0012, [95% CI: −0.0024, −0.0001]). Magnitude of lead associations was expressed as the number of years of increased age at baseline that was equivalent to an increase of lead from the 25th to 75th percentile. At baseline, these lead associations were equivalent to 3.8 years of age for cross-sectional blood lead, 0.9 years of age for historical tibial lead and 4.8 years of age for longitudinal blood lead. Analyses showed decline in performance over time related to tibia lead.

Table AX6-3.13 (cont'd). Neurobehavioral Effects Associated with Occupational Lead Exposure in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Hwang et al. (2002) South Korea	From the above cohort of 803 Korean lead workers, 212 consecutively enrolled workers, were examined for protein kinase C (PKC) activity and the relations between blood lead and neurobehavioral performance. PKC activity assessed by measuring levels of phosphorylation of three erythrocyte membrane proteins. Seventy-four percent of workers were men, mean age 36(0.8)years, duration of exposure 9 (0.6) and education 93% had high school or less. For the female workers, mean age 47 (0.9) years, duration of exposure 6 (0.5), and education 95% had high school or less.	Male workers Mean blood lead 32 (13.0) µg/dL Mean tibia lead 38 (39.6) µg/g Mean ZPP 69(47.8) µg/dL Female workers Mean blood lead 20 (9.2) µg/dL Mean tibia lead 26 (14.7) µg/g Mean ZPP 72 (29.7) µg/dL	Blood lead was associated significantly with decrements in Trails B ($\beta = -0.003$ [SE 0.002], $p < 0.10$), SRT ($\beta = -0.0005$ [SE 0.0003], $p < 0.10$) and Purdue Pegboard (dom $\beta = -0.21$ [SE 0.010], $p < 0.05$); non-dom ($\beta = -0.021$ [SE 0.010], $p < 0.05$); both ($\beta = -0.021$ [SE 0.009], $p < 0.05$). PKC activity as measured by back-phosphorylation of erythrocyte membrane proteins was not associated with neurobehavioral test scores. Addition of the interaction term of blood lead by back-phosphorylation dichotomized at the median found significant effect modification with the association of higher blood lead and poorer neurobehavioral performance occurring only among workers with lower back-phosphorylation levels that corresponds to higher in vivo PKC activity. Association of blood lead and SRT for the 52 kDa subunit with high in vivo PKC activity (adjusted $\beta = -0.001$, $p < 0.01$) and for low in vivo PKC (adjusted $\beta = -0.0001$, $p = 0.92$). The authors suggest that PKC activity may identify a subpopulation at increase risk of neurobehavioral effects of lead.
Chia et al. (2004) Singapore	120 workers from lead stabilizer factories, mean age 40 (10.7) years, duration of exposure 10.2 (7.9) were given a neurobehavioral battery. Genotyping of ALAD polymorphisms was performed. ANCOVA used to test for differences in neurobehavioral performance among ALAD polymorphism types adjusting for age, exposure duration and blood lead.	Mean blood lead 22 (9.4) µg/dL ALAD0.6 (0.25) µm of porphobilinogen/h/ml of RBC ALAU0.9 (0.56) mg/g cr	Frequency of ALAD1 1, 87%, ALAD1 2, 12%, and ALAD2 2, 1%. Mean blood lead adjusting for age and exposure duration was 20 µg/dL for ALAD1 1 (n = 107) and 20.4 µg/dL for ALAD1 2and 2 2 (n = 13). However ALAU was significantly higher in ALAD1 1 ($p = 0.023$). After adjusting for the covariates significant differences for grooved pegboard dominant hand ($p = 0.01$), non-dominant hand ($p = 0.04$), and grooved pegboard mean time ($p = 0.006$) were found between ALAD1 1 and ALAD1 2 & 2 2. Considering cognitive tests were part of battery it is surprising education was ignored. As noted by the authors the study only had 13 in the group with better performance and the ALAD1 2 or 2 2 genotypes limiting the power.

Table AX6-3.13 (cont'd). Neurobehavioral Effects Associated with Occupational Lead Exposure in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Chia et al. (1997) Singapore	50 lead battery manufacturing workers, mean age 36 (10.6) years, education 8.6 (2.1) years duration of employment 9 (7.4) years and 97 controls, mean age 34 (3.7) years, and education 12 (1.8) years were administered a neurobehavioral battery. ANCOVA and linear regression used to assess relationship of lead dose and performance.	Median blood lead of 38 (13.2 - 64.6) µg/dL Median IBL 264 (10.0 - 1146.2) µg-yr/dL Controls Median blood lead 6 (2.4 - 12.4) µg/dL	Significant group differences for Santa Ana, grooved pegboard, digit symbol, pursuit aiming and Trails A and B after adjusting for age, education, smoking, ethnic group and alcohol use. When the exposed group was stratified by age, in the group >35 years the poorer performance on digit symbol and Trails A was significantly associated with cumulative lead and not blood lead after adjusting for age and education.
Niu et al. (2000) China	44 lead-exposed workers (17 men, 27 women) from lead printing houses, mean age 35 (4.9) and education 9.3 (no SD) years and 34 controls (19 men and 15 women), mean age 33 (7.4) years and education 9.5 (no SD) years completed the NCTB. ANCOVA controlling for age, sex and education examined group differences and linear regression for dose-response relationship.	Mean blood lead 29 (26.5) µg/dL (8 workers blood lead exceeded 50 µg/dL) Controls Mean blood lead 13 (9.9) µg/dL (1 control blood lead exceeded 50 µg/dL)	SRT ($F = 2.30$, $p < 0.05$), digit symbol ($F = 4.81$, $p < 0.01$) pursuit aiming # correct ($F = 7.186$, $p < 0.01$) and pursuit aiming total ($F = 6.576$, $p < 0.01$) had significantly poorer performance compared to controls. No repression analyses provided.
Boey et al. (1988) Singapore	49 lead -exposed workers, mean age 26 (7.6) years and 36 controls, mean age30 (6.4) years completed SRT and 8 psychological tests covering attention, vigilance, visual-motor speed, short-term memory, visuomotor tracking, visual scanning, and manual dexterity. Control group was matched for education level. Discriminate analysis of neurobehavioral tests performed to determine which best discriminate the groups.	Mean blood lead 49 (15) µg/dL Controls Mean blood lead 15 (3) µg/dL	Six tests were significantly different between the two groups-Digit Symbol, Bourdon-Wiersma, Trails A, Santa Ana dominant, Flicker Fusion and SRT. The group of tests that best differentiates lead-exposed workers from nonexposed workers were Simple Reaction Time, Digit Symbol (WAIS) and Trail Making Test (Part A) with long latency in reaction time contributing three times more to the derived function than Digit Symbol (WAIS) or Trails A.

Table AX6-3.14. Meta-analyses of Neurobehavioral Effects with Occupational Lead Exposure in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States			
Davis et al. (1990)	Meta-analysis of 32 studies of nerve conduction studies and lead exposure.		Presented 41 effect sizes with the overall effect size for all studies $D = -0.369$ ($p \leq 0.001$). All median nerves combined was $D = -0.481$ ($p \leq 0.001$) and for all ulnar nerves $D = -0.211$ ($p \leq 0.001$). The median motor was most sensitive with an effect size of $D = -0.553$ ($p \leq 0.001$). Overall blood lead was a weak measure of exposure for the peripheral nervous system. Paradoxical association found effect size smaller with increasing blood lead but increased with duration of exposure.
Meyer-Baron et al. (2000)	Meta-analysis of studies with blood lead <70 µg/dL found 12 studies with comparable test procedure and sufficient documentation of results. Thirteen tests from the 12 studies examined.	Exposed group Range of mean blood lead 31 to 49 µg/dL Controls Range of mean blood lead 6 to 18 µg/dL	Block Design, Logical Memory, and Santa Ana had performance deficits with small effect size. For Block Design the effect size was comparable to changes observed with 20 years of aging. Aiming, SRT, Trials A and B, Digit Span and Digit Symbol also had poorer performance but the large variance for effect sizes suggest other factors besides lead exposure influenced performance. The authors conclude, "that the evidence of neurobehavioral deficits at a blood lead of approximately 40 µg/dL is obvious."
Goodman et al. (2002)	Meta-analysis of 22 studies with median blood lead <70 µg/dL, numbers of exposed and unexposed workers given with scores and dispersion on neurobehavioral tests.	Exposed group Range blood lead 24 to 63 µg/dL Unexposed group Range blood lead 0 to 28 µg/dL	Digit symbol and D-2 errors significant effect for fixed effects, weighted random effects and unweighted random effects. Simple reaction time, grooved pegboard, Trails A and B, picture completion visual reproduction, eye-hand coordination and vocabulary had significant effects for the fixed effects model only. The authors conclude none of the individual studies were adequate or conclusive of subclinical neurobehavioral effects of exposure to lead as the biological effects of blood lead <70 µg/dL are inconsistent. (See Schwartz et al. (2002) for comments).

Table AX6-3.14 (cont'd). Meta-analyses of Neurobehavioral Effects with Occupational Lead Exposure in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Schwartz et al. (2002)	Letter to the Editor commenting on shortcomings in the Goodman et al. (2002) meta-analysis on studies of neurobehavioral testing in workers occupationally exposed to lead.		The six points regarding problems with the methodology included: (1) no evaluation of quality of study design or statistical methods, (2) data from poorly done and well done studies are combined, (3) included 6 studies with no age adjustment and 3 with no adjustment for education, (4) confounding of age and education when addressed the variation across studies not discussed, (5) main effect only examined exposed versus nonexposed comparisons that are known to have the lowest power, cannot evaluated dose-effect relationships and have a tendency for selection bias, and (6) few of the 22 studies included contributed to effect size.
Seeber et al. (2002)	A comparison of the two meta-analyses Meyer-Baron and Goodman) performed to evaluate recommendations of a German BEI of 40 µg/dL.		Effect size calculated for 12 tests in two meta-analyses and 10 tests from one meta-analyses found subtle impairments associated with blood lead between37 µg/dL and 52 µg/dL for Logical Memory, Visual Reproduction, Simple Reaction Time, Attention Test d2, Block Design, and Picture Completion, Santa Ana, Grooved Pegboard and Eye-hand Coordination. Effect sizes related to age norms between approximately 40 to 50 years. For example, −3 score on Block Design = 10 to 15 years; −3.5 score on Digit Symbol = 10 years; −21 score on Cancellation d2 = 10 years; and +5 to +6 on Trails A = 10 to 20 years. This analyses concluded that both meta-analyses supported recommendation for German BEI of 40 µg/dL.
Graves et al. (1991)	A meta-analysis on 11 case-control studies of Alzheimer's disease for occupational exposure to solvents and lead.		Four studies had data for lead exposure with a pooled analysis of relative risks for occupational lead of 0.71 (95% CI: 0.36, 1.41). The exposure frequencies was 16/261 for the cases and 28/337 for the controls.

Table AX6-3.15. Neurophysiological Function and Occupational Lead Exposure in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Canada			
Bleecker et al. (2005b) New Brunswick 1992-=1993	74 current smelter workers, mean age 44 (8.4) years, education 8 (2.8) years and employment duration 20 (5.3) year had current perception threshold (CPT) measured for large and small myelinated and unmyelinated nerve fibers in the finger. Linear regression modeled CPT on metrics of lead dose after adjusting for covariates. Interaction of lead dose and ergonomic stressor on peripheral nerve function was assessed.	Mean blood lead 26 (7.1) µg/dL Mean IBL 891 (298.8) µg-yr/dL Mean TWA 42 (8.4)µg/dL Mean tibia bone 40 (23.8) µg/g 5 metrics relating to IBL cumulated only exposure above increasing blood lead ranging from 20 to 60 µg/dL	Blood lead and tibial bone lead were not associated with any of the three nerve fiber populations. IBL and TWA accounted for a significant percentage of the variance only for the large myelinated nerve fibers (ΔR^2 = 3.9%, ΔR^2 = 8.7% respectively). The relationship of CPT and TWA was curvilinear with a minimum at a TWA of 28 µg/dL. Unique variance of CPT for large myelinated fibers explained by different thresholds of IBL were IBL - 3.9%, $p = 0.08$; IBL20 - 5.8%, $p < 0.03$, IBL30 - 7.8%, $p < 0.02$; IBL40, $p < 0.005$; IBL50, $p < 0.005$; and IBL60, $p < 0.005$. IBL60 also explained significant variance of CPT for small myelinated nerve fibers demonstrating an increased impairment in peripheral nerve function. This effect on myelinated sensory nerve fibers was enhanced when a measure of ergonomic stress was added to the model for IBL60.
Europe			
Kovala et al. (1997) Finland	60 workers in a lead battery factory with a mean age of 43 (9) years and mean exposure duration of 16 (8) years. Nerve conduction studies, vibration thresholds, and quantitative EEG were performed. Relationship of lead exposure with peripheral nerve function and quantitative EEG were examined by partial correlation and regression analyses adjusting for age.	Mean Tibial lead 26 (17) mg/kg Mean Calcaneal lead 88 (54) mg/kg Mean IBL 546 (399) µg-yr/dL Mean TWA 34 (8.4) µg/dL, Mean Max blood lead 53 (19) µg/dL, Mean blood lead 27 (8.4) µg/dL	The sensory amplitude of the median and sural nerves had a negative correlation with IBL and duration of exposure that was not related to age. Vibration threshold at the ankle related significantly to IBL and duration of exposure after adjusting for age. Vibration threshold in the finger was associated with blood lead and blood lead averages over the past three years. The alpha and beta frequencies were more present in workers with higher long term lead exposure such as tibial and calcaneal, IBL and TWA. Overall historical blood lead measures were more closely associated with peripheral nerve function than bone lead concentrations. The study had no comparison group and did not account for the effect of smoking and alcohol use or give their usage in this population.

Table AX6-3.15 (cont'd). Neurophysiological Function and Occupational Lead Exposure in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia			
Schwartz et al. (2001) South Korea 1997-1999	804 workers from 26 different lead using facilities and 135 controls with a mean age of 40 (10.1) and 35 (9.1) years respectively, job duration of 8 (6.5) and 9 (5.3) years respectively, and education level 42 % and 69% completed high school respectively had comparable alcohol and smoking use. Linear regression used to compare vibration threshold in lead exposed and controls controlling for potential confounders.	Lead-exposed workers Mean blood lead 32 (15) µg/dL Tibia bone lead 37 (40.3) µg/g DMSA-chelatable lead level 186(208.1) µg (4 hour collection)	After adjustment for age, gender, education and height, tibia lead but not blood lead was significantly associated with poorer vibration threshold in the dominant great toe but not the finger ($\beta = -0.0020$ [SE 0.0007], $p < 0.01$). These results contrast with those for neurobehavioral measures (see above) performed in the same study where tibial lead was not a predictor of performance.
Schwartz et al. (2003) South Korea 1997-2001	Longitudinal decline in neurobehavioral performance examined in 576 of the above group of lead exposed workers who completed 3 visits at one year intervals. Mean age at baseline was 41 (9.5) years and job duration 9 (6.3) years and 76% were men. Compared to non-completers lead workers who completed 3 visits were 3.3 years older, baseline mean blood lead was 2.0µg/dL lower, on the job 1.6 years longer, 24% women vs. 10% of noncompleters, and usually had less than high school education. Models examined short-term versus long-term effects. Final model had current blood lead, tibia bone lead and longitudinal blood lead and covariates.	Baseline mean blood lead 31 (14.2) µg/dL Tibia lead 38 (43) µg/g	After adjustment for age, visit number, education, gender, height (for vibration) and BMI (for grip strength and pinch) vibration threshold in the dominant great toe and not the finger was associated with tibia lead ($\beta = -0.0006$ [95% CI: −0.0010, −0.0002]) and longitudinal blood lead ($\beta = -0.0051$ [95% CI: −0.0078, −0.0024]) in one Model and blood lead ($\beta = -0.0019$ [95% CI: −0.0039, 0.0001]) in another model.

Table AX6-3.15 (cont'd). Neurophysiological Function and Occupational Lead Exposure in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Chuang et al. (2000) Taiwan	206 lead battery workers, mean age 41 years, with annual blood lead for the previous five years had vibration perception measured in hand and foot. Relationship of lead exposure term and vibration perception threshold assessed with multiple regressions, hockey stick regression analysis after adjusting for potential confounders.	Mean blood lead 28 µg/dL, Mean blood lead over past 5 years 32 µg/dL Mean maximum blood lead 39 µg/dL Mean index of cumulative exposure 425 µg-yr/dL, Mean TWA 32 µg/dL Mean working duration 13 years and life span in work 31%.	After adjustment for age, sex, body height, smoking, alcohol consumption, and use of vibrating hand tools, significant association between mean blood lead and mean TWA and vibration perception in the foot were found. After adjustment for the covariates, a hockey stick regression analysis of foot vibration threshold versus mean blood lead concentration for 5 years found an inflection point around 30 µg/dL with a positive linear relation above this point suggesting a potential threshold.
Chia et al. (1996a) Singapore	72 workers in a lead battery manufacturing factory with a mean age of 30 years and reference group of 82 workers had nerve conduction studies and blood lead performed every 6 months over the course of three years. Only 28 lead battery workers completed the program. At the end of the first year of the 82 workers in the comparison group only 26 remained and by year 3 this had decreased to 4. Mean nerve conduction values examined by ANCOVA between the exposed and reference after adjustment for age, ethnic group, smoking and drinking habits. Analysis of serial nerve conduction values and blood lead treated as a clustered sample had the within-cluster regression coefficient examined. The 28 exposed workers were stratified by blood lead level and the relationship between nerve conduction values and blood tested within the cluster.	The geometric mean blood lead concentrations for the 6 testing periods were 37, 41, 42, 40, 41, and 37 µg/dL. The overall range for blood lead was 16-73 µg/dL.	The relationship between blood lead levels and nerve conduction values for the 28 exposed workers was significant for all outcomes except median motor conduction velocity and ulnar sensory nerve conduction velocity and ulnar sensory amplitude. The regression correlation coefficients for blood lead >40 µg/dL was significant for all parameters except the median sensory conduction velocity and for blood lead <40 µg/dL there was no association with nerve conduction values. Therefore the blood lead level associated with no change in nerve conduction studies was <40 µg/dL.

Table AX6-3.15 (cont'd). Neurophysiological Function and Occupational Lead Exposure in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Chia et al. (1996b) Singapore	Extension of above study - 72 workers in lead battery manufacturing and 82 controls. Mean duration of exposure 5.3 years.	Mean blood lead 37 µg/dL Mean blood lead cumulative 137 µg-yr/dL	ANCOVA found significant differences for all nerve conduction parameters except three for the ulnar nerve, after adjusting for age, ethnic groups, smoking and drinking habits. There was no significant correlation between blood lead and blood leadCum with nerve conduction values after linear regression with adjustment for confounders. When blood leadCum was stratified- 12 workers <40 µg-yr/dL, 28 workers 40-300 µg-yr/dL, 21 workers >300 µg-yr/dL ANCOVA found significant differences for 5 nerve conduction parameters. The strongest dose effect relationship was for sensory nerve conduction velocity.
Chuang et al. (2004) Taiwan	181 lead battery manufacture workers were stratified by milk drinkers, n = 158 and non- or rare mild drinkers n = 23. Mean age in the two groups was 40 and 36 years and working duration 10/8 years respectively. Peripheral nerve evaluation was with current perception threshold at 3 frequencies 5Hz = C fibers, 250 Hz = A-delta fibers and 2000 Hz = A-beta fibers. Linear regression estimated the association of CPT and lead exposure variable and adjustment of milk intake and potential confounders.	Blood lead 25 µg/dL milk drinkers 30 µg/dL non or rare milk drinkers TWA 28 µg/dL milk drinkers 32 µg/dL non or rare milk drinkers IBL 316 µg-yr/dL milk drinkers 245 µg-yr/dL non or rare mild drinkers	Age was significantly different but distributions of gender, smoking, alcohol use, use of hand vibration tool, working history and height were not different. Linear regressions found association of 5 Hz CPT and 250 Hz CPT in hand and foot with blood lead and TWA but not IBL. However the protective effects of drinking milk was present for all fiber populations only in the hands. This paper presents an unusual finding of subclinical lead neuropathy involving the unmyelinated and small myelinated fibers. Toxic axonopathies classically involve the large nerve fibers. The main group difference may be related to other nutritional deficiencies associated with the malabsorption syndrome that lead to the non-milk drinking status.
Yokoyama et al. (1998) Japan	17 gun-metal workers, mean age 48 years and a 20 controls with a mean age of 45 years had distribution of conduction velocities (DCV) measured and the maximum median sensory conduction velocity (SVC) performed twice at a year interval. Group differences controlling for confounders and dose-effect relationships were examined.	Mean blood lead 40 µg/dL Mean mobilized Pb (CaEDTA) in urine 1 mg/24 h	ANCOVA controlling for age and alcohol found mobilized lead was associated with significant slowing in the large nerve fibers while blood lead was not. Workers with increased change in mobilized lead over 1 year interval (mean 0.44 mg/24hr) had significant reduction in large fiber (V95) conduction velocity while those workers with less change in mobilized lead (0.08 mg/24hr) did not have significant change in DCV or SVC. It appears that larger faster conducting nerve fibers are susceptible to lead and a measure of body burden (readily mobilized lead from soft tissue) is a stronger predictor of this change than blood lead.

Table AX6-3.15 (cont'd). Neurophysiological Function and Occupational Lead Exposure in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
He et al. (1988) China	40 workers in a lead smelter with age range 20 to 45 years (no mean provided) and duration of exposure 5.4 years. Fifty controls age 20 to 55 years. Nerve conduction studies examined 11 parameters. Student = s t-test examined for differences between exposed and controls.	Mean blood lead 40 µg/dL Mean urinary lead 71 µg/dL Mean ALAU5 µg/dL	There were no symptoms or signs of peripheral nerve disorder. Both motor and sensory conduction velocities were slowed in the lead exposed groups. 10 nerve conduction parameters were significant in the group with blood lead >40 µg/dL and 6 parameters were significant in the group with blood lead <40 µg/dL. An unusual finding in this study was the lack of age association with nerve conduction values and therefore it was not controlled for in the analyses.
Niu et al. (2000) China	44 lead-exposed workers (17 men, 27 women) from lead printing houses, mean age 35 (4.9) and education 9.3 (no SD)years and 34 controls (19 men and 15 women), mean age 33 (7.4) years and education 9.5 (no SD) years had nerve conduction studies for maximal motor nerve conduction velocity. ANCOVA controlling for age, sex and education examined group differences and linear regression for dose-response relationship.	Mean blood lead 29 (26.5) µg/dL (8 workers blood lead exceeded 50 µg/dL) Controls Mean blood lead 13 (9.9) µg/dL (1 control blood lead exceeded 50 µg/dL)	Only 12 lead exposed workers and 24 controls examined for NCV. Left ulnar nerve was significantly slower but the left median and right ulnar were faster in the lead exposed and the right median was slightly slower. This appears to be a finding of chance due to the small n. For the lead exposed group mean left ulnar CV was 52 while the mean right ulnar CV was 59 while for the controls left ulnar CV was 58 while the mean right ulnar CV was 55.

Table AX6-3.16. Evoked Potentials and Occupational Lead Exposure in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Canada			
Bleecker et al. (2003) New Brunswick 1992-1993	359 currently employed smelter workers, mean age 41 years, had brainstem auditory evoked potentials (BAEP) measured. Relationship between absolute latencies and interpeak latencies assessed using linear regression after adjusting for potential confounders. Exposure was assessed in cases with clinical abnormalities in Wave I and I-V interpeak latency compared to those workers with normal BAEP using post-hoc analysis.	Mean blood lead 28 µg/dL Mean TWA 39 µg/dL Mean IBL 719 µg-yr/dL	Linear regression after the contribution of age found blood lead and TWA were significantly associated with Wave I while IBL was significantly associated with Wave III and I-III interpeak interval. Four groups created with increasing abnormalities based upon clinical cut-off scores for Wave I and I-V interpeak interval had similar age. blood lead, TWA and IBL were all significantly higher in the group with prolonged Wave I and I-V interpeak interval compared to the group with normal BAEP = s. These findings support involvement of the brainstem and auditory nerve with lead exposure.
Europe			
Abbate et al. (1995) Italy	300 lead exposed men ages 30 to 40 years in good health with no other neurotoxic exposure had P100 latency measured for visual evoked potentials (VEP) for 15 and 30 minute of arc. Groups created based upon blood lead had VEPS examined followed by linear regression for each group.	Blood lead 17 to 60 µg/dL range Mean blood lead for 4 groups n = 39 23 µg/dL n = 113 30 µg/dL n = 89 47 µg/dL n = 59 56 µg/dL	ANOVA of the blood lead and P100 latencies were significantly prolonged for 15 and 30 minutes of arc. Linear regression found the association of blood lead and P100 were significant in each group but the relationship was not proportional (angular coefficient). Effect of blood lead on VEP began at 17-20 µg/dL. With age limited to one decade, contribution from age was not a concern. Even though no comparison group, careful screening ruled out other medical and eye conditions and other potential exposures.
Discalzi et al. (1992) Italy	49 lead exposed workers and 49 age and sex matched controls had BAEPs measured. Relationship of 6 BAEP outcome variables and lead exposure examined with analysis of variance and linear regression.	Mean blood lead 55 µg/dL and TWA for previous 3 years 54 µg/dL	Latencies for waves I, III, V and interpeak latencies, I-V, I-III, and III-V were all significantly prolonged in the lead-exposed workers ($p < 0.04$). No significant association found with linear regression between BAEP outcomes and exposure variables. In those workers with TWA >50 µg/dL, I-V latency was significantly prolonged compared to workers with TWA <50 µg/dL.
Discalzi et al. (1993) Italy	22 battery storage workers, mean age 35 years and 22 control group, age and sex matched, with normal hearing had BAEPs recorded. Latencies I and V and lead exposure examined by ANOVA after stratifying blood lead.	Mean blood lead 48µg/dL	Interpeak latency I-V was significantly prolonged in lead exposed workers ($p = 0.001$). No significant associations by linear regression between I-V and lead exposure. Stratifying lead exposed workers by blood lead 50 µg/dL found I-V interpeak latency significantly prolonged ($p = 0.03$) in subgroup with higher blood lead.

Table AX6-3.16 (cont'd). Evoked Potentials and Occupational Lead Exposure in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Latin America			
Counter et al. (2002) Ecuador	30 lead-glazing workers, median age 35 years, had pure-tone thresholds and BAEPs performed. Regression analyses examined relations between auditory outcomes and blood lead.	Mean blood lead 45 µg/dL (range 11 to 80 µg/dL)	Sixty percent of the men and 20 percent of the women had abnormal high-frequency thresholds, however there was no significant relationship with blood lead and pure tone threshold at all frequencies. Analysis of BAEPs found agreement between latencies for Waves I, III and V and peripheral hearing status. Interpeak latencies were within normal limits but no analysis provided with lead exposure. Workers lived in a lead contaminated environment from discarded lead-acid storage batteries. Therefore a measure of chronic lead exposure may have been more appropriate.
Asia			
Holdstein et al. (1986) Israel	20 adults and 8 children (mean age 27 years, range 8 - 56 years) accidentally exposed to lead through food until one year prior to measurement of BAEP.	Adult mean blood lead 31 µg/dL Children mean blood lead 22 µg/dL In the adults 10 month average blood lead in adults 43 µg/dL and in children 36 µg/dL	In adults, latencies I, III and I-III and I-V interpeak intervals were significantly longer than the control group ($p < 0.05$). When group stratified by 10 month average blood lead I-III interpeak interval was longer in the high group. Age and blood lead were not studied due to few subjects. The I-III interpeak interval reflects transmission in the lower brainstem and VIIIth nerve.
Hirata et al. (1993) Japan	41 lead-exposed men from lead-glass-based colors manufacturing (n = 20), production of lead electrode plates (n = 8), casting of lead-bronze (n = 4) and casting of lead pipes and plates (n = 9) had mean age 41 years, mean duration of exposure 13 years. A battery of tests administered including radial nerve conduction study, electroretinogram (ERG), visual evoked potential (VEP), brainstem auditory evoked potential (BAER), and short-latency somatosensory evoked potential (SSEP). Comparison group of 39 unexposed used only for BAER analysis by Student's t test. Correlation and linear regression controlling for age examined the relationship of lead and the other variables.	Mean blood lead 43 µg/dL (13-70) Mean TWA based upon previous 5 years 43 µg/dL (13-70) Mean duration of exposure 13 (0.6-29) years.	Significant partial correlation after adjusting for age included TWA and radial motor conduction velocity, blood lead and sensory conduction velocity, exposure duration and VEP, blood lead and SSEP-N20. Comparison of BAERs of 15 lead exposed and 39 controls found interpeak interval III-V was prolonged significantly. It is not clear why comparison group only used for BAERs. Considering the large number of variables examined with three exposure terms some of the findings could be by chance alone.

Table AX6-3.16 (cont'd). Evoked Potentials and Occupational Lead Exposure in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Murata et al. (1993) Japan	22 gunmetal foundry workers with age range of 32 to 59 years and work duration of 1 to 19 years and control group matched for age, no chronic disease and no lead exposure participated. No significant difference between groups for age, height, skin temperature, alcohol consumption, and years of schooling. The test battery consisted of visual evoked potential (VEP), brainstem auditory evoked potential (BAEP), short latency somatosensory-evoked potential (SSEP), event related potential (P300) and EKG R-R interval variability. Paired-sample t test examined for differences between the matched groups. Dose-effect relationships examined with partial correlation adjusting for age and stepwise linear regression.	Blood lead 12 to 64 µg/dL (no mean provided)	For VEPs, N75 and N145 were significantly prolonged in the lead exposed workers. N9-N13 interpeak latency of the SSEP was significantly prolonged. BAEP latencies showed no significant differences. P300 believed to reflect cognitive function was prolonged in the lead workers and correlated with blood lead, and PbU. Autonomic nervous system effects were significantly diminished for $CV_{R\text{-}R}$ and for a measure of parasympathetic activity $C\text{-}CV_{RSA}$. Fifty percent of the outcome variables showed significant group differences but there is limited dose effect for any outcome within the exposed group. Small sample size limited conclusions with 20 outcome variables and 8 biomarkers of lead exposure.

Table AX6-3.17. Postural Stability, Autonomic Testing, Electroencephalogram, Hearing Thresholds, and Occupational Lead Exposure in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe			
Kovala et al. (1997) Finland	60 workers in a lead battery factory with a mean age of 43 (9) years and mean exposure duration of 16 (8) years. Quantitative EEG were performed. Relationship of lead exposure with quantitative EEG were examined by partial correlation and regression analyses adjusting for age.	Mean tibial lead 26 (17) mg/kg Mean calcaneal lead 88 (54) mg/kg Mean IBL 546 (399) µg-yr/dL, Mean TWA 34 (8.4) µg/dL, Mean max blood lead 53 (19) µg/dL, Mean blood lead 27 (8.4) µg/dL	The alpha and/or beta frequencies were more present in workers with higher long term lead exposure such as tibial ($p < 0.05$) and calcaneal ($p < 0.05$), IBL ($p < 0.01$) and TWA ($p < 0.05$). Slow alpha in workers was believed to correlate with increased episodes of 'microdrowsiness'. The study had no comparison group and did not account for the effect of smoking and alcohol use or give their usage in this population.
Asia			
Yokoyama et al. (1997) Japan	49 chemical workers exposed to lead stearate, mean age 48 (1.3) years and 23 controls, mean age 47 (2.5) had postural sway evaluated. ANCOVA examined group differences after adjusting for covariates.	Mean blood lead 18 (1.0) µg/dL Mean maximum blood lead 48 (3.8) µg/dL TWA 24 (1.3) µg/dL Cumulative blood lead 391 (48.2) µg-yr/dL	There were significant increases in sway in all directions at high and low frequencies with eyes open and eyes closed ($p < 0.05$). Regression analysis found blood lead associated with sway in the anterior-posterior direction, .5-1Hz (0.321, $p = 0.03$), 1-2Hz (0.313, $p = 0.04$) and TWA associated with right to left sway (0.326, $p = 0.02$) after adjustment for the covariates age, height, weight and alcohol consumption. The authors conclude that change in the vestibulo-cerebellum is affected by blood lead while in the anterior cerebellar lobe is affected by past lead exposure.
Chia et al. (1994) Singapore	60 lead storage workers, mean age 32 (7.7) years and 60 controls, mean age 35 (7.4) had postural sway parameters measured. ANCOVA used to examine group differences after adjusting for covariates. Linear regression examined relationship between lead exposure and postural sway.	Mean blood lead 36 (11.7) µg/dL Controls Mean blood lead 6 (2.4) µg/dL	Computerized postural sway measurements found lead workers have poorer postural stability that increased with eyes closed ($p < 0.01$). Regression analysis adjusting for age, height, and weight found no significant association with blood lead.
Chia et al. (1997) Singapore	The same 60 lead storage workers as above and 60 control had postural sway data examined for contribution of cumulative blood lead fractionated over 10 years of exposure.	Mean blood lead 36 (11.7) µg/dL Controls Mean blood lead 6 (2.4) µg/dL	The lead exposed group had significantly poorer performance on all postural sway parameters with eyes closed compared to controls after adjusting for height, weight, age and drinking habits ($p < 0.01$). All postural sway parameters with eyes closed were significantly associated with IBL for the 2 years prior to testing ($n = 23$, $p < 0.05$).

Table AX6-3.17 (cont'd). Postural Stability, Autonomic Testing, Electroencephalogram, Hearing Thresholds, and Occupational Lead Exposure in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Ratzon et al. (2000) Israel	63 lead battery workers, mean age 39 (8.7) years and 48 controls mean age 36 (11.8) years, matched for age with similar sex and education, had postural control measured. Group differences examined with t test. Dose-effect relations assessed with Pearson = s correlation coefficients. Linear regression done with exposure category as major predictor.	Mean past blood lead 38 µg/dL, mean years employed 11 and cumulative lead determined by average blood lead X years employed	Using a computerized sway measurement system the exposed workers had significantly increased mean body oscillations with eyes closed ($p < 0.01$) and head tilted forward ($p < 0.001$). Partial correlation adjusting for education, coffee consumption, hours of sleep and estimate of health was significant only for total lead exposure and increased body oscillations with head tilted forward ($\beta = 2.25$, $p = 0.0089$). In order to maintain balance lead exposed workers required increased oscillations when visual and vestibular inputs were altered.
Teruya et al. (1991) Japan	172 lead exposed workers, mean age 34 (18.4-57.4)years had cardiac autonomic nervous system evaluated by R-R intervals variation with respiration measured.	Mean blood lead 36 (5-76) µg/dL	Age adjustment controlled for by use of ratios of predicted to observed values. A significant dose related decrease of R-R interval variation during deep breathing was present in 132 workers with stable blood lead over the past year ($p < 0.01$). This finding was more prominent in younger workers with blood lead ≥30 µg/dL but a mild decrease present at blood lead ≥20 µg/dL. A decrease in R-R interval variation indicates decreased cardiac parasympathetic function.
Ishida et al. (1996) Japan	128 workers in the ceramic painting industry, 58 men, mean age 55 (11.7) years and 70 women, mean age 52 (9.2) years had measures of sympathetic function by variations in R-R interval on EKG and changes in finger blood flow with postural changes using Doppler flowmetry. Correlation analyses and linear regression examined relationship of finger blood flow and lead exposure after adjusting for covariates.	Men Mean lead 17 (2.1) µg/dL ALAD62 (28.3)5 Women Mean blood lead 11 (1.7) µg/dL ALAD73 (20.8)%	22% had blood lead >20 µg/dL, and 43% had ALAD% <60%. The 46 workers in the lowest group with blood lead <10 µg/dL had ALAD%>80% equivalent to nonoccupational exposure and therefore served as the control group. Blood lead ($\beta = 0.205$, $p = 0.02$), smoking ($\beta = -0.464$, $p < 0.01$), and BMI ($\beta = 0.213$, $p = 0.01$) were significant predictors of change in finger blood flow with postural change. Decrease in change of finger blood flow is compatible with a peripheral sympathetic nerve impairment.

Table AX6-3.17 (cont'd). Postural Stability, Autonomic Testing, Electroencephalogram, Hearing Thresholds, and Occupational Lead Exposure in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Niu et al. (2000) China	44 lead-exposed workers (17 men, 27 women) from lead printing houses, mean age 35 (4.9) and education 9.3 (no SD) years and 34 controls (19 men and 15 women), mean age 33 (7.4) years and education 9.5 (no SD) years had autonomic nervous system examined. ANCOVA controlling for age, sex and education examined group differences and linear regression for dose-response relationship.	Mean blood lead 29 (26.5) µg/dL (8 workers blood lead exceeded 50 µg/dL) Controls Mean blood lead 13 (9.9) µg/dL (1 control blood lead exceeded 50 µg/dL)	Niu et al. (2000) examined autonomic nervous system in 44 lead exposed workers, mean blood lead 29 µg/dL, and 34 controls, mean blood lead, 13 µg/dL. Linear regression found association between blood lead and decreased R-R interval with valsalva (F/T2.349, $p < 0.05$) and duration of lead exposure and decreased R-R interval with deep breathing (F/T 3.263, $p < 0.01$) after adjusting for age, sex, education, smoking and drinking. In the same study, quantitative EEG found significant abnormalities in the lead-exposed workers, dominant low amplitude in 59%, dominant beta frequency in 42% and abnormalities in 81%.

Table AX6-3.18. Other Neurological Outcomes Associated with Lead Exposure in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States			
Louis et al. 2005 New York	63 cases of essential tremor (ET) and 101 controls, similar for age, 67 (16.6) and 65 (11.1) years, education, gender and ethnicity were examined for interaction of blood lead and ALAD gene polymorphisms and increased odds of ET.	ET Mean blood lead 4 (2.2) µg/dL Controls Mean blood lead 3 (1.5) µg/dL 2 ET cases but no controls had blood lead >10 µg/dL	Of the 63 ET cases 18 (29%) vs. 17 (17%) of 101 controls had an ALAD-2 allele (OR 1.98 [95% CI: 0.93, 4.21]; p = 0.077). When log blood lead was examined by presence of ALAD2 allele in ET, log blood lead was highest in ET cases with and ALAD2 allele, intermediate in ET cases without an ALAD2 allele and lowest in controls (test for trend, β = 0.10; p = 0.001). When ALAD2 allele was present, blood lead was significantly associated with odds of ET (OR 80.29 [95% CI: 3.08, 2.096]; p = 0.008). This increased odds of ET with an ALAD-2 allele was 30 times greater than in an individual with only an ALAD-1 alleles. In the highest log blood lead tertile, ALAD2 allele was present in 22% of ET cases and 5% of controls. It was proposed that increased blood lead along with the ALAD2 allele could affect the cerebellum and thereby increase the risk of tremor.
Louis et al. 2003 New York	100 cases of ET and 143 controls matched for age, sex, and ethnicity. The relationship between blood lead and ET was examined.	ET Mean blood lead 3 µg/dL Controls Mean blood lead 2 µg/dL	Ten cases and 7 controls had bone lead levels measured that were significantly correlated with blood lead suggesting that higher blood lead may have occurred in the past. Total tremor score was correlated with blood lead (r = 0.14, p = 0.03). Logistic regression adjusting for age and current cigarette smoking found the odds ratio for ET was 1.19 (95% CI: 1.03, 1.37) per unit increase in blood lead. Blood lead was higher in those 39 ET cases with no family history. Both current and lifetime prevalence of occupational lead exposure was the same in ET cases and controls but those with history of occupational exposure did have a higher blood lead than those without this history (median, 3.1 µg/dL vs. 2.4 µg/dL, p = 0.004).

Table AX6-3.18 (cont'd). Other Neurological Outcomes Associated with Lead Exposure in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Kamel et al. (2002) Massachusetts	109 cases of ALS and 256 controls matched for age, sex and region of residence examined the relation of lead and ALS.	Cases/controls Mean blood lead 5(0.4)/3(0.4) µg/dL 3 cases and no controls had blood lead >10 µg/dL . Patella lead 21 (2.1)/17 (2.0) µg/g 5 cases and 1 control had patella lead levels >50 µg/g Tibia lead 15(1.6)/11(1.6) µg/g 2 cases and no controls had tibia lead >50 µg/g.	Increased risk of ALS was found for history of occupational lead exposure (adjusted OR 1.9 [95% CI: 1.1, 3.3]) increased lifetime days of exposure (adjusted OR 2.3 [95% CI: 1.1, 4.9]). Association of blood lead and ALS (adjusted OR 1.9 [95% CI: 1.4, 2.6]). Elevation in both blood lead and patella and tibia bone lead was found in ALS cases though the precision of these measurements was questioned (Patella lead adjusted OR 3.6 [95% CI: 0.6, 20.6] and tibia lead adjusted OR 2.3 [95% CI: 0.4, 14.5]). Therefore, this study found lead exposure from historical questionnaire data and biological markers associated with ALS.
Kamel et al. (2003) Massachusetts	As above, the same data was used to determine the associations of ALS with polymorphism in ALAD and the vitamin D receptor (VDR) and the influence of genotype.	Same as above	The ALAD2 allele was associated with a 2-fold increase risk of ALS after adjustment for the covariates, age, sex, region, education and physical activity adjusted (OR 1.9 [95% CI: 0.60, 6.3]). Additionally adjusting for blood lead strengthened the association of ALAD2 and ALS risk adjusted (OR 3.6 [95% CI: 0.9, 15]). This was not found for bone lead or occupational history of lead exposure (Patella adjusted OR 2.1 [95% CI: 0.61, 6.9]; tibial adjusted (OR 2.2 [95% CI: 0.66, 7.3]; occup his adjusted (OR 2.4 [95% CI: 0.67, 8.7]). VDR was not associated with lead or ALS risk.
Armon et al (1991) Minnesota	A case-control design with 47 ALS patients, mean age 61 with involvement of upper and lower motor neurons and 201 controls, mean age 62. For the lead exposure analysis 45 male matched pairs were examined.	Lifetime exposure to lead of 200 hours or more (years on job x hours spent per week)	Of 13 discordant pairs for lead exposure, 11 were in ALS patient. The relative risk was 5.5 (95% CI: 1.44, 21.0). A dose-response was weakened by 3 controls with highest lifetime exposure. Men with ALS worked more often at blue collar jobs and significantly more time welding ($p < 0.01$). These results expanded a prior pilot study that found a higher incidence of heavy metal exposure in ALS cases.

Table AX6-3.18 (cont'd). Other Neurological Outcomes Associated with Lead Exposure in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe			
Chancellor et al. (1993) Scotland 1990-1991	A case-control design 103 ALS patients from the Scottish Motor Neuron Disease Register and matched community controls. Differences in potential occupational exposures were determined between cases and controls.	Exposure to lead obtained by lifetime employment history from Office of Population and Censuses and Surveys. Physician's record review and direct interview questionnaire.	Odds ration for manual labor in ALS patients was 2.6 (95% CI: 1.1, 6.3). Occupational exposure to lead was more common in ALS patients (OR 5.7 [95% CI: 1.6, 30]).
Gunnarsson et al. (1992) Sweden 1990	A case-control study of 92 cases of MND and 372 controls. MND included ALS, progressive bulbar paresis (PBP), and progressive muscular atrophy (PMA). Relation of MND to risk factors including occupational exposure examined.	Exposure information obtained by self-administered questionnaire.	Exposure to heavy metals primarily from welding had an increased Mantel-Haenszel odds ratio of 3.7 [95% CI: 1.1, 13.0].
Guidetti et al. (1996) Italy	A retrospective incidence, prevalence, and mortality survey of ALS in northern Italy was performed.	Mean air lead 3μg/m^3 in 1975 to 1μg/m^3 in 1985; blood lead in monitored children decreased 18, 14, and 11 μg/dL in same time period.	The area studied had documented lead pollution for years. Based upon 79 cases incidence and prevalence rate were comparable to the surrounding area.
Vinceti et al. (1997) Italy	19 ALS cases, mean age 66 (14) years and 39 controls, mean age 64 (12.9) years.	Sporadic ALS Mean blood lead of 13 (6.8) μg/dL Controls mean blood lead 11 (4.4) μg/dL	There were no cases familial ALS. Blood lead between ALS cases and controls was not significantly different. Blood lead was associated with disability due to ALS but no support was found for involvement of lead in the etiology of sporadic ALS.

Table AX6-3.19. Occupational Exposure to Organolead and Inorganic Lead in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States			
Schwartz et al. (1993)	Two hundred and twenty-two current employees that manufactured tetraethyl lead participated in a study to determine if there was impairment on a neurobehavioral battery associated with a measure of cumulative exposure to organic and inorganic lead derived from 12 years of air sampling. Mean age was 44 (8.7) years, education 13 (1.7) years.	Cumulative lead exposure, inorganic and organic 869 (769) µg-yr/m^3 Mean years of exposure 13 (9.5)	Exposure was divided into 4 groups with the lowest for years of exposure and cumulative lead exposure serving as the reference group. After adjustments for premorbid intellectual ability, age, race, and alcohol consumption, cumulative lead exposure had differential association poorer performance in many cognitive domains but most often in manual dexterity and verbal memory/learning. Performance on tests associated with exposure was 5 to 22% lower in the highest groups when compared with the low exposure reference group.
Stewart et al. (1999)	543 former organolead workers, mean years since last exposure18, examined for ongoing neurobehavioral impairment related to past lead exposure. Thirty-eight % were age 60 or older, predominantly white, 93% had at least a high school degree. Linear regression assessed the relationship between lead dose and neurobehavioral function adjusting for the covariates.	Mean tibial lead 14 (9.3) µg/g Peak tibial bone lead extrapolated back using a clearance half-time of lead in tibia of 27 years 24 (17.4) µg/g DMSA chelatable lead level 19 (17.2) µg (urine collected for 4 hours)	Peak tibial lead was a significant predictor of poorer performance on vocabulary ($\beta = -0.063$, $p = 0.02$), serial digit learning ($\beta = -0.043$, $p = 0.04$), RAVLT trial 1 ($\beta = -0.054$, $p = 0.03$), RAVLT recognition ($\beta = -0.019$, $p = 0.03$), Trails B ($\beta = -0.002$, $p = 0.03$), finger tapping nondominant ($\beta = -0.042$, $p = 0.02$), Purdue pegboard dominant ($\beta = -0.043$, $p = 0.00$); nondominant ($\beta = -0.49$, $p = 0.00$), both ($\beta = -0.038$, $p = 0.00$) assembly ($\beta = -0.133$, $p = 0.00$) and Stroop ($\beta = -0.014$, $p = 0.00$). Current tibial lead had similar associations Vocabulary ($\beta = 0.103$, $p = 0.04$), Digit Symbol ($\beta = -0.095$, $p = 0.05$), finger tapping dominant ($\beta = -0.87$, $p = 0.02$), Finger tapping nondominant ($\beta = 00.102$, $p = 0.00$), Purdue Pegboard dominant ($\beta = -0.065$, $p = 0.01$), nondominant ($\beta = -0.091$, $p = 0.00$), both ($\beta = -0.068$, $p = 0.00$), assembly ($\beta = -0.197$, $p = 0.03$), Stroop ($\beta = 0.017$, $p = 0.01$). DMSA-chelatable lead was only significantly associated with choice reaction time ($\beta = -0.001$, $p = 0.01$).

Table AX6-3.19 (cont'd). Occupational Exposure to Organolead and Inorganic Lead in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Stewart et al. (2002)	From the above group of former organolead workers 535 were re-examined twice or four times over a four year period. Also a nonexposed control group of 118 had repeat examinations. Mean age at first visit exposed/controls 56 (7.4)/59 (7.0), percentage with at least a high school education 66/71.2.	First examination Mean blood lead 5 (2.7) µg/dL Mean tibia lead 14 (9.3) µg/g Mean peak tibia lead 23 (16.5) µg/g Mean exposure duration 8 (9.7) years Mean duration since last exposure 16 (11.7) years	On 17 of 19 neurobehavioral tests, former organolead workers demonstrated greater annual decline in adjusted test scores compared to controls with significant differences for Rey complex Figure copy, RAVLT Trial 1 and RAVLT recognition. Annual declines in performance showed greater age-related change in lead workers compared to controls for block design, digit symbol, serial digit learning, finger tapping and Trails A. Blood lead did not predict annual change scores but peak tibial lead did for symbol digit, Rey Complex Figure delayed recall, RAVLT trial1 , RAVLT delayed recall, Purdue pegboard (1 measure) and the Stroop. For these 6 tests it was determined that an increase of 15.7 µg/g bone mineral of peak tibia lead was equivalent in its effect on annual test decline to 5 more years of age at baseline. Authors conclude that data supports ongoing cognitive decline associated with past occupational exposure to lead.
Balbus et al. (1997)	222 organolead manufacturing workers, mean age 44 (8.7) years and 62 nonexposed referents, mean age 43 (10) years performed simple visual reaction time (SVRT). Linear regression examined relationship between lead exposure and mean RT, median RT and standard deviation of RT after controlling for covariates.	Mean blood lead 20 (9.5) µg/dL Mean peak urine lead level 143 (130)µg/L	
Balbus et al. (1998)	A second publication further examined the above data for relationship of interstimulus interval (ISI) and lead exposure.	Same as above	Short ISIs,1-3 seconds, had no relationship with lead exposure while ISIs of 4-6 seconds were significantly associated with blood lead ($\beta = 0.06$ [SE 0.02], $p = 0.02$ along with ISIs of 7-10 seconds ($\beta = 0.05$ [SE 0.02], $p = 0.03$). ISIs 7-10 seconds with peak urine lead levels ($\beta = 64.29$ [SE 21.86], $p < 0.01$).

Table AX6-3.19 (cont'd). Occupational Exposure to Organolead and Inorganic Lead in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Stewart et al. (2002)	Population as described in Stewart et al. (1999) and Schwartz et al. (2000). Data on 20 neurobehavioral tests from 529 former organolead workers were evaluated to determine if the previously described relationship with bone lead levels is influenced by the apolipoprotein E (ApoE) genotype.		In 20 linear regression models, coefficients for the ApoE and tibia lead interaction term were negative in 19 with significance reached for digit symbol ($\beta = -0.109$ [SE 0.054], $p \leq 0.05$), Purdue pegboard dominant ($\beta = 0.068$ [SE 0.028], $p \leq 0.05$) and complex reaction time ($\beta = -0.003$ [SE 0.001], $p \leq 0.05$) and borderline significance existed for symbol digit ($\beta = -0.046$ [SE 0.026], $p \leq 0.10$), Trails A ($\beta = -0.303$, [SE 0.164] $p \leq 0.10$) and Stroop ($\beta = -0.013$ [SE 0.008], $p \leq 0.10$). The slope of the relation between tibia lead and neurobehavioral outcome was more negative in those individuals with at least one ε4 allele than individuals without this allele. It is suggested that the presence of one Apo-ε-4 allele increases the risk of persistent central nervous system effects of lead.
Tassler et al. (2001)	490 former organolead workers, mean age 58 (7.5) years. The peripheral nervous system was examined with sensory pressure thresholds, and pinch and grip strength.	Mean blood lead 5 (2.6) µg/dL Mean DMSA-Chelatable lead 19 (16.3) µg, Mean current tibia lead 15 (9.4) µg/g Peak tibia lead 24 (17.6) µg/g	No strong association was found between lead biomarkers and measures of sensory and motor function after adjusting for age. The authors attributed the findings to decreased sensitivity of the peripheral nerves in this dose range of inorganic lead or the possibility of differential repair in the peripheral nervous system compared to the central nervous system.
Bolla et al. (1995)	190 current workers in organolead manufacturing (from the 222 described in Schwartz et al. 1993) mean age 45 (8) years compared to 52 referents, mean age 45 (8) years and 144 solvent exposed workers, mean age 42 (8) years.	IH found organic lead was 65 to 70% of exposure in production area. Weighted average blood lead 24 (9.4) µg/dL	Lead and solvent exposure associated with adverse effects on tests of manual dexterity. When compared to the solvent group lead exposure had greater impairment on memory and learning and less on executive/motor tests. An elevated neuropsychiatric score was present in 43% of the lead group, 15% of the solvent and 7% of the referent group.

Table AX6-3.19 (cont'd). Occupational Exposure to Organolead and Inorganic Lead in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Mitchell et al. (1996)	58 organolead workers, self-selected for a clinical evaluation. Mean age 45 (7.1) years.	Mean blood lead 19 (6.5) µg/dL Mean lifetime blood lead 26 (9.1) µg/dL Mean lifetime urine lead 51 (18.8) µg/L	The most common symptoms were memory loss 74%, joint pain 56%, trouble sleeping 54%, irritability 51%, paresthesias 49%, fatigue 49%, nightmares 35%, moodiness 28%, headaches 21% and depression 21%. Of the 31 workers receiving nerve conduction studies, 29% were normal, carpal tunnel syndrome 36%, cubital tunnel syndrome 3%, median neuropathy 3%, ulnar neuropathy 23%, mononeuropathy in lower extremity 5%, tarsal tunnel syndrome 7% and sensorimotor polyneuropathy 36%. 39 workers had neurobehavioral evaluation with 64% had abnormal tests of which 46% were considered to be consistent with a toxic exposure.

CHAPTER 6 ANNEX

ANNEX TABLES AX6-4

Table AX6-4.1. Renal Effects of Lead – General Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States			
Kim et al. (1996) Boston, MA 1979 - 1994	459 men in the Normative Aging Study; periodic exams every 3-5 years Mean serum creatinine at baseline 1.2 mg/dL Random effects modeling, adjusting for baseline age, time since initial visit, body mass index, smoking status, alcohol ingestion, education level, hypertension (defined as blood pressure ≥160 or 95 mmHg or anti-hypertensive medication use), and, in longitudinal analysis, baseline serum creatinine and time between visits.	Mean (SD) blood lead at baseline 9.9 (6.1) µg/dL Blood lead levels from stored red blood cells were adjusted for hematocrit; the assay and adjustment procedure were validated against freshly collected samples. Storage tubes were shown to be lead free.	Cross-sectional Positive association between log transformed blood lead and concurrent serum creatinine. 10-fold higher blood lead level associated with 0.08 mg/dL higher serum creatinine (95% CI: 0.02, 0.13 mg/dL). Association stronger in participants with lower peak blood lead levels. β coefficient (95% CI) in the 141 participants whose peak blood lead ≤10 µg/dL: 0.06 (0.023, 0.097) Longitudinal Positive association between log transformed blood lead and change in serum creatinine over subsequent follow-up period in participants whose peak blood lead was ≤25 µg/dL β coefficient (95% CI: 0.027 [0.0, 0.054]) Slope of age-related increase in serum creatinine steeper in group with highest quartile of time weighted average lead exposure compared to the lowest quartile

Table AX6-4.1 (cont'd). Renal Effects of Lead – General Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Muntner et al. (2003) U.S. 1988-1994	Blood lead levels measured in 15,211 adult subjects enrolled in the NHANES III study. Study cohort representative of U.S. population; non-Hispanic African Americans, Mexican Americans, the elderly and children over-sampled to allow stable estimates in these groups. Hypertension defined as blood pressure ≥140 and/or 90 mmHg and/or current antihypertensive medication use. Based on evidence of interaction between blood lead and hypertension, the population was stratified by hypertension for further analysis. 4,813 hypertensives; 10,398 normotensives. Elevated serum creatinine (%) defined as ≥99th percentile of each race-gender specific distribution for healthy young adults [age 20-39 without hypertension or diabetes] 11.5 % (hypertensives) 1.8 % (normotensives) Chronic kidney disease (%) chronic kidney disease defined as GFR <60 mL/min/1.73 m^2; estimated by MDRD equation (Levey et al. [1999]) 10 % (hypertensives) 1.1% (normotensives) Multiple logistic regression Age, race, gender, diabetes, systolic blood pressure, smoking status, history of cardiovascular disease, body mass index, alcohol consumption, household income, marital status, and health insurance	Mean blood lead 4.21 (0.14) µg/dL (hypertensives) 3.30 (0.10) µg/dL (normotensives)	Higher odds ratios of both increased serum creatinine and chronic kidney disease by quartile of blood lead in hypertensives but not in normotensives Hypertensives Odds ratios for elevated serum creatinine after full adjustment: Blood lead (range, µg/dL) % Odds ratio (95% CI) Quartile 1 (0.7 to 2.4) 7.2 1.00 Quartile 2 (2.5 to 3.8) 12.1 1.47 (1.03, 2.10) Quartile 3 (3.9 to 5.9) 12.4 1.80 (1.34, 2.42) Quartile 4 (6.0 to 56.0) 16.3 2.41 (1.46, 3.97) p < 0.001 for chi-squared test for trend Odds ratios for chronic kidney disease after full adjustment: Blood lead % Odds ratio (95% CI) Quartile 1 6.1 1.00 Quartile 2 10.4 1.44 (1.00, 2.09) Quartile 3 10.8 1.85 (1.32, 2.59) Quartile 4 14.1 2.60 (1.52, 4.45) p < 0.001 for chi-squared test for trend Associations were similar when lead was entered as a log transformed continuous variable. In non-hypertensives, higher blood lead was associated with a higher prevalence of chronic kidney disease, but not elevated serum creatinine, in diabetics.

Table AX6-4.1 (cont'd). Renal Effects of Lead – General Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Payton et al. (1994) Boston, MA 1988 - 1991	Blood lead levels measured in 744 men enrolled in the Normative Aging Study Serum creatinine 1.3 mg/dL Measured creatinine clearance 88.2 mL/min Calculated creatinine clearance 71 mL/min Multiple linear regression adjusting for age, body mass index, analgesic and diuretic use, alcohol consumption, smoking status, systolic/ diastolic blood pressure	Mean blood lead 8.1 µg/dL Blood lead levels below the limit of detection of 5 µg/dL were recoded as 4 µg/dL (n not stated).	ln blood lead negatively associated with ln measured creatinine clearance β coefficient (95% CI) −0.04 (−0.079, −0.001) 10 µg/dL higher blood lead associated with a 10.4 mL/min lower creatinine clearance Borderline significant associations ($p < 0.1$) between blood lead and both serum creatinine ($\beta = 0.027$; neither SE nor CI provided) and estimated creatinine clearance ($\beta = -0.022$; neither SE nor CI provided)
Shadick et al. (2000) Boston, MA 1991-1996	777 participants in all male Normative Aging Study	Mean blood lead 5.9 µg/dL Mean Tibia Lead 20.8 µg/g bone mineral Mean Patella Lead 30.2 µg/g bone mineral	A significant association between patella lead and uric acid (β [95% CI: 0.007 [0.001, 0.013]); $p = 0.02$) was found, after adjustment for age, BMI, diastolic blood pressure, alcohol ingestion, and serum creatinine. Borderline significant associations between tibia ($p = 0.06$) and blood lead ($p = 0.1$) and uric acid were also observed. Notably these associations were significant even after adjustment for blood pressure and renal function, providing further evidence that low level lead increases uric acid. Fifty-two participants had gout; lead dose was not associated with risk for gout.

Table AX6-4.1 (cont'd). Renal Effects of Lead – General Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Tsaih et al. (2004) Boston, MA 1991-~2001	448 men enrolled in the Normative Aging Study Baseline Serum Creatinine 1.3 mg/dL Longitudinal analysis of data from 2 evaluations a mean of 6 years apart Annual change in serum creatinine = (follow-up serum creatinine – baseline serum creatinine) / years of follow up Covariates assessed = age, age squared, body mass index, hypertension (defined as blood pressure ≥160 or 95 mmHg or physician diagnosis with use of antihypertensive medication), diabetes (defined as use of oral hypoglycemic drugs or insulin or reported physician diagnosis), smoking status, alcohol consumption, analgesic use, and, in longitudinal models, baseline serum creatinine and its square Six percent and 26% of subjects had diabetes and hypertension, at baseline, respectively.	Baseline blood lead 6.5 (4.2) μg/dL Baseline tibia lead 21.5 (13.5) μg/g bone mineral Baseline patella lead 32.4 (20.5) μg/g	Mean blood lead levels and serum creatinine decreased significantly over the follow-up period in the group. Lead dose not associated with change in creatinine overall Significant interaction of blood and tibia lead with diabetes in predicting annual change in serum creatinine Beta coefficient (95% CI) for natural ln baseline blood lead 0.076 (0.031, 0.121) compared to 0.006 (−0.004, 0.016) for non-diabetics Beta coefficient (95% CI) for natural ln baseline tibia lead 0.082 (0.029, 0.135) compared to 0.005 (−0.005, 0.015 for non-diabetics Significant interaction of tibia lead with hypertensive status in predicting annual change in serum creatinine Beta coefficient (95% CI) for natural ln baseline tibia lead 0.023 (0.003, 0.019) compared to 0.0004 (-0.001,0.002 for non-hypertensives Follow-up serum creatinine was also modeled separately in longitudinal analyses; diabetes modified the association between baseline tibia lead and follow-up serum creatinine.

Table AX6-4.1 (cont'd). Renal Effects of Lead – General Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Wu et al. (2003) Boston, MA 1991-1995	709 men enrolled in the Normative Aging Study Serum creatinine 1.2 mg/dL Calculated creatinine clearance 71.3 mL/min Serum uric acid 6.5 mg/dL Multiple linear regression, adjusting for age, body mass index, blood pressure or HTN (depending on model), and alcohol ingestion. Uric acid models also adjusted for serum creatinine, other outcome models adjusted for smoking status and analgesic medication use.	Blood lead 6.2 (4.2) µg/dL Tibia lead 22 (13.4) µg/g bone mineral Patella lead 32.1 (19.5) µg/g bone mineral	Significant inverse association between patella lead and creatinine clearance Beta coefficient = −0.069, SE not provided Borderline significant ($p = 0.08$) inverse association between tibia lead and creatinine clearance. Borderline significant ($p = 0.08$) positive associations between tibia and patella lead and uric acid. No lead measure significantly associated with serum creatinine. ALAD gene polymorphism also assessed. 114 participants had the ALAD2 variant allele (7 were homozygous). None of the three renal outcomes differed by genotype. Effect modification by genotype on the association between tibia lead and serum creatinine was observed; the beta coefficient (and slope) was greater in the with group with the variant allele ($\beta = 0.002$; $p = 0.03$ [SE not provided]). Effect modification of borderline significance ($p < 0.1$) on relations between of patella and tibia lead with uric acid was observed; this was significant in participants whose patella lead levels were above 15 µg/g bone mineral ($\beta = 0.016$; $p = 0.04$ [SE not provided]). Similar to the serum creatinine model, patella lead was associated with higher uric acid in those with the variant allele. Genotype did not modify lead associations in models of estimated creatinine clearance.

Table AX6-4.1 (cont'd). Renal Effects of Lead – General Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe			
De Burbure et al. (2003) France Study date not provided	600 adults (399 exposed, 201 age and gender matched controls) 400 children (200 exposed, 200 age and gender matched controls). Age ranged from 8.5 to 12.3 years. Exposure from residence near smelters Exclusion criteria for children included obesity, diabetes and puberty; for adults included pregnancy, cancer, diabetes and kidney disease. Serum creatinine 1.43 mg/dL (adult male controls) 1.38 mg/dL (exposed adult males) 1.33 mg/dL (adult female controls) 1.26 mg/dL (exposed adult females) Urinary β_2-microglobulin 68.16 µg/g cr (adult male controls) 76.29 µg/g cr (exposed adult males) 63.79 µg/g cr (adult female controls) 71.98 µg/g cr (exposed adult females) 87.8 µg/g cr (boy controls) 97.3 µg/g cr (exposed boys) 88.2 µg/g cr (girl controls) 94.8 µg/g cr (exposed girls) Urinary NAG 1.12 IU/g cr (adult male controls) 1.24 IU/g cr (exposed adult males) 0.98 IU/g cr (adult female controls) 1.28 IU/g cr (exposed adult females) 2.29 IU/g cr (boy controls) 1.70 IU/g cr (exposed boys) 2.21 IU/g cr (girl controls) 1.07 IU/g cr (exposed girls) Urinary RBP 82.8 µg/g cr (adult male controls) 85.8 µg/g cr (exposed adult males) 83.42 µg/g cr (adult female controls) 95.81 µg/g cr (exposed adult females) 94 µg/g cr (boy controls) 99 µg/g cr (exposed boys) 110 µg/g cr (girl controls) 109 µg/g cr (exposed girls) Renal outcome measures also included urinary total protein, albumin, transferrin, and brush border antigens Multiple linear regression adjusting for age, sex, body mass index, area of residence, smoking, alcohol ingestion, mercury, cadmium and urinary creatinine level	Geometric mean blood lead 7.13 µg/dL (adult male controls) 6.78 µg/dL (exposed adult males) 4.17 µg/dL (adult female controls) 5.25 µg/dL (exposed adult females) 3.42 µg/dL (boy controls) 4.22 µg/dL (exposed boys) 2.74 µg/dL (girl controls) 3.69 µg/dL (exposed girls)	Adults Mean blood lead level higher in exposed women but not men. None of the renal outcomes analyzed showed any significant difference between exposed and unexposed groups. After adjustment for covariates, blood lead was not associated with any renal outcomes. Children Mean blood lead levels higher in exposed. The highest geometric mean blood cadmium was 0.52 µg/L. None of the renal outcomes were significantly higher in exposed. After adjustment for covariates, blood lead was not associated with any renal outcomes, however, blood cadmium was positively associated with NAG. This association was present in both control and exposed areas. Participants with extremes of urinary creatinine excluded from data analyses. As a result, number of subjects in data tables substantially less than in study.

Table AX6-4.1 (cont'd). Renal Effects of Lead – General Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Factor-Litvak et al. (1993) Kosovo, Yugoslavia 1985-1986	1447 Yugoslavian women in prospective study of environmental lead exposure and pregnancy Exposure from Kosovska Mitrovica with a lead smelter, refinery and battery plant. Controls from Pristina, 25 miles away Renal outcome = Proteinuria assessed with a dipstick Exclusionary criteria included HTN (n = 37 excluded, similar blood lead levels to remaining participants) Multiple logistic regression adjusting for age (linear and quadratic), height (linear and quadratic), cigarette smoking, gestational age (linear and quadratic), daily milk consumption, no. of previous live births, average weekly meat consumption, hemoglobin level and ethnic group.	Blood Lead 17.1 µg/dL (582 exposed) 5.1 µg/dL (865 controls)	Proteinuria (negative, trace, or ≥1+) Exposed = 16.2% negative, 74.1% trace and 9.7% with ≥1+ proteinuria. Controls = 32.4% negative, 60.6% trace and 7.1% with ≥1+ proteinuria. Authors attributed overall high proportion of proteinuria to pregnancy. Higher blood lead associated with increased odds ratio for trace and ≥1+ proteinuria. Comparing women in upper 10th percentile of exposure to lower 10th percentile of exposure, adjusted odds ratios (95% CI) for trace and ≥1+ proteinuria was 2.3 (1.3, 4.1) and 4.5 (1.5, 13.6), respectively. Limitations = limited renal outcomes assessed.
Staessen et al. (1990) London, England Study date not provided	531 London civil servants (398 male, 133 female) Exclusionary criteria = occupational exposure to heavy metals Serum creatinine 1.10 mg/dL (men) 0.88 mg/dL (women)	Mean blood lead 12.4 µg/dL (men) 10.2 µg/dL (women)	After removal of 2 outliers, the study found no significant correlation between serum creatinine and log blood lead in men. No correlation between serum creatinine and log blood lead in women Limitations = lack of adjustment in data analysis, limited lead dose and renal outcome assessment, loss of power by analyzing gender in separate models

Table AX6-4.1 (cont'd). Renal Effects of Lead – General Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Staessen et al. (1992) Belgium 1985-1989	Blood lead levels were measured in 1981 adult subjects (965 males, 1016 females) enrolled in the Cadmibel study of general Belgian population in four cadmium polluted and unpolluted areas. Inclusion criteria included age ≥20 years and residence in one of four study areas for ≥8 years. Participants were randomly selected from the study areas; participation rates were 78% in the two rural areas but only 39% in the urban areas (one area from each category was known to be cadmium polluted). Measured creatinine clearance 99 mL/min (males) 80 mL/min (females) Calculated creatinine clearance 80 mL/min (males) 69 mL/min (females) Multiple linear regression Covariates assessed included age, age squared, gender (by stratifying), body mass index, blood pressure, ferritin level, smoking status, alcohol ingestion, rural vs. urban residence, analgesic and diuretic use, blood and urinary cadmium, diabetes, occupational exposure to heavy metals, and gamma glutamyl transpeptidase	Blood lead 11.4 µg/dL (males) 7.5 µg/dL (females) Zinc protoporphyrin also assessed	After adjustment, log transformed blood lead negatively associated with measured creatinine clearance β coefficient (95% CI) −9.5 (−0.9, −18.1) males −12.6 (−5.0, −20.3) females A 10 fold increase in blood lead associated with a decrease in creatinine clearance of 10 and 13 mL/min in men and women respectively Log transformed blood lead also negatively associated with calculated creatinine clearance β coefficient (95% CI) −13.1 (−5.3, −20.9) males −30.1 (−23.4, −36.8) females Log transformed zinc protoporphyrin negatively associated with measured and calculated creatinine clearances and positively associated with serum β_2- microglobulin in both sexes and with serum creatinine in men Blood lead positively associated with serum β_2-microglobulin in men

Table AX6-4.1 (cont'd). Renal Effects of Lead – General Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia			
Lin et al., Am J Nephrol. (1993) Taiwan Study date not provided	123 adults living near a lead battery factory for more than 10 years Divided into 3 groups by proximity to the factory Group 1 ≤500 m (n = 49) Group 2 1000-1500 m (n = 47) Group 3 farther away (n = 27) Exclusionary criteria included history of exposure to nephrotoxicants and nephrotoxicant medications, such as NSAIDs. 24 hour urinary NAG excretion 3.3 U/day (Group 1) 2.4 U/day (Group 3) Multiple linear regression with adjustment for age	Blood lead 16.6 µg/dL (Group 1) 13.5 µg/dL (Group 2) 7.9 µg/dL (Group 3) EDTA diagnostic chelation (done in Group 1) 126.1 µg/24 hrs	Significantly higher prevalence of abnormal urinary NAG found in the exposed group 1 compared to the control group 3 (55.6% compared to 11.1%; $p < 0.001$). However, mean NAG not significantly higher in Group 1. In all 45 participants in whom both measures were obtained, EDTA chelatable lead was not correlated with urinary NAG excretion. However, a significant correlation between EDTA chelatable lead ≤200 µg/24 hrs and urinary NAG excretion was observed in the 39 participants in this group. Further evaluation with multiple linear regression, adjusting for age, revealed a βcoefficient (95% CI: 0.034 [0.009, 0.059]); $p = 0.01$. No correlation noted between blood lead level and urinary NAG. Limitations = small sample size, plots indicate potential for influential outliers.
Satarug et al., EHP (2004) Bangkok, Thailand Study date not provided	118 Thai adults (53 men, 65 women) Renal outcome measures noted below, also include BUN and total urinary protein. Serum creatinine 0.94 mg/dL (males) 0.66 mg/dL (females) Urinary NAG 4.4 U/g cr (males) 4.6 U/g cr (females) Urinary β_2-microglobulin 51 µg/g cr (males) 29 µg/g cr (females)	Mean "serum" lead 0.42 µg/dL (males) 0.3 µg/dL (females) Note – cannot determine from article if actually serum lead (much less commonly used) or blood lead Mean urinary lead 1.3 µg/g cr (males) 2.4 µg/g cr (females) Urinary cadmium (CdU) also assessed	In men, urinary lead excretion correlated only with urinary protein at borderline significance ($r = 0.22$, $p < 0.06$), In women, urinary lead excretion correlated with urinary NAG ($r = 0.5$, $p < 0.001$), protein ($r = 0.31$, $p = 0.01$) and β_2-microglobulin ($r = 0.36$, $p = 0.002$) excretion. After adjustment for CdU, only association between urinary lead and NAG remained significant. Three urinary renal biomarkers correlated with CdU, although only at borderline significance ($p = 0.06$) for β_2-microglobulin. Limitations = small sample size, lead dose assessment since only urine lead used in renal analyses, limited data analysis

Table AX6-4.1 (cont'd). Renal Effects of Lead – General Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Satarug et al., Toxicology (2004) Bangkok, Thailand Study date not provided	96 Thai men Subjects subdivided into nonsmokers (n = 53), current smokers (n = 27), and ex-smokers (n = 16). Renal outcome measures noted below, also include BUN and total urinary protein. <u>Serum creatinine</u> 0.94 mg/dL (nonsmokers) 0.93 mg/dL (smokers) 0.96 mg/dL (ex-smokers) <u>Urinary NAG</u> 4.4 U/g cr (nonsmokers) 4.2 U/g cr (smokers) 3.8 U/g cr (ex-smokers) <u>Urinary β_2-microglobulin</u> 51 µg/g cr (nonsmokers) 95 µg/g cr (smokers) 98 µg/g cr (ex-smokers)	<u>Mean "serum" lead</u> 0.42 µg/dL (nonsmokers) 0.9 µg/dL (smokers) 0.61 µg/dL (ex-smokers) <u>Mean urinary lead</u> 1.3 µg/g cr (nonsmokers) 1.4 µg/g cr (smokers) 1.4 µg/g cr (ex-smokers) Urinary cadmium (CdU) also assessed	Urinary lead correlated with urinary protein ($r = 0.49$, $p < 0.01$) in smokers and at borderline significance ($r = 0.22$; $p = 0.06$) in never smokers. Also correlated with β_2-microglobulin in ex-smokers at borderline significance ($r = 0.39$; $p = 0.06$) CdU correlated with urinary NAG in current and never smokers and at borderline significance ($p = 0.07$) in ex-smokers. Also correlated with urinary protein and β_2-microglobulin in current smokers and, at borderline significance, in never smokers. Limitations = small sample size, lead dose assessment since only urine lead used in renal analyses, limited data analysis
Middle East			
Mortada et al. (2004) Egypt Study date not provided	68 Egyptian men (35 smokers, 33) Renal outcomes included serum creatinine, BUN, and β_2-microglobulin and urinary albumin, NAG, β_2-microglobulin, alkaline phosphatase, and γ-glutamyl transferase.	<u>Blood lead</u> 14.4 µg/dL (smokers) 10.2 µg/dL (nonsmokers) Lead also measured in urine, hair, and nails Also measured cadmium, and mercury	Blood and hair lead levels significantly higher in smokers as compared to nonsmokers. No significant differences in renal outcome measures by smoking status. No correlation between exposure indices and renal outcome measures. Limitations: small sample size, data analysis – no adjustment.

Table AX6-4.2. Renal Effects of Lead – Occupational Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States			
Smith et al. (1995) U.S. Study date not provided	691 construction workers 96 participants with the ALAD2 allele	Mean blood lead 7.8 µg/dL (ALAD11) 7.7 µg/dL (ALAD12 or 22)	Higher mean BUN ($p = 0.03$) in participants with the ALAD2 allele compared to those with the ALAD11 genotype. However, after adjustment for age, alcohol ingestion and blood lead, the association was no longer significant. Effect modification was not evaluated.
Europe			
Bergdahl et al. (1997) Sweden Study date not provided	89 lead workers; 7 had the ALAD2 allele 34 controls; 10 had the ALAD2 allele	Median blood lead 31.1 µg/dL in lead workers with ALAD11 28.8 µg/dL in lead workers with ALAD12 or 22 3.7 µg/dL in control workers with ALAD11 3.7 µg/dL in control workers with ALAD12 or 22	Higher crude mean serum creatinine ($p = 0.11$) in participants with the ALAD2 allele compared to those with the ALAD11 genotype. Adjusted data not presented.
Cardenas et al. (1993) Belgium Study date not provided	N = 41 lead smelter workers, 41 controls (all males) Study started with 50 lead smelter workers and 50 controls. Blood lead level >35 µg/dL and exposure >1 year were required in exposed workers. Participants with renal disease, renal risk factors, such as diabetes or regular analgesic medication use, or urinary cadmium >2 µg/g creatinine, were excluded. Multiple linear regression; adjusted for urinary creatinine and, in some cases, BMI Serum creatinine 1.02 mg/dL (workers) 1.03 mg/dL (controls) Battery of more than 20 renal biomarkers obtained including: RBP 68 µg/L (workers) 64 µg/L (controls) NAG 1.56 U/L (workers) 1.21 U/L (controls)	Mean Blood lead 48.0 µg/dL (workers) 16.7 µg/dL (controls) Mean duration of lead exposure = 14 years Urinary cadmium also measured as potential confounder	Serum creatinine was not increased in lead workers compared to controls; associations between lead dose and serum creatinine, if assessed, were not specifically reported. In all 82, blood lead: -associated with thromboxane B_2 ($\beta = 0.36$, $p < 0.01$) -negatively associated with 6-keto-prostaglandin $F_{1\ alpha}$ ($\beta = -0.179$, $p < 0.01$) -neither SE β nor CI provided Zinc protoporphyrin positively associated with sialic acid excretion NAG increased in lead workers but associated with CdU Limitations = sample size, potential for healthy worker bias, limited statistical analysis

Table AX6-4.2 (cont'd). Renal Effects of Lead – Occupational Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Coratelli et al. (1988) Study location and date not provided; authors from Italy	20 lead battery factory workers 20 controls 12 month longitudinal study Renal outcomes = urinary alanine aminopeptidase, NAG and lysozyme	Initial mean blood lead 47.9 µg/dL (workers) 23.6 µg/dL (controls)	NAG and lysozyme higher in exposed compared to controls throughout study. A statistically significant decline in urinary NAG was noted in association with a one month period of decreased occupational exposure in the lead workers. NAG correlated with time of exposure (nonlinear) but not blood lead. Clinical renal function measures were not studied.
Fels et al. (1994) Study location and date not provided	81 male lead workers; 45 age matched controls Extensive exclusionary criteria <u>Renal outcomes</u> Serum creatinine Glomerular markers = 6-keto-prostaglandin $F_{1\ alpha}$, thromboxane B_2, and fibronectin Proximal tubular markers = brush border antigens (BBA, BB50, HF5) and intestinal alkaline phosphatase Distal nephron markers = prostaglandin E_2, prostaglandin $F_{2\ alpha}$	<u>Median blood lead</u> 42.1 µg/dL (workers) 7.0 µg/dL (controls)	Serum creatinine similar in exposed compared to controls. Medians of several markers statistically greater in workers compared to controls. After adjustment for age and erythrocyte protoporphyrin, several renal marker outcomes showed "some relation" to blood lead. The table of these data shows r and r^2 but not beta coefficients making the actual statistical method used unclear. Study limitations include lack of adjustment in statistical analysis, potential for healthy worker bias.
Garcon et al. (2004) France Study date not provided	Thirty-five male nonferrous metal smelter workers Renal outcomes = α_1-microprotein, β_2-microglobulin, retinol binding protein, α and π glutathione S transferases (GST) Oxidative stress markers also measured. All variables log transformed	<u>Mean blood lead</u> = 39.6 µg/dL <u>Mean blood cadmium</u> = 5.8 µg/L <u>Mean urine cadmium</u> = 4.7 µg/g creatinine	Correlations between urine lead and cadmium and the renal outcomes assessed (not blood lead or cadmium). Significant positive correlations included: urine lead and α GST ($p < 0.01$) urine cadmium and RBP ($p < 0.05$) Also, urine cadmium and 8-OhdG negatively correlated Limitations = use of urine lead, lack of adjustment for other covariates, sample size Significant correlations between blood lead and two markers of oxidative stress were observed along with a correlation between blood cadmium and one marker of oxidative stress

Table AX6-4.2 (cont'd). Renal Effects of Lead – Occupational Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Gennart et al. (1992) Study location and dates not provided; authors from Belgium	98 lead workers and 85 controls from initial group of 221 Renal outcomes = urinary retinol-binding protein, β-2 microglobulin, albumin, NAG, and serum creatinine and β-2 microglobulin and estimated creatinine clearance Exclusionary criteria included lack of exposure to other metals or solvents, urinary cadmium < 2 µg/g creatinine, neurologic or renal disease, certain medications, blood lead level >40 µg/dL (workers) and < 40 µg/dL for controls.	Mean Blood lead 51 µg/dL (workers) 20.9 µg/dL (controls) Mean duration of employment 10.6 years	Mean renal outcomes were not different in workers compared to controls. Prevalence of abnormal values was not greater in workers compared to controls. An analysis of variance, in all participants, by categorical blood lead, duration of employment, ZPP, and delta-aminolevulinic acid showed no relations with any of the outcomes (data were not shown). Limitations include high lead levels in controls, adjustment only for age in statistical analysis, potential healthy worker bias
Gerhardsson et al. (1992) Sweden Study date not provided	70 current lead smelter workers 30 retired lead smelter workers 31 active and 10 retired truck assembly workers (controls) Renal outcomes = serum creatinine, urinary β-2 microglobulin, NAG, and albumin, clearances of creatinine, albumin, relative albumin, β-2 microglobulin and relative β-2 microglobulin Blood lead measured annually since 1950; time integrated blood lead index = summation of annual blood lead measurements	Median Values Blood lead 31.9 µg/dL (current lead workers) 9.9 µg/dL (retired lead workers) 4.1 µg/dL (current control workers) 3.5 µg/dL (retired control workers) Time integrated blood lead index 369.9 µg/dL (current lead workers) 1496.1 µg/dL (retired lead workers) Calcaneus lead 48.6 µg/g bone mineral (current lead workers) 100.2 µg/g bone mineral (retired lead workers) Tibia lead 13.0 µg/g bone mineral (current lead workers) 39.3 µg/g bone mineral (retired lead workers) 3.4 µg/g bone mineral (current control workers) 12.0 µg/g bone mineral (retired control workers)	Creatinine clearance was higher in lead workers; p-values not reported for this or other median values between lead workers and controls. In current lead workers, blood lead was positively correlated with urinary β-2 microglobulin and time integrated blood lead index was correlated with NAG (data not shown). Strengths include assessment of cumulative lead, inclusion of former workers Limitations = statistical analysis, lack of power by stratifying

Table AX6-4.2 (cont'd). Renal Effects of Lead – Occupational Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Pergande et al. (1994) Study location and date not provided; research team is German	82 male lead workers 44 age-matched healthy male volunteers without known exposure to lead and living "in areas distant from the exposed people" Renal outcomes = serum creatinine and β_2 microglobulin, urinary albumin and 14 other early biological effect markers Exclusion criteria included prescription medication use and many diseases; 11 workers and 3 controls excluded.	Mean blood lead 42.1 µg/dL (workers) 7.0 µg/dL (controls) Erythrocyte protoporphyrin also measured	Serum creatinine and β_2 microglobulin not increased in exposed compared to control participants; correlations with these outcomes not reported. Blood lead and/or erythrocyte protoporphyrin correlated with 9 of the urinary renal outcomes. Study limitations include lack of adjustment in statistical analysis, potential for healthy worker bias, potential for differences between exposed and control groups.
Roels et al. (1994) Belgium Study date not provided	76 lead smelter workers (including 21 participants from Cardenas et al. [1993] [Dr. Roels, email communication]) 68 controls All males Matched for age, sex, socioeconomic status, residence, and workshift characteristics. Extensive exclusionary criteria included renal disease, analgesic abuse, chronic medication for gout, diabetes, occupational exposure to other nephrotoxicants, and prior EDTA chelation. Renal outcomes included serum creatinine and urea nitrogen, measured creatinine clearance, NAG, RBP, serum and urinary β_2-microglobulin, as well as other renal early biological effect markers. Measured creatinine clearance 121.3 mL/min/1.73 m^2 (workers) 115.5 mL/min/1.73 m^2 (controls) Multiple linear regression, adjusted for age, urinary cadmium, hypertension, serum gamma-glutamyl transpeptidase, smoking, exposure status (exposed vs. control), and interaction between exposure variables and hypertension	Blood lead 43.0 µg/dL (workers) 14.1 µg/dL (controls) Tibia Lead 66 µg/g bone mineral (workers) 21 µg/g bone mineral (controls) CdU also measured	Creatinine clearance measured before and after an oral protein load to determine if eicosanoid changes in Cardenas et al. (1993) had clinical implications (Acute protein ingestion causes increased renal perfusion and transient hyperfiltration thought to be mediated by changes in vasodilator prostanoids. Therefore, it was hypothesized that, if the changes noted in Cardenas et al. (1993) were clinically significant, the hyperfiltration response would be diminished in the lead workers.) All participants had normal baseline creatinine clearances (>80 mL/min/1.73 m^2). Both control and lead-exposed workers showed a similar increment in creatinine clearance after protein load. However, mean creatinine clearance was statistically higher in lead workers compared to controls. Log tibia lead was positively correlated with log measured creatinine clearance in the combined group ($\beta = 0.0319$, SE not provided). This was unexpected as the change in eicosanoids found in the initial study would not seem to result in vasodilatation with increased GFR. Unfortunately, it was not possible to measure eicosanoid levels in the follow-up study. No other significant associations between lead measures and renal outcomes were observed. CdU associated with NAG.

Table AX6-4.2 (cont'd). Renal Effects of Lead – Occupational Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Verschoor et al. (1987) Study location and date not provided; authors from The Netherlands	155 lead workers (lead battery and plastic stabilizer) 126 control industrial workers Workers with renal disease, HTN, prescription medications excluded Renal outcomes = BUN, serum creatinine, uric acid, β_2-microglobulin, and RBP, and urinary RBP, NAG, albumin, uric acid, β_2-microglobulin, IgG, and total protein. Urine protein electrophoresis performed on subset (n = 25) Cadmium in blood and, in a subset of exposed workers, in urine was also assessed due to this exposure in one plant each from which lead exposed and control workers were drawn	Blood lead 47.5 µg/dL (workers) 8.3 µg/dL (controls) Zinc protoporphyrin also used as lead dose measure	Mean renal outcomes in all participants shown by categorical lead levels. NAG and RBP higher at blood lead levels >21 µg/dL compared to those below this level (statistical significance not reported). Serum β_2-microglobulin and urinary total protein lower at blood lead levels >21 µg/dL compared to those below this level (again, statistical significance not reported). In simple linear regression models of log transformed urinary total protein, urinary RBP, NAG and serum β_2-microglobulin, higher log transformed blood lead was significantly associated with lower serum β_2-microglobulin and higher RBP and NAG. A matched pair analysis of 55 pairs matched for age within 5 years, smoking, socioeconomic status, and duration of employment found no differences in renal outcomes between exposed and controls. Limitations = lack of adjustment, potential for healthy worker bias, occupational cadmium exposure (including in controls) not adequately adjusted for
Latin and South America			
Cardozo dos Santos et al. (1994) Study location and date not provided; authors from Brazil	166 lead battery workers 60 control workers Renal outcomes = serum creatinine, NAG, urine albumin, and total urinary protein, γ-glutamyl-transpeptidase, alanine-aminopeptidase	Median blood lead 36.8 µg/dL (workers) 11.6 µg/dL (controls)	Significant results Median NAG higher in exposed group ($p < 0.001$). Blood lead level and duration of exposure correlated with NAG in combined group (Spearman's correlation coefficients = 0.32 and 0.22, respectively, $p < 0.001$ for both). No results mentioned for serum creatinine. Limitations = statistical analysis (no regression for renal outcomes)

Table AX6-4.2 (cont'd). Renal Effects of Lead – Occupational Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Latin and South America (cont'd)			
Pinto de Almeida et al. (1987) Northeast Brazil Study date not provided	52 primary lead smelter workers (had to have worked ≥ 5 years on production line) 44 control paper mill workers in same city All males Renal outcomes = BUN, serum creatinine, uric acid, proteinuria, creatinine clearance Only 2 participants excluded for medical reasons	Mean blood lead 64.1 µg/dL (workers) 25.5 µg/dL (controls) Also measured zinc protoporphyrin and delta-aminolevulinic acid	Mean serum creatinine and uric acid higher in exposed than controls (1.23 vs. 1.1 mg/dL; $p < 0.05$ and 6.6 vs. 4.7 mg/dL; $p < 0.001$, respectively) Serum creatinine ≥1.5 mg/dL present in 32.7% lead workers compared to only 2.3% controls. Serum creatinine correlated with duration of employment. Limitations = data analysis including lack of adjustment, several outcomes not analyzed.
Australia			
Pollock and Ibels (1988) Harbor Bridge workers in Sydney, Australia Study date not provided	Thirty-eight bridge workers Twenty-four hour urine lead excretion following 1 g of EDTA Renal outcomes = serum creatinine, creatinine clearance, and 24 hour urine protein excretion	Blood lead mean & range 34.8; 21.8 to 56.2 µg/dL (lead intoxication) 19.9; 9.5 to 26.1 µg/dL (nontoxic) EDTA chelatable lead range 443 to 2366 µg/24 hrs (lead intoxication) 131 to 402 µg/24 hrs (nontoxic)	No significant differences in renal outcomes by lead exposure group. Two workers in high exposure group had evidence of lead nephropathy.
Asia			
Chia et al., OEM (1994) Study location not provided; authors from Singapore 1982-1992 (blood lead measurements obtained every 6 months over this time)	128 lead workers 152 control workers without lead or cadmium exposure Renal outcomes = total NAG, NAG-B isoenzyme (released with lysosomal breakdown assoc with cell membranes, thought to indicate proximal tubular cell toxicity), NAG-A (released by exocytosis). Cross-sectional outcomes but longitudinal exposure data.	Median blood lead 33.8 µg/dL (workers) 8.7 µg/dL (controls) Median cumulative blood lead (mean of 3.6 blood lead levels per worker) 208.3 µg-yr/dL Change in blood lead (in 6 months preceding NAG measurement) Mean = 5.8%	NAG not different in exposed compared to control workers. After adjustment for race, recent change in blood lead was significantly associated with all NAG outcomes (standardized partial regression coefficients ranged from 0.31 for NAG-A to 0.64 for total NAG; neither SE nor CI provided). In contrast, current blood lead was inversely associated with NAG-A and NAG-B separately but, oddly, not with total NAG. Authors do not comment on these inconsistencies. NAG not associated with cumulative lead dose. Strengths = longitudinal exposure data Limitations = data analysis clarity and adjustment

Table AX6-4.2 (cont'd). Renal Effects of Lead – Occupational Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			

Chia et al., Ann Acad Med Singapore (1994)
 	
Study location not provided; authors from Singapore
 	
1982-1992 (blood lead measurements obtained every 6 months over this time)
	63 lead workers of >6 months work duration (median = 3 years)
	91 lead workers of <6 months work duration were considered controls
	Renal outcomes = urinary BB-50 (brush border antigen in proximal tubule), total NAG, NAG-B isoenzyme, RBP, α-1-microgobulin, albumin and urine and serum β_2-microglobulin.
	Cross-sectional outcomes but longitudinal exposure data.
		Lead Dose Measures
		(means or medians not stated)
		
		Most recent blood lead, time integrated blood lead index, relative % change in blood lead, absolute change in blood lead, # of times blood lead level >40, 50, and 60 µg/dL.
			Urinary BB-50 higher in exposed compared to recent hire "control" workers. Time integrated blood lead, # times blood lead >40 µg/dL, and relative change in recent blood lead were associated with urinary BB-50.
			Strengths = longitudinal exposure data
			Limitations = data analysis content (lead dose means not reported), clarity and adjustment.

Chia et al., Toxicol Letters (1995) Study location not provided; authors from Singapore 1982-1993 (blood lead measurements obtained every 6 months over this time)	137 lead stabilizer workers Control group of 153 postal workers (older than lead workers) Renal outcomes = serum creatinine, four hour creatinine clearance, serum β-2 microglobulin, serum α-1 microglobulin, urine albumin Longitudinal blood lead data (mean of 4.5 measurements per lead worker)	Lead Dose Measures (means or medians not stated) Most recent blood lead, time integrated blood lead index, relative % change in recent blood lead, absolute change in recent blood lead, # of times blood lead level >40, 50, and 60 µg/dL.	In analysis of covariance modeling, adjusted for age and race, mean serum α-1 microglobulin and urine albumin were significantly higher in control compared to lead workers. Serum β-2 microglobulin was significantly higher in lead workers ≥ 30 years of age. After adjustment for age, race, and smoking, prevalence rates for abnormal values of serum creatinine and β-2 microglobulin were higher in the highest category of time integrated blood lead index in workers ≥30 years of age (PRR [95% CI: 3.8 [1.1, 13.3] and 10.3 [3.9, 26.9], respectively). Strengths = longitudinal exposure data Limitations = data analysis content (lead dose means not reported), clarity and adjustment

Table AX6-4.2 (cont'd). Renal Effects of Lead – Occupational Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Chia et al., AJIM (1995) Study location not provided; authors from Singapore 1982-1993 (blood lead measurements obtained every 6 months over this time)	128 lead stabilizer factory workers 93 unexposed control subjects (evaluated at pre-employment examination; all quit within 1 month of hire) Blood and urinary cadmium also measured on random subset (40 controls and 31 lead workers) Renal outcomes = serum β-2 microglobulin and urinary α-1 microglobulin, β-2 microglobulin, albumin, RBP	Mean recent blood lead 32.6 µg/dL (workers) 9.0 µg/dL (controls) Mean time integrated blood lead index 119.9 (µg/dL) × yr (workers) 0.05 (µg/dL) × yr (controls) Mean relative change in recent blood lead 28.2 % (workers) Mean absolute change in recent blood lead 6.4 (µg/dL)/year (workers) # of times blood lead level >40, 50 and 60 µg/dL	Only urinary α-1 microglobulin was significantly higher in lead workers compared to controls. In multiple linear regression analysis, adjusted only for ethnicity and smoking, at least one lead measure was significantly associated with each of the five renal outcomes. Outcome / Lead measure / β (95% CI) U α-1 MG / cum. blood lead / 0.10 (0.06, 0.14) U α-1 MG / # blood lead >50 / 0.43 (0.04, 0.82) U β-2 MG / cum. blood lead / 0.05 (0.01, 0.09) U RBP / # blood lead >50 / 0.35 (0.12, 0.59) S β-2 MG / # blood lead >60 / 0.47 (0.29, 0.65) U Alb / # blood lead >60 / 0.66 (0.13, 1.19) Cadmium dose measures reportedly not significant in these models (although power would have been reduced as cadmium measured only in a subset). Strengths = longitudinal exposure data Limitations = data analysis clarity and adjustment. Overlap in populations between this study and earlier ones possible
Endo et al. (1990) Study location not provided; authors from Japan 1987	39 male workers 7 female workers (none directly exposed to lead) secondary lead refinery, mean job duration = 10.5 years Renal outcomes = BUN, serum creatinine and uric acid, urinary NAG, and tubular reabsorption of phosphate	Mean blood lead Ranged from 24.1 to 67.6 µg/dL (males) 19.6 µg/dL (females) Other lead measures included urinary lead, delta-aminolevulinic acid, and coproporphyrin.	Significant correlations of blood lead and delta-amino-levulinic acid with BUN and NAG were observed. The correlation between blood lead and NAG was dependent on a small number of workers whose blood lead levels were above 80 µg/dL. Limitations include absence of adjustment in statistical analysis, small sample size.
Endo et al. (1993) Study location and date not provided; authors from Japan	99 male lead workers Renal outcomes = serum creatinine and serum and urine alpha-1-microglobulin	Median blood lead Ranged from 7.9 µg/dL in category I consisting of 16 office workers who did not work directly with lead to 76.2 µg/dL in 16 workers in the highest exposure group (category V).	Median urinary alpha-1-microglobulin significantly higher in categories III – V compared to the low exposure group of office workers. This was also the only renal outcome to be significantly correlated with blood lead (Spearman rank correlation). After alpha-1-microglobulin adjusted for age and blood lead (by stratifying); few significant differences noted. However, analysis approach resulted in substantial loss of power.

Table AX6-4.2 (cont'd). Renal Effects of Lead – Occupational Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Hsiao et al. (2001) Taiwan, PR China 1991-1998	N = 30 lead battery workers Mean serum creatinine at baseline ~ 1.0 mg/dL (based on figure; exact values not provided) Longitudinal Analysis, 8 annual evaluations. Generalized estimating equations used to adjust for autocorrelation in multiple datapoints from each participant. Adjusted for age, gender, and, in models of change in serum creatinine, creatinine at beginning of interval.	Mean blood lead at baseline ~35 µg/dL (based on figure; exact values not provided) Mean duration of exposure at baseline 13.1 years	Cross-sectional higher blood lead associated with lower concurrent serum creatinine. Longitudinal Change in blood lead negatively associated with concurrent change in serum creatinine ($p = 0.07$). Blood lead at the beginning of the interval not associated with change in serum creatinine in the following year. Associations may represent lead-related hyperfiltration. However, as noted by the authors, cumulative lead dose may also be a factor. Mean blood lead declined greatly just before renal data collection started. Therefore, the inverse longitudinal associations could be due to persistently elevated cumulative dose (which was unmeasured but, as evidenced by the long half-life of bone lead, likely did not decline as much as blood lead). However, authors did not model cumulative blood lead or analyze effect modification by time period, age, or exposure duration to determine if these associations changed in a pattern consistent with hyperfiltration. The small sample size also limits conclusions that may be drawn from these results since a small number of individuals may be overly influential. Strengths = longitudinal data Limitations = data analysis content (lead dose means not reported), clarity and adjustment
Huang et al. (1988) Beijing, China Study date not provided	40 lead workers (4 women) Control group not described Renal outcomes = serum beta-2-microglobulin and urinary beta-2-microglobulin, total protein, IgG	Geometric mean blood lead 40 µg/dL	Increased urinary β_2 microglobulin in workers compared to controls Multiple limitations including lack of information on control group, data analysis

Table AX6-4.2 (cont'd). Renal Effects of Lead – Occupational Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Jung et al. (1998) Korea Study date not provided	75 randomly selected male lead workers 64 male office workers (controls) Renal outcomes = BUN, serum creatinine, uric acid and urinary NAG, albumin, α_1 microglobulin and β_2 microglobulin	Mean Blood lead Means ranged from 24.3 to 74.6 µg/dL (workers) 7.9 µg/dL (controls) Other lead measures included zinc protoporphyrin, δ-aminolevulinic acid activity and urinary lead, coproporphyrin, and δ-aminolevulinic acid	Blood lead, zinc protoporphyrin, and urinary δ-aminolevulinic acid significantly correlated with BUN, NAG, and α_1 microglobulin (appears to be combined group analysis) Limitation = statistical analysis - lack of adjustment
Konishi, et al. (1994) Study location not provided; research team from Japan 1991	99 male lead workers, including 16 office workers to serve at controls renal outcomes = fractional clearances of α_1 microglobulin and β_2 microglobulin (utilizing serum and urinary levels of both biomarkers), BUN, serum creatinine, uric acid and urinary NAG	Median blood lead Range from 7.9 µg/dL in controls to 76.2 µg/dL in Category V	Urinary NAG, α_1 microglobulin and fractional clearance of α_1 microglobulin increased with higher blood lead category. Spearman rank correlation between fractional clearance of α_1 microglobulin and blood lead was significant. This relation also assessed by multiple linear regression with adjustment for age; both independent variables were significantly associated with the fractional clearance of α_1 microglobulin. Limitation = statistical analysis - lack of adjustment
Kumar and Krishnaswamy (1995) India Study date not provided	22 auto mechanics volunteers 27 male control workers (from Institute performing study) Renal outcomes = serum creatinine, 4 hour creatinine clearance and urinary NAG and β-2 microglobulin Renal disease, diabetes, HTN and occupational exposures excluded in controls, possibly excluded in workers	Blood lead range 24.3 - 62.4 µg/dL (exposed) 19.4 - 30.6 µg/dL (controls)	Urinary NAG and β_2 microglobulin levels were significantly higher in exposed compared to controls. However, only NAG was significantly correlated with blood lead ($r = 0.58$, $p < 0.01$). Limitations = study size and lack of adjustment in analysis, values for 4 hour creatinine clearance in abnormal low range in both exposed and controls

Table AX6-4.2 (cont'd). Renal Effects of Lead – Occupational Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Lim et al. (2001) Singapore 1999 Blood lead levels every 6 months from 1982 to 1999	55 male lead workers workers followed since 1982, many of same workers as in Chia et al., AJIM (1995) Renal outcomes = 4 hour creatinine clearance and urinary albumin, RBP, α_1 microglobulin, β_2 microglobulin, NAG, NAG-A, and NAG-B Exclusionary criteria included diabetes, HTN, recent ingestion of analgesics, antipyretics, or antibiotics, and thalassemia; 24 participants of the original 80 were excluded as a result. One female also excluded.	<u>Mean current blood lead</u> 24.1 µg/dL <u>Cumulative blood index</u> 880.6 µg × yrs/dL (geometric mean) Number of times blood lead exceeded 40 µg/dL 1.9 (geometric mean)	In separate models, after adjustment for age and smoking, higher categorical cumulative blood index and number of times blood lead exceeded 40 µg/dL were associated with lower creatinine clearance ($P < 0.001$). After adjustment, higher number of times blood lead exceeded 40 µg/dL was associated with higher urinary albumin, α_1 microglobulin, RBP, NAG, and NAG-B. Similarly, cumulative blood index was associated with higher urinary albumin, α_1 microglobulin, RBP, and β_2 microglobulin. No associations between recent blood lead and any of the renal outcomes was observed. Analysis of covariance was used to adjust for smoking and age Limitation = statistical analysis - lack of adjustment, small sample size, potential for healthy worker bias
Ong et al. (1987) Singapore and Japan Study date not provided	209 lead workers (51 females) 30 control workers from research staff Renal outcomes = BUN, serum creatinine, calculated creatinine clearance, and urinary NAG	Mean blood lead 42.1 µg/dL (males) 31.9 µg/dL (females) Urine lead also measured	Blood lead correlated with BUN($r = 0.16$; $p < 0.01$), serum creatinine ($r = 0.26$; $p < 0.001$) and creatinine clearance ($r = -0.16$; $p < 0.01$). Blood lead associated with NAG after adjustment for age (method not specified). Higher NAG in exposed compared to controls when stratified by categorical age. Strengths = sample size Limitations = statistical analysis - lack of adjustment, urinary NAG not adjusted for urine dilution
Wang et al. (2002) Taiwan Study date not provided	229 lead battery workers, including 109 females Renal outcomes = BUN, serum creatinine, serum uric acid Multiple linear & logistic regression Adjustment for age, gender, smoking, alcohol ingestion, milk ingestion.	Mean blood lead 67.7 µg/dL (males) 48.6 µg/dL (females)	β coefficient (95% CI) for blood lead in model of BUN, after adjustment for lead job duration/age = 0.062 (0.042, 0.082). β coefficient (95% CI) for blood lead in model of uric acid, after adjustment for gender and weight = 0.009 (0.001, 0.016). Blood lead not associated serum creatinine

Table AX6-4.2 (cont'd). Renal Effects of Lead – Occupational Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Weaver et al. (2003a) South Korea 1997-1999	N = 803 lead workers including 164 females and 94 former lead workers Serum Creatinine: 0.90 mg/dL Calculated creatinine clearance 94.7 mL/min 4-hr measured creat. clearance 114.7 mL/min RBP 63.6 µg/g creatinine NAG 215.3 µmol/h/g creatinine Multiple linear regression, adjusting for age, gender, BMI, work status (current vs. former worker), HTN or blood pressure (depending on model), and, for the EBE markers, alcohol ingestion and diabetes. 42 associations modeled (7 lead measures with 6 renal outcomes) Interaction models that assessed effect modification by age in tertiles in 24 associations (4 lead exposure/dose measures with 6 renal outcomes).	Blood lead 32.0 µg/dL Tibia Lead 37.2 µg/g bone mineral DMSA-chelatable lead 767.8 µg/g creatinine Lead exposure also assessed with job duration and three hematologic measures as surrogates for lead dose (aminolevulinic acid in plasma, zinc protoporphyrin, and hemoglobin). Mean CdU measured in n = 191 subset 1.1 µg/g creatinine	After adjustment, higher lead measures associated with worse renal function in 9 of 42 models. Associations in the opposite direction (higher lead measures associated with lower serum creatinine and higher creatinine clearances) in five models. Opposite direction (inverse) associations observed only in models of the clinical outcomes whereas the associations between higher lead dose and worse renal function were predominantly among the biomarker models. In three of 16 clinical renal interaction models, positive associations between higher lead measures and worse renal function in participants in the oldest age tertile were significantly different from associations in those in the youngest age tertile which were in the opposite direction - this pattern was observed at borderline significance ($p < 0.1$) in 3 other models - pattern was not observed in the EBE marker models CdU associated with NAG. Authors concluded that occupational lead exposure in the moderate dose range has an adverse effect on renal function. Inverse associations may represent hyperfiltration. Environmental cadmium may have an adverse impact, at least on NAG.

Table AX6-4.2 (cont'd). Renal Effects of Lead – Occupational Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Weaver et al. (2003b) Korea lead workers 1997-1999	798 lead workers with genotype information in same population as in Weaver et al. (2003a) 79 (9.9%) participants were heterozygous for the ALAD2 allele (none was homozygous). 89 (11.2%) had VDR genotype Bb or BB	Blood lead 31.7 µg/dL (ALAD11) 34.2 µg/dL (ALAD12) 31.6 µg/dL (VDR bb) 34.8 µg/dL (VDR Bb or BB) Tibia Lead 37.5 µg/g (ALAD11) 31.4 µg/g (ALAD12) 37.1 µg/g (VDR bb) 38.1 µg/g (VDR Bb or BB)	Data were analyzed to determine whether polymorphisms in the genes encoding δ-aminolevulinic acid dehydratase (ALAD), endothelial nitric oxide synthase (eNOS), and the vitamin D receptor (VDR) were associated with renal outcomes or modified relations of lead exposure and dose measures with renal outcomes. After adjustment, participants with the ALAD2 allele had lower mean serum creatinine and higher calculated creatinine clearance. Effect modification by ALAD on associations between blood lead and/or DMSA-chelatable lead and three of six renal outcomes was observed. Among those with the ALAD12 genotype, higher lead measures were associated with lower BUN and serum creatinine and higher calculated creatinine clearance. No significant differences were seen in renal outcomes by VDR genotype nor was consistent effect modification observed.

Table AX6-4.2 (cont'd). Renal Effects of Lead – Occupational Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Weaver et al. (2005a) Korea 1997-1999	N = 803 current and former lead workers; 164 females Serum Uric acid 4.8 mg/dL Other renal outcomes as listed in Weaver et al. 2003a Multiple linear regression Interaction models that assessed effect modification by age in tertiles	Blood lead 32.0 µg/dL Tibia Lead 37.2 (40.4) µg/g bone mineral DMSA-chelatable lead 767.8 µg/g creatinine	Work to address whether one mechanism for lead-related nephrotoxicity, even at current lower levels of lead exposure, is via increasing serum uric acid. Assessed 1) whether lead dose was associated with uric acid and 2) whether previously reported associations between lead dose and renal outcomes (Weaver et al., 2003) were altered after adjustment for uric acid. After adjustment for age, gender, body mass index, and alcohol use, lead biomarkers not associated with uric acid in all participants. However, in interaction models, both blood and tibia lead were significantly associated in participants in the oldest age tertile (β coefficient and 95% CI: 0.0111 (0.003, 0.019) and 0.0036 (0.0001, 0.007) for blood and tibia lead, respectively). These models were further adjusted for blood pressure and renal function. Hypertension and renal dysfunction are known to increase uric acid. However, they are also risks associated with lead exposure. Therefore, adjustment for these variables in models of associations between lead dose and uric acid likely results in over-control. On the other hand, since non-lead related factors contribute to both renal dysfunction and elevated blood pressure, lack of adjustment likely results in residual confounding. Therefore, as expected, associations between lead dose and uric acid decreased after adjustment for systolic blood pressure and serum creatinine, although blood lead remained borderline significantly associated (β (95% CI) = 0.0071 (−0.001, 0.015). However, when the population was restricted to the oldest tertile of workers with serum creatinine greater than the median (0.86 mg/dL), likely the highest risk segment of the population, blood lead remained significantly associated with uric acid even after adjustment for systolic blood pressure and serum creatinine (β = 0.0156) Next, in models of renal function in all workers, uric acid was significantly ($p < 0.05$) associated with all renal outcomes except NAG. In models in the oldest tertile of workers (266 workers, median age 51.1 years, range 46.0 to 64.8 years), after adjustment for uric acid, associations between lead dose and NAG were unchanged, but fewer of the previously significant ($p \leq 0.05$) associations noted between lead dose and the clinical renal outcomes in Weaver et al. (2003a) remained significant.

Table AX6-4.2 (cont'd). Renal Effects of Lead – Occupational Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Weaver et al. (2005b) South Korea 1999-2001	N = 652 lead workers including 149 females and 200 former workers Patella lead measured in the third evaluation of the same study reported in Weaver et al. (2003a). Data collection performed a mean of 2.2 years after collection of the data presented in Weaver et al. (2003a). Same renal outcomes as Weaver et al. (2003a) Serum Creatinine: 0.87 mg/dL Calculated creatinine clearance 97.0 mL/min Multiple linear regression, adjusting for age, gender, BMI, work status (current vs. former worker), HTN or blood pressure (depending on model), diabetes, smoking status, and, for the clinical measures, use of analgesics Interaction models assessed effect modification by age, dichotomized at the 67th percentile	Mean blood lead 30.9 µg/dL Mean Tibia Lead 33.6 µg/g bone mineral Mean Patella Lead 75.1 µg/g bone mineral Mean DMSA-chelatable lead 0.63 µg Pb/mg creatinine	All 4 lead measures were correlated (Spearman's r = 0.51 – 0.76). Patella, blood and DMSA-chelatable lead levels positively associated with NAG Higher DMSA-chelatable lead associated with lower serum creatinine and higher calculated creatinine clearance Interaction models All four lead measures associated with higher NAG among participants in oldest age tertile Higher blood, tibia, and patella lead associated with higher serum creatinine among older participants -beta coefficients less in the lead workers whose ages were in the younger two-thirds of the age range; difference between slopes in the two age groups was statistically significant only for association of blood lead and serum creatinine Inverse DMSA associations (higher DMSA-chelatable lead associated with lower serum creatinine and higher calculated creatinine clearance) significant in younger workers Patella lead associations were consistent with those of blood and tibia lead; DMSA-chelatable lead associations unique. Authors hypothesized that similarities between patella, blood, and tibia lead associations could be due, in part, to high correlations among the lead biomarkers in this population. Despite similar high correlations, DMSA-chelatable lead associations with serum creatinine and calculated creatinine clearance were unique. This biomarker is dependent on renal function and the collection time was only 4 h. Therefore, the amount of lead that is excreted in this relatively short time period after chelation may be influenced not only by bioavailable lead burden, but also by high-normal as well as actual supranormal glomerular filtration which are more common in the younger workers.

Table AX6-4.2 (cont'd). Renal Effects of Lead – Occupational Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Weaver et al. (2005c) Korea 1997-1999	798 current and former lead workers. same population as in Weaver et al. (2003a,b)		Data were analyzed to determine whether polymorphisms in the genes encoding δ-aminolevulinic acid dehydratase (ALAD), endothelial nitric oxide synthase (eNOS), and the vitamin D receptor (VDR) were associated with uric acid or modified relations of lead exposure and dose measures with uric acid. Uric acid not different by ALAD or VDR genotype. Among older workers (age ≥ median of 40.6 years), ALAD genotype modified associations between lead dose and uric acid levels. Higher lead dose was significantly associated with higher uric acid in workers with the ALAD11 genotype; associations were in the opposite direction in participants with the variant ALAD12 genotype.
Ye et al. (2003) Chinese lead workers Study date not provided	216 lead workers Renal outcomes = urinary NAG and albumin	Geometric mean blood lead 37.8 µg/dL (n = 14 workers with the ALAD12 genotype) 32.4 µg/dL (n = 212 workers with the ALAD11 genotype) 31.9 µg/dL (VDR bb) 41.7 µg/dL (in 20 participants with VDR Bb or BB)	After adjustment for age, NAG was borderline higher in those with the ALAD variant allele whose blood lead levels were ≥40 µg/dL (p = 0.06). In all lead workers, after adjustment for age, gender, smoking and alcohol ingestion, a statistically significant positive association between blood lead and creatinine adjusted NAG was observed in the workers with the ALAD12 genotype but not in lead workers with the ALAD11 genotype (the groups were analyzed separately rather than in an interaction model). No effect modification by VDR genotype on associations between blood lead and urinary albumin and NAG observed (separate analysis reduced power).
Middle East			
Al-Neamy et al. (2001) United Arab Emirates Feb-June, 1999	100 "industrial" workers exposed in a range of industries 100 working controls matched for age, sex, and nationality Renal Outcomes = BUN, serum creatinine	Blood lead 77.5 µg/dL (workers) 19.8 µg/dL (controls)	Mean BUN and serum creatinine not statistically different between exposed workers and controls Limitations = data analysis

Table AX6-4.2 (cont'd). Renal Effects of Lead – Occupational Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Middle East (cont'd)			
Ehrlich et al. (1998) South Africa Study date not provided	382 lead battery factory workers Mean age = 41.2 years All males Multiple linear regression adjusted for age, weight, and height (Covariates assessed for inclusion also included smoking, alcohol ingestion, and diabetes) Clinical renal outcomes included serum creatinine, uric acid, and BUN. Mean serum creatinine 1.13 mg/dL Renal early biological effect markers (NAG, RBP, intestinal alkaline phosphatase, tissue nonspecific alkaline phosphatase, Tamm-Horsfall glycoprotein, epidermal growth factor, and microalbuminuria) were measured in 199 participants randomly selected by tertiles of current blood lead.	Mean blood lead 53.5 µg/dL Mean exposure duration 11.6 years Mean cumulative blood lead (defined as sum of the average blood lead in each year over all years of employment; done in subset of 246 with past blood lead data) 579.0 (µg × yr)/dL Mean historical blood lead (defined as cumulative blood lead divided by years of exposure) 57.3 µg/dL Mean tibia lead 69.7 µg/g bone mineral (measured 2 years after initial study on random sample of 40)	After adjustment for age, weight, and height, categorical current and historical blood lead and zinc protoporphyrin were associated with serum creatinine and uric acid, in separate models. Associations between cumulative blood lead or exposure duration and the renal outcomes were not observed. Among the EBE markers, only current blood lead was borderline associated with NAG ($p = 0.09$). Associations with renal dysfunction were observed at blood lead levels <40 µg/dL. Not explained by an effect on blood pressure since lead measures not associated with blood pressure. Blood cadmium measured in 56 participants 2 years after the initial study. All low (≤1.2 µg/L) suggesting that occupational level cadmium exposure was not a contributing factor. The authors did implicate lead body burden which was substantial based on mean tibia lead. However, cumulative blood lead was not associated in this study and mean tibia lead in Roels et al. (1994) was similar (in that study a positive association with creatinine clearance was observed).
El-Safty et al. (2004) Egypt Study date not provided	45 lead workers with lead job duration <20 years 36 lead workers with lead job duration ≥20 years 75 control workers Renal outcomes = urinary α_1-microglobulin, NAG, and glutathione S-transferase	Median urine lead Ranged from 15.4 µg/g creatinine in nonsmoking control workers to 250.4 µg/g creatinine in smoking lead workers with ≥20 years lead job duration	Medians of all 3 renal outcomes significantly higher in lead workers irregardless of smoking status (analysis stratified by smoking status). Urine lead significantly correlated with urinary α_1-microglobulin and glutathione S-transferase in nonsmoking lead workers and with NAG as well in smoking lead workers. Limitations include using urine lead as sole lead dose measure and data analysis.

Table AX6-4.2 (cont'd). Renal Effects of Lead – Occupational Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Middle East (cont'd)			
Mortada et al. (2001) Egypt Study date not provided	43 traffic policemen 52 matched control office workers (similar in terms of age, gender, smoking, and "social life"). Renal outcomes = serum creatinine, beta-2 microglobulin, BUN and urinary β-2- microglobulin, NAG, alkaline phosphatase, γ-glutamyl transferase, and albumin. Exclusionary criteria included diabetes, HTN, hepatic, renal or urologic diseases.	Blood lead 32.1 µg/dL (exposed) 12.4 µg/dL (controls) Lead also measured in hair, urine and nails	NAG and albumin significantly higher in policemen compared to controls. NAG positively correlated (Pearson's) with job duration and blood and nail lead. Urinary albumin positively correlated with job duration and blood and hair lead. Limitations: data analysis – no adjustment, use of parametric correlation techniques with data likely to be nonparametric; study size

Table AX6-4.3. Renal Effects of Lead – Patient Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States			
Osterloh et al. (1989) Northern CA Study date not provided	40 male subjects with hypertensive nephropathy (hypertension preceded renal insufficiency; serum creatinine 1.8-4 mg/dL) 24 controls with renal dysfunction from other causes Patients recruited from the Kaiser Permanente Regional Laboratory database (large health maintenance organization) in northern California	Mean blood lead 7.3 µg/dL (in both hypertensive nephropathy and controls CRI from other causes) Mean EDTA chelatable lead levels 153.3 µg/72 hours (hypertensive nephropathy) 126.4 µg/72 hours (control CRI)	No significant difference in EDTA chelatable lead levels; highest chelatable lead level was 609.2 µg/72 hours. Lead dose and serum creatinine were not correlated. Blood and chelatable lead levels much lower than those reported by Wedeen et al. (1983) and Sanchez-Fructuoso et al. (1996). Only 17% of their study participants had a history of possible lead exposure based on questionnaire, again much lower than the two other studies.
Steenland et al. (1990) Michigan Diagnosis from 1976-1984	325 men with ESRD (diabetes, congenital and obstructive nephropathies excluded) controls by random digit dialing, matched by age, race, and place of residence.		Risk of ESRD significantly related to moonshine alcohol consumption (OR = 2.43), as well as analgesic consumption, family history of renal disease, and occupational exposure to silica or solvents.
Europe			
Behringer et al. (1986) Germany Study date not provided	16 patients with CRI (median serum creatinine = 2.2 mg/dL) and gout 19 patients with CRI (median serum creatinine = 5.1 mg/dL) without gout 21 healthy controls Lead excretion in the 96 hours after administration of 1 g EDTA iv	Median blood lead 7.2 µg/dL (controls) 11.5 µg/dL (CRI, no gout) 15.3 µg/dL (CRI & gout) Median EDTA chelatable lead (µg/4 days/1.73 m^2) 63.4 (controls) 175.9 (CRI, no gout) 261.3 (CRI & gout)	EDTA chelatable lead higher in gout patients who developed gout after CRI than those in which gout preceded CRI (statistical test results not mentioned or shown). Authors conclude a role for lead in patients with gout occurring in setting of CRI and that lead may contributes to renal function decline in established renal disease from other causes. Limitations = small groups, limited data analysis
Colleoni and D'Amico (1986) Italy (~1982-1985)	12 consecutive patients with CRI (mean serum creatinine = 3.3 mg/dL) and gout, renal diagnosis consistent with chronic interstitial nephritis in all; 7 had history of occupational lead exposure 12 controls with chronic glomerulonephritis and no history of lead exposure or gout Lead excretion in the 48 hours after administration of 1.5 g EDTA im	Mean EDTA chelatable lead (µg/48 hrs) 180 (CRI, no gout) 505 (CRI & gout)	Significantly higher EDTA chelatable lead in the group with CRI and gout compared to CRI alone. EDTA chelatable lead significantly correlated with serum creatinine in patients with CRI and gout but not CRI alone. Authors conclude that lead is cause of CRI with gout but renal insufficiency alone not responsible for increased lead body burden (absence of evidence for reverse causation). Limitations = small sample size, limited data analysis

Table AX6-4.3 (cont'd). Renal Effects of Lead – Patient Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Colleoni et al. (1993) Italy Study date not provided	All 115 patients on hemodialysis at the time of the study; 41 women Blood lead data from prior study of 383 healthy controls in same geographical area served as comparison	Mean blood lead (corrected for hemoglobin) 19.9 µg/dL (patients) 14.7 µg/dL (controls)	Significantly higher mean blood lead in hemodialysis patients compared to healthy controls. 13% had blood lead levels >30 µg/dL. Blood lead level was not associated with duration of hemodialysis. Mean lead levels higher in smokers and in relation to alcohol ingestion. Lead not detectable in dialysis fluids. Limited data analysis
Creswell et al. (1987) Germany and Australia Study date not provided	See discussion below under Australia		
Fontanel's et al. (2002) Spain Study date not provided	ALAD/restored ALAD as a possible index of lead poisoning in chronic renal failure patients.		Restored ALAD was measured after the addition of zinc and dithiothreitol (DTT) to the incubation media. The ALAD/restored ALAD ratio was found to correlate with the results of the EDTA lead mobilization test. Patients excreting I,115 to 3860 µg lead per 72 hours had a ratio of 0.19 while chronic renal failure patients excreting an average of 322 µg lead (range 195 to 393) had a ratio of 0.47. In comparison, normal controls had a ratio of 0.5.
Jones et al. (1990) Study location and date not provided; authors from UK	27 dialysis patients 59 healthy controls	Mean blood lead 8.1 µg/dL (patients) 10.0 µg/dL (controls)	Tibia lead levels not correlated with blood lead but were correlated with lead in bone biopsy measurements ($r = 0.42$). Limitations = data analysis
Koster et al. Study location and date not provided; authors from Germany	91 patients with CRI (median serum creatinine = 2.5 mg/dL) 46 age-matched normal controls. Lead excretion in the 4 days after 1 g EDTA iv	Mean Blood lead (corrected for hemoglobin) 11.2 µg/dL (patients) 7.6 µg/dL (controls) EDTA chelatable lead 164.7 µg/4 days /1.73 m² (patients) 63.6 µg/4 days /1.73 m² (controls)	CRI patients had significantly higher blood and EDTA chelatable lead levels than controls. In 13% of the CRI patients, EDTA chelatable lead exceeded the highest value in controls (328.8 µg). EDTA chelatable lead levels were correlated with serum creatinine in patients ($r = 0.37$; $p < 0.007$). Limitations = data analysis

Table AX6-4.3 (cont'd). Renal Effects of Lead – Patient Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Miranda-Carus et al. (1997) Spain 1990-1994	27 patients with gout and CRI 50 patients with gout only 26 controls with normal renal function and no gout Multiple purine metabolism measures including serum urate, hypoxanthine, and xanthine, as well as their excretion, clearance and fractional excretion measures	Mean blood lead 17.8 µg/dL (gout & CRI) 14.9 µg/dL (gout only) 12.4 µg/dL (controls) EDTA chelatable lead 845 µg/120 hrs (gout & CRI) 342 µg/120 hrs (gout only) 215 µg/120 hrs (controls)	Lead dose measures significantly higher in patients with gout and CRI compared to the other two groups. EDTA chelatable lead inversely correlated with creatinine clearance. Each of the 2 patient groups were dichotomized by EDTA-chelatable lead level of 600 µg/120 hours, resulting in 3 small groups (n ranging from 6 to 14) and one group of 44 participants with gout and EDTA chelatable lead below the cut-off. No significant differences in mean purine metabolism measures were observed. It is not clear whether correlations between EDTA-chelatable lead and the purine measures were assessed and if so whether the small groups were combined for this analysis. Thus lack of power may be one reason for the inconsistency with Lin's work. Different lead body burdens may be a factor as well. Uric acid parameters were unchanged following chelation in 6 participants with EDTA-chelatable above 600 µg/120 hours. Again higher lead body burdens may be a factor but the small number and limited details on the group make firm conclusions difficult.
Nuyts et al. (1995) Belgium Study date not provided	Case-control study 272 cases with chronic renal failure (all types) matched to 272 controls by age, sex and residence Exposure assessed by 3 industrial hygienists blinded to case or control status		Significantly increased odds ratio for chronic renal failure with lead exposure (odds ratio = 2.11 [95% CI: 1.23, 4.36]) as well as several other metals. Increased risk with diabetic nephropathy.

Table AX6-4.3 (cont'd). Renal Effects of Lead – Patient Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Sanchez-Fructuoso et al. (1996) Spain Study date not provided	296 patients: Group I = 30 normal control subjects Group II = 104 patients with essential HTN & normal renal function Group III-A = 68 patients with HTN and CRI of uncertain etiology but presumed nephroangiosclerosis Group III-B = 64 patients with HTN, CRI, and gout Group IV =30 patients with CRI of known etiology	Mean blood and EDTA-chelatable lead levels: Group I 16.7 µg/dL 324 µg/72 hrs Group II 16.8 µg/dL 487 µg/72 hrs Group III-A 18.5 µg/dL 678 µg/72 hrs Group III-B 21.1 µg/dL 1290 µg/72 hrs Group IV 16.5 µg/dL 321 µg/72 hrs	EDTA chelatable lead >600 µg/72 hrs in 16 patients in group II, 30 patients in group III-A, 44 patients in group III-B, but no patients in either group I and IV. Mean blood and EDTA chelatable lead levels in the patients with CRI of known cause were not statistically different from controls with normal renal function. However, baseline urinary lead excretion was lower in group IV. This provides conflicting evidence regarding the "reverse causality" hypothesis of increased lead burden due to decreased excretion in CRI Significant correlations noted between bone lead levels (assessed by biopsy) and EDTA chelatable lead level in 12 patients whose chelatable lead levels were >600 µg/72 hours; provides support for validity of chelatable lead levels in CRI. A positive correlation was observed between serum creatinine levels and EDTA-chelatable lead levels >600 µg/72 hrs but not below this level. In group III, mean measured creatinine clearance was significantly lower in those with EDTA chelatable lead levels >600 µg/72 hrs compared to participants with chelatable lead < 600 µg/72 hrs.

Table AX6-4.3 (cont'd). Renal Effects of Lead – Patient Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Van de Vyver et al. (1988) Belgium, France and Germany Study date not provided	Transiliac bone biopsies obtained from: 11 cadavers without known lead exposure and with normal renal function 13 patients with CRI, gout and/or HTN 22 lead workers 153 dialysis patients	Mean transiliac lead levels 5.5 µg/g (153 dialysis pts) 20.6 µg/g (in highest 5% dialysis pts) 3.7 µg/g (in 10 pts on dialysis due to analgesic nephropathy) 6.3 µg/g (11 cadavers) 30.1 µg/g (22 lead workers)	In 5% of the hemodialysis patients studied, bone lead concentrations approximated the levels found in active lead workers, suggesting lead as a primary cause of their renal failure. Levels in the 10 patients with analgesic nephropathy were the lowest (all <7 µg/g), evidence against reverse causality. In the combined group of 13 patients with CRI, gout and/or HTN and 22 lead workers, EDTA chelatable lead correlated with lead in bone biopsies (r = 0.87).
Winterberg et al. (1991) Study location and date not provided; authors from Germany	Iliac crest bone lead measured by biopsy in: 8 controls 8 patients with CRI 14 dialysis patients	Mean iliac crest bone lead levels 1.63 µg/g (8 controls) 2.18 µg/g (8 patients with CRI) 3.59 µg/g (in 14 dialysis pts)	Noted that the bone lead levels in patients with analgesic nephropathy and cadaver controls in Van de Vyver et al. (1988) were much higher than in control groups of other researchers. They reiterated the concern that lead did accumulate due to decreased renal excretion.
Latin and South America			
Navarro et al. (1992) Venezuela Study date not provided	18 dialysis patients 14 controls Bone (biopsy) and blood levels of lead and several other metals	Mean blood lead 5.2 µg/dL (patients) 11.5 µg/dL (controls) Mean lead in bone 9.7 µg/g (patients) 7.0 µg/g (controls)	Blood but not bone lead significantly higher in patients compared to controls. Authors concluded that bone accumulation of aluminum, iron and vanadium, but not lead, occurred in dialysis patients. Limitations = sample size, data analysis including lack of adjustment

Table AX6-4.3 (cont'd). Renal Effects of Lead – Patient Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Australia			
Craswell et al. (1987) Germany and Australia Study date not provided	German participants from industrialized area where chronic lead nephropathy not previously observed Gp 1 = 8 healthy controls (from hospital staff) Gp 2a = 12 CRI patients, no gout or lead exposure Gp 2b = 7 CRI patients, no gout but + lead exposure Gp 3a = 7 CRI patients with gout but no lead exposure Gp 3b = 6 CRI patients with gout and lead exposure Australian participants from Queensland site of known chronic lead nephropathy Gp 1 = 9 healthy controls (from hospital staff) Gp 2a = 14 CRI patients, no gout or lead exposure Gp 2b =11 CRI patients, no gout but + lead exposure Gp 3a = 25 CRI patients with gout but no lead exposure Gp 3b = 11 CRI patients with gout and lead exposure CRI defined as serum creatinine ≥ 1.5 mg/dL "excess" EDTA chelatable lead defined as lead excreted over 4 days after EDTA minus twice baseline lead excreted pre-EDTA	Median blood lead (hemoglobin corrected) Gp 1 German = 6.8 µg/dL Australian = 11.0 µg/dL Gp 2a German = 6.2 µg/dL Australian = 9.1 µg/dL Gp 2b German = 8.5 µg/dL Australian = 16.2 µg/dL Gp 3a German = 10.6 µg/dL Australian = 12.8 µg/dL Gp 3b German = 12.0 µg/dL Australian = 27.1 µg/dL Median "excess" EDTA chelatable lead Gp 1 German = 68.4 µg Australian =82.9 µg Gp 2a German = 126.4 µg Australian = 393.7 µg Gp 2b German = 489.0 µg Australian = 1181.1 µg Gp 3a German = 227.9 µg Australian = 808.1 µg Gp 3b German = 422.7 µg Australian = 1077.5 µg	Using nonparametric statistical techniques due to skewed data, German participants excreted statistically less lead than their Australian counterparts. Mean EDTA chelatable lead levels were significantly higher in German patients with gout than in those without gout; the observed increase in the Australian patients was of borderline significance ($p < 0.1$). Limitations = small groups, limited data analysis
Price et al. (1992) Queensland, Australia 1981-1986	8 renal patients compared with age-matched controls X-ray fluorescence of finger bone lead conducted twice 5 years apart		Authors conclude that lead in bone half-life is similar in renal patients compared to age-matched controls. Study limitations substantial, however. Limitations = small numbers (although bone lead measured in more patients, many were below the limit of detection, inclusion of outliers without formal statistical analysis.

Table AX6-4.3 (cont'd). Renal Effects of Lead – Patient Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia			
Lin et al. (1992) Chinese population (likely in Taiwan) Study date not provided	10 healthy controls 10 patients with CRI but no gout 8 patients with gout and subsequent CRI 6 patients with CRI and subsequent gout Exclusionary criteria included + history of occupational or environmental lead exposure	Mean EDTA chelatable lead in µg/72 hrs/1.73 m^2 90.2 (controls) 98 (CRI, no gout) 171.6 (gout, then CRI) 359.8 (CRI, then gout)	Lead body burden higher in patients with CRI and gout, especially when CRI precedes gout. Limitations = small sample sizes, statistical analysis
Lin and Huang (1994) Taiwan Study date not provided	Group 1 = 10 patients with normal renal function and no gout; Group 2 = 10 patients with CRI (serum creatinine >1.4 mg/dL) and subsequent gout; Group 3 = 20 patients with CRI but no gout All males Lead body burden assessed with 1 g EDTA iv followed by 72 hr urine collection	Mean EDTA chelatable lead Gp 1 = 60.55 µg/ 72 hrs Gp 2 = 252.24 µg/ 72 hrs Gp 3 = 84.86 µg/ 72 hrs	Mean EDTA chelatable lead and serum urate significantly higher in the patients with gout. After adjustment for creatinine clearance, log transformed EDTA chelatable lead was significantly associated with serum urate levels (β [95% CI: 0.757 [0.142, 1.372]; $p < 0.05$), daily urate excretion (β [95% CI: −60.15 [−118.1, −2.16]); $p < 0.05$), urate clearance (β [95% CI: −0.811 [−1.34, −0.282]; $p < 0.05$), and fractional urate excretion (β [95% CI: −1.535 [−2.723, −0.347]; $p < 0.05$). EDTA chelatable lead not associated with creatinine clearance. Limitations = small sample sizes, limited adjustment in regression analyses.
Lin and Lim (1994) Taiwan Study date not provided	Gp 1 = 12 healthy controls Gp 2 = 10 patients with HTN Gp 3 = 12 patients with HTN, then CRI (hypertensive nephropathy) Gp 4 = 12 patients with CRI only Gp 5 = 12 patients with CRI not due to HTN, but subsequent HTN	Mean EDTA chelatable lead Gp 1 = 76.6 µg/ 72 hrs Gp 2 = 67.96 µg/ 72 hrs Gp 3 = 182.9 µg/ 72 hrs Gp 3 = 84.46 µg/ 72 hrs Gp 3 = 92.86 µg/ 72 hrs	Higher mean EDTA chelatable lead level in Gp 3; 5 of 12 had history of gout developing after CRI Limitations = small sample sizes, limited analyses

Table AX6-4.3 (cont'd). Renal Effects of Lead – Patient Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Lin et al. (1999) Taiwan Study date not provided	32 patients selected from 102 patients with serum creatinine from 1.5 –4.0 mg/dL who were followed in the Institution's outpatient clinics Eligibility criteria included serum creatinine from 1.5 – 4.0 mg/dL, stable renal function over 6 months before study entry; controlled blood pressure and cholesterol; daily protein intake <1 g/kg body wt; no known history of exposure to lead or other heavy metals and EDTA chelatable lead >150 but <600 µg/72 hour. Exclusionary criteria included potentially reversible or unstable renal disease (i.e., due to systemic diseases such as lupus and diabetes), and nephrotoxicant medications. Patients divided into 16 patients receiving 1 g EDTA i.v. weekly for two months and a control group of 16 patients who received no therapy	Mean EDTA chelatable lead levels pre-chelation 254.9 µg/ 72 hrs in group receiving subsequent chelation 279.7 µg/ 72 hrs in control group Blood lead levels not mentioned	Rates of progression of renal insufficiency were followed by reciprocal of serum creatinine during the 12 months prior to therapy and for 12 months following therapy. Rates of progression of renal insufficiency were similar in the treatment group and the control group during the baseline observation. However, improvement in renal function was observed during EDTA chelation. Following chelation, renal function stabilized in the treated group but continued to decline in the control group. At 12 months after treatment, the mean difference in the change in the reciprocal of serum creatinine between the two groups was 0.000042 L/µmol per month (95% CI: 0.00001, 0.00007). Results using a sensitivity analysis for patients lost to follow-up (only one in each group) gave similar results.

Table AX6-4.3 (cont'd). Renal Effects of Lead – Patient Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Lin et al., Arch Intern Med. (2001) Taiwan Study date not provided	24 month prospective observational study 110 patients with CRI dichotomized by EDTA chelatable lead level of 80 µg / 72 hrs into two groups of 55 each Eligibility criteria included serum creatinine from 1.5 – 4.0 mg/dL, stable renal function (decrease in GFR <5 mL/min over 6 months); blood pressure <140/90 mmHg; cholesterol level <240 mg/dL; daily protein intake <1 g/kg body wt; no known history of exposure to lead or other heavy metals and EDTA chelatable lead <600 µg/72 hour. Exclusionary criteria included potentially reversible or unstable renal disease (i.e., due to systemic diseases such as lupus and diabetes), nephrotoxicant medications, and drug allergies. 196 patients initially screened for study; details on reasons for non-eligibility not provided. Primary outcome = 1.5 times increase in the initial creatinine level or need for dialysis; secondary outcome = change in creatinine clearance Cox proportional-hazards model analysis for primary outcome. Mean differences in creatinine clearance compared at sequential time points with t or Mann-Whitney U tests. Adjustment for age, gender, baseline BMI, smoking, proteinuria, hypertension, hyperlipidemia, daily protein intake, and underlying renal disease Intention-to-Treat and sensitivity analyses compared creatinine clearance a by time period in high and low lead groups. 3 month clinical trial of chelation with 1 year follow-up At 24 months, 36 patients whose EDTA chelatable lead levels were 80 - 600 µg/72 hours and serum creatinine levels of <4.2 mg/dL were randomized; 24 to a 3-month treatment period consisting of weekly chelation with 1 g EDTA iv until their excreted lead levels fell below 80 µg/72 hours and 12 to placebo infusion. Intention-to-Treat and sensitivity analyses compared creatinine clearance by time period in treated and control groups.	Mean blood lead levels 6.6 µg/dL in high normal lead body burden group (n = 55) 3.9 µg/dL in low normal lead body burden group (n = 55) Mean EDTA chelatable lead levels pre-chelation 182.9 µg/ 72 hrs in high normal lead body burden group (n = 55) 37.9 µg/ 72 hrs in low normal lead body burden group (n = 55)	24 month prospective observational study Lead dose measures were only significant differences between high and low normal lead body burden groups. Of the 96 participants who completed the observation study, 14 patients in the high normal body lead burden group reached the primary endpoint compared to 1 patient in the low body lead burden group ($p < 0.001$ by log-rank test). From month 12 to month 24, creatinine clearance in high normal body lead burden patients was at least borderline statistically lower than in low body lead burden patients; from 18-24 months, 95% CI excluded 0. 95% CI for the difference at 24 months was (–15.0, –3.8); difference in creatinine clearance between groups was 0.15 mL/s at that point. In a Cox multivariate regression analysis, chelatable lead was significantly associated with overall risk for the primary endpoint (relative risk = 41.5 [95% CI: 3.9, 440.8]; $p = 0.002$]). In this model, age, basal BMI, and basal daily proteinuria were also associated with increased risk. 3 month clinical trial of chelation with 1 year follow-up The two groups were similar in baseline renal risk factors (although numbers small so beta error possible). Mean EDTA dose during the 3 month period was 5 µg. After three months of lead chelation therapy, the body lead burden of the patients in the chelation group decreased from 198 to 39.2 µg. After 3 months of chelation and 3 months of follow-up, creatinine clearance increased by 0.08 mL/s in the treated group but declined by 0.04 mL/s in the controls. At the end of the study period, mean creatinine clearance was 0.68 mL/s in the chelated group compared to 0.48 mL/s in the control group ($p < 0.05$; 95% CI for the difference between groups = –25.0 to –0.2).

Table AX6-4.3 (cont'd). Renal Effects of Lead – Patient Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Lin et al., Kidney International (2001) Study location and date not provided; authors from Taiwan	101 patients with CRI (defined as serum creatinine between 1.5 and 3.0 mg/dL) 67 with CRI and gout 34 with CRI only Eligibility criteria included no known history of lead exposure, certain diagnoses and medications. CRI must have preceded gout diagnosis. Randomized chelation trial 30 participants with CRI, gout, and EDTA-chelatable lead levels between 80.2 and 361 µg/72 hours randomized to either a treatment group receiving 1 gram EDTA iv per week for 4 weeks (N = 20) or to a control group who received glucose in normal saline iv.	Mean blood lead 5.4 µg/dL (CRI and gout) 4.4 µg/dL (CRI only) Mean EDTA-chelatable lead 138.1 µg/ 72 hrs (CRI and gout) 64.2 µg/ 72 hrs (CRI only) ($p < 0.01$)	In 101, EDTA-chelatable lead higher in patients with CRI and gout compared to those with CRI only. EDTA-chelatable lead, but not blood lead, was associated positively with serum urate and negatively with daily urate excretion, urate clearance, and fractional urate excretion. Randomized chelation trial The two groups had similar uric acid, renal function, and lead measures pre-chelation. In the treated group, mean EDTA-chelatable lead declined from 159.2 to 41 µg/72 hours; mean serum urate decreased from 10.2 to 8.6 mg/dL (% change compared to the control group = −22.4; [95% CI: −46.0, −1.5]; $p = 0.02$), and mean urate clearance increased from 2.7 to 4.2 mL/min ((% change compared to the control group = 67.9; [95% CI: 12.2, 121.2]; $p < 0.01$). Daily and fractional urate excretion were also significantly different between the two groups. Mean measured creatinine clearance increased from 50.8 to 54.2 mL/min (% change compared to the control group = 8.0; [95% CI: −0.4, 20.1]; $p = 0.06$).
Lin et al. (2002) Study location and date not provided; authors from Taiwan	84 healthy participants 27 participants with gout All with normal renal function (defined as serum creatinine ≤1.4 mg/dL) Participants with a history of occupational heavy metal exposure, EDTA-chelatable lead levels >600 µg/72 hours, or systemic diseases were excluded. Randomized chelation trial 24 participants with EDTA-chelatable lead levels between 75 and 600 µg/72 hours randomized to either a treatment group receiving 1 gram EDTA iv per week for 4 weeks (N = 12) or to a control group who received glucose in normal saline i.v. Multiple linear regression, adjustment for age, sex, BMI, daily protein intake, and creatinine clearance.	Mean blood lead 3.9 µg/dL (controls) 4.2 µg/dL (gout) Mean EDTA-chelatable lead 45 µg/ 72 hrs (controls) 84 µg/ 72 hrs (gout) ($p < 0.0001$)	Significantly higher mean EDTA-chelatable lead and lower urate clearance were present in patients with gout compared to those without (3.7 versus 6.0 mL/min /1.73 m^2; $p < 0.001$ for urate clearance) After adjustment, EDTA-chelatable lead associated with all four uric acid measures (serum urate, daily urate excretion, urate clearance, and fractional urate excretion). Blood lead associated with serum urate. All associations in same direction as in Lin et al. (2001). Randomized chelation trial. The two groups had similar urate, renal function, and lead measures pre-chelation. In the treated group, mean blood and EDTA-chelatable lead levels declined (from 5.0 to 3.7 µg/dL and 110 to 46 µg/72 hours, respectively). Statistically significant improvement observed in all four urate measures in the treated group compared to the control group.

Table AX6-4.3 (cont'd). Renal Effects of Lead – Patient Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Lin et al. (2003) Study location and date not provided; authors from Taiwan	24 month prospective observational study 202 patients with CRI Eligibility criteria included serum creatinine from 1.5 - 3.9 mg/dL, stable renal function (decrease in GFR <5 mL/min over 6 months); blood pressure <140/90 mmHg; cholesterol level <240 mg/dL; daily protein intake <1 g/kg body wt; no known history of exposure to lead or other heavy metals and EDTA chelatable lead <600 µg/72 hour. Exclusionary criteria included potentially reversible or unstable renal disease (i.e., due to systemic diseases such as lupus and diabetes), nephrotoxicant medications, and drug allergies. 250 patients initially observed, loss due to noncompliance or unstable renal function, baseline data on the 48 who left or were removed from the study not provided. Cox proportional-hazards model analysis for primary outcome. Generalized estimating equations (GEE) for associations between baseline chelatable lead or blood lead level and longitudinal change in GFR (estimated by an MDRD equation [Levey et al., 1999]) and by measurement of creatinine clearance. Adjustment for age, gender, baseline BMI, smoking, baseline serum creatinine, proteinuria, hypertension, hyperlipidemia, daily protein intake, and underlying renal diseases.	Mean blood lead levels 5.3 µg/dL in total group (n = 202) 6.1 µg/dL pre-chelation in chelated group (n = 32) 5.9 µg/dL pre-chelation in control group Mean EDTA chelatable lead levels pre-chelation 104.5 µg/72 hrs in total group (n = 202) 150.9 µg/72 hrs pre-chelation in chelated group 144.5 µg/72 hrs pre-chelation in control group	24 month prospective observational study Primary endpoint = increase in serum creatinine to 1.5 times baseline or need for hemodialysis; occurred in 24 participants. Secondary endpoint = change in estimated glomerular filtration rate (GFR) In a Cox multivariate regression analysis, chelatable lead was significantly associated with overall risk for the primary endpoint (hazard ratio for each 1 µg chelatable lead was 1.00 [95% CI: 1.00, 1.01]; p = 0.03). In this model, baseline serum creatinine was also associated (hazard ratio for each 1 mg/dL was 2.75 [95% CI: 1.46, 5.18]; p = 0.002) and, at borderline significance (p < 0.1), baseline daily protein excretion and smoking were as well. The association between baseline chelatable lead and change in GFR was modeled using GEE. Estimate = −0.003 (p = <0.001) (neither SE nor CI provided). In this model, gender and daily protein intake were associated with increased GFR; baseline serum creatinine level, daily urinary protein excretion, and the presence of polycystic kidney disease were significant predictors of a progressive decline in glomerular filtration rate. Based on this model, a 10 µg higher baseline chelatable lead level was associated with a GFR decrease of 0.03 mL per minute per 1.73 m^2 of body-surface area during the 2 year observation period. Although statistically significant, this effect is clinically small. Furthermore, it is 40 fold lower than that reported in Yu et al. (2004) over a follow-up period that is only two-fold shorter.

Table AX6-4.3 (cont'd). Renal Effects of Lead – Patient Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Lin et al. (2003) (cont'd) Study location and date not provided; authors from Taiwan	27 month clinical trial of chelation At 24 months, 64 patients whose EDTA chelatable lead levels were 80 - 600 µg/72 hours and serum creatinine levels of <4.2 mg/dL were randomized; half to a 3-month treatment period consisting of weekly chelation with 1 g EDTA iv until their excreted lead levels fell below 60 µg/72 hours and half to five weeks of placebo infusion. Intention-to-Treat analysis compared creatinine clearance and GFR by time period in treated and control groups		27 month clinical trial of chelation The two groups were similar in baseline renal risk factors (although numbers small so beta error possible). After three months of lead chelation therapy, the body lead burden of the patients in the chelation group decreased from 150.9 to 43.2 µg and their mean blood lead levels decreased from 6.1 to 3.9 µg/dL. GFR increased by 3.4 mL/min/1.73 m^2 in the treated group; in contrast, it decreased by 1.1 mL/min/1.73 m^2 in the control group. Mean EDTA dose during the 3 month period was 5.2 µg. In the subsequent 24 months, chelation in 19 (59%) participants was repeated due to increases in serum creatinine in association with rebound increases in EDTA chelatable lead levels. Each received one additional chelation series (mean = 4.1 g EDTA) a mean of 13.7 months after the first chelation period. Control patients receiving placebo weekly for five weeks every six months. At the end of the study period, mean estimated glomerular filtration rate increased by 2.1 mL/min/1.73 m^2 of body-surface area in the chelated group compared to a decline of 6.0 in the controls ($p < 0.01$; 95% CI for the difference between groups = –11.0 to –5.1).

Table AX6-4.3 (cont'd). Renal Effects of Lead – Patient Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Yu et al. (2004) Study location and date not provided; authors from Taiwan	121 patients followed over a four year observational period Eligibility criteria included serum creatinine from 1.5 -3.9 mg/dL, stable renal function (decrease in GFR <5 mL/min over 6 months); blood pressure <140/90 mmHg; cholesterol level <240 mg/dL; daily protein intake <1 g/kg body wt; no known history of exposure to lead or other heavy metals and EDTA chelatable lead <600 µg/72 hour. Exclusionary criteria included potentially reversible or unstable renal disease (i.e., due to systemic diseases such as lupus and diabetes), medical noncompliance (patients were followed for 6 months to assess compliance before enrollment in the study), nephrotoxicant medications, and drug allergies. Cox proportional hazards model analysis for primary outcomes and generalized estimating equations (GEE) for associations between baseline chelatable lead or blood lead level and longitudinal change in GFR (estimated by an MDRD equation [Levey et al., 1999]) Adjustment for age, gender, baseline BMI, smoking, baseline serum creatinine, proteinuria, hypertension, hyperlipidemia, daily protein intake, use of ACE inhibitor or angiotensin-receptor antagonists (since not all patients were on these), and chronic glomerulonephritis (other underlying renal diseases included in GEE as well)	Mean (SD) blood lead at baseline 3.4 (1.3) µg/dL in 58 patients with "low-normal" EDTA chelatable lead levels (<80 µg lead/72 hours) 4.9 (2.6) µg/dL in 63 patients with "high-normal" EDTA chelatable lead levels (≥80 but <600 µg/72 hours)	The two groups (dichotomized by diagnostic EDTA chelatable lead of 80 µg lead/72 hours) were similar in most baseline risk factors other than lead body burden. Borderline statistically significant ($p < 0.1$) differences included mean older age in the high chelatable lead group and certain renal diagnoses (chronic glomerulosclerosis, chronic interstitial nephritis, hypertensive nephropathy; surprisingly both of the latter two diagnoses were less common in the lower lead body burden group). Fifteen patients in the "high-normal" chelatable lead group reached the primary endpoint (doubling of serum creatinine over the 4 year study period or need for hemodialysis) compared to only two in the "low-normal" group ($p = 0.001$ by Kaplan-Meier analysis). In a Cox multivariate regression analysis, chelatable lead was significantly associated with overall risk for the primary endpoint (hazard ratio for each 1 µg chelatable lead was 1.01 [95% CI: 1.00-1.01; $p = 0.002$]). In this model, the only other variable reaching at least borderline significance ($p < 0.1$) was baseline serum creatinine. The associations between baseline chelatable lead or blood lead level and change in GFR were modeled separately using GEE. Estimates = −0.1295 ($p = 0.002$) for lead body burden (neither SE nor CI provided) −4.0123 ($p = 0.02$) for blood lead (neither SE nor CI provided) Based on these models, a 10 µg higher baseline chelatable lead level or 1µg/dL higher blood lead level predicted 1.3 and 4.0 mL/min declines in GFR, respectively, during the four year study period. Similar to the primary outcome analysis, of the many traditional renal risk factors adjusted for in these models, only diagnosis of chronic interstitial nephritis was significantly associated, in this case with an increase in GFR. Of note, chronic interstitial nephritis was also a more frequent diagnosis in the group with the low-normal chelatable lead levels ($p = 0.09$). The authors stated that these patients were not included in earlier publications (which are described below in Section 6.4.4.3.3 Therapeutic EDTA Chelation in Patients).

Table AX6-4.4. Renal Effects of Lead – Mortality

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States			
Cooper (1988); Cooper, Wong and Kheifets (1985) 16 U.S. plants Employment between 1946 and 1970; mortality from 1947 to 1980	4519 male battery plant workers 2300 male lead production workers Employed for at least one year between 1946 and 1970 Cause of death per death certificate (extrapolated when missing) Standardized mortality ratios (SMRs) compared with national age-specific rates. PMR also assessed Analyzed separately by battery and lead production, by hire date before and after 1/1/1946, and by cumulative years of employment (1-9, 10-19, 20+)	Mean blood lead 63 µg/dL in n = 1326 battery workers 80 µg/dL in n = 537 production workers Past lead exposures poorly documented prior to 1960	Follow-up >90% in both groups; 2339 deaths observed "chronic or unspecified nephritis" SMR 222 (95% CI: 135, 343) in battery workers 265 (95% CI: 114, 522) in lead production workers "other hypertensive disease" SMR ("includes HTN and related renal disease without mention of heart disease)" 320 (95% CI: 197, 489) in battery workers 475 (95% CI: 218, 902) in lead production workers Race adjusted proportionate mortality ratios analyses similar. Nephritis deaths observed primarily in workers hired before 1946. Limitations = due to mortality analysis (inaccuracies of death certificates, exposure assessment generally limited)
Steenland et al. (1992) Idaho Employed between 1940 and 1965; mortality up to 1988	1990 male lead smelter workers employed in a lead-exposed department for at least one year between 1940 and 1965 Vital status was determined using records from the Social Security Administration and the National Death Index.	Mean blood lead 56.3 µg/dL (n = 173, measured in 1976) High lead exposure defined as workers from departments with an average >0.2 mg/m^3 airborne lead or ≥50% of jobs had average levels more than twice that level (1975 survey). n = 1,436 in this category.	Compared to the U.S. white male population, the standardized mortality ratio (SMR) for chronic kidney disease, based on only 8 deaths, was 1.26 (95th CI = 0.54, 2.49). SMR = 1.55 in high lead exposure group, also not significant. The SMR for chronic kidney disease increased with duration of exposure from 0.79 in workers exposed 1-5 years to 2.79 in workers exposed >20 years; however SMR was not significant.

Table AX6-4.4 (cont'd). Renal Effects of Lead – Mortality

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe			
Bernard et al. (1995) Czech Republic Study date not provided	144 children living close to a lead smelter (exposed groups 1 and 2) 51 controls living in a rural area presumed to be relatively unpolluted with lead. Mean age = 13.5 years. Renal outcome measures included urinary albumin, RBP, NAG, Clara cell protein and β_2-microglobulin. Retinol binding protein 73.8 µg/g cr (controls) 109.4 µg/g cr (exposed group 1) 117.8 µg/g cr (exposed group 2) β_2-microglobulin 60.3 µg/g cr (controls) 89.1 µg/g cr (exposed group 1) 66.4 µg/g cr (exposed group 2) NAG 1.56 IU/g cr (controls) 2.32 IU/g cr (exposed group 1) 1.46 IU/g cr (exposed group 2) Multiple linear adjusting for age and gender.	Blood lead 8.7 µg/dL (control boys) 8.39 µg/dL (control girls) 10.9 µg/dL (exposed boys 1) 9.4 µg/dL (exposed girls 1) 14.9 µg/dL (exposed boys 2) 12.9 µg/dL (exposed girls 2)	Mean blood lead levels significantly higher in both exposed groups compared to the control group. In contrast, blood cadmium levels were similar among all groups. After adjustment for age, sex, blood cadmium, and zinc protoporphyrin, log transformed blood lead was associated with log transformed RBP (β coefficient = 0.302, p = 0.005 [SE nor CI provided]).
Fanning (1988) UK Deaths from 1926-1985	Deceased males identified through pension records of lead battery and other factory workers 867 deaths of mean with high lead exposure compared to 1206 men with low or no lead exposure	Range of blood lead 40-80 µg/dL since ~1968 in high lead exposure group; thought not to have had clinical lead poisoning due to medical surveillance <40 µg/dL since ~1968 in little or no exposure group	Odds ratio for renal disease = 0.62, not significant, based on only 11 deaths. Similar for diagnosis of nephritis. Possible decreasing odds ratio over time of deaths with mention of nephritis on death certificate but not significant and numbers still quite small. Limitations = standard mortality study issues although deaths compared with other workers and not general population which is a strength in this type of study.

Table AX6-4.5. Renal Effects of Lead – Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States			
Hu (1991) U.S. Study date not provided	21 of 192 adults who were hospitalized at Boston Children's Hospital between 1932 to 1942 for childhood lead poisoning were traced to a Boston area address. Matched on age, sex, race, and neighborhood to 21controls.	Mean (SD) blood lead 6.0 μg/dL (lead poisoned) 7.5 μg/dL (controls)	No significant differences in blood lead level, serum creatinine, or BUN. Mean measured creatinine clearance higher in the previously lead poisoned group compared to controls (112.8 vs. 88.8 mL/min/1.73 m2 [$p < 0.01$]). Mean in the lead exposed group was also higher than the predicted value of 94.2 mL/min/1.73 m2 from the nomogram of Rowe et al. (1976). Suggests lead-related hyperfiltration. As noted in section 6.4, one survivor, identified but not included in the study, had disease consistent with lead nephropathy. Limitations = small study size and concern for survivor bias in the study group.
Loghman-Adham (1998) Chicago, IL Study date not provided	134 children and young adults, 8 to 13 years after chelation therapy for severe lead poisoning Mean age at poisoning = 2.3 years Mean age at follow-up = 13.4 years	Mean peak blood lead level 121 μg/dL Mean blood lead level at time of study 18.6 μg/dL	Mean serum creatinine was normal (0.8 mg/dL). Calculated creatinine clearance normal in all but 3 children. No correlation between either initial or current blood lead and serum creatinine or calculated creatinine clearance. Urinary α-amino nitrogen concentrations were significantly increased compared with 19 healthy age matched controls and were correlated with current blood lead levels. Thirty-two children (24%) had glycosuria. Fractional excretion of phosphate, however, was normal in all children. The author concluded that a partial Fanconi syndrome could persist for up to 13 years after childhood lead poisoning. The author notes that the prognostic significance of this is unknown at present.
McDonald and Potter (1996) Boston, MA 1991	454 pediatric hospital patients who were diagnosed with lead poisoning between 1923 and 1966 were traced through 1991 Mortality study, comparison with U.S. population		Chronic nephritis was not a significant cause of death. Mortality from all cardiovascular disease was elevated (observed/expected = 2.1 [95% CI: 1.3, 3.2]) and cerebral vascular deaths were particularly common among women (observed/expected = 5.5 [95% CI: 1.1, 15.9]).
Moel and Sachs (1992) Chicago, IL 1974-1989	62 participants with blood lead >100 μg/dL, diagnosed and chelated between 1966 and 1972, together with 19 age-matched control siblings with initial blood leads less than 40 μg/dL. Mean age at follow-up = 22 years. Renal outcomes = serum creatinine, uric acid, and β_2-microglobulin, fractional excretion of β_2-microglobulin, urinary protein:creatinine ratio, and tubular reabsorption of phosphate.	Mean initial blood lead 150.3 μg/dL (highly poisoned as children) Data for siblings not available as levels <40 μg/dL not quantified.	There were no statistical differences in either renal function or blood pressure between study subjects and control siblings. Initial blood lead level was not associated with serum creatinine, after adjustment for age, gender and body mass index. A modest increase in serum creatinine values was observed over a nine-year period in four of the 62 study subjects (up to 1.6 mg/dL).

Table AX6-4.5 (cont'd). Renal Effects of Lead – Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe			
Factor-Litvak et al. (1999) Kosovo, Yugoslavia 1985-1993	577 children followed at 6 month intervals through 7.5 years of age. Divided into a high exposure and a low exposure group, based on residence in Kosovska Mitrovica with a lead smelter, refinery and battery plant or in Pristina, 25 miles away. Renal outcome = Proteinuria assessed with a dipstick. Multiple logistic regression modeling of proteinuria dichotomized as either any or none, adjusting for socioeconomic status, maternal education/ intelligence, and quality of childrearing environment.	Mead blood lead from graph peaked at ~38 µg/dL between ages 3-5 in Kosovska Mitrovica and at ~10 µg/dL in controls. Blood lead level (not means) range = 1 – 70 µg/dL	In higher exposed group, adjusted OR for proteinuria was 3.5 (CI = 1.7 – 7.2); adjusted odds of proteinuria increased by 1.15 (CI = 1.1 – 1.2) per unit increase in blood lead in the higher exposed group. Proteinuria unrelated to blood lead in lower exposed control group. Limitations = limited renal outcomes assessed, high dropout rate in the study.
Fels et al. (1998) Poland 1995	112 children (50 controls, 62 exposed) Mean age = 9.9 years and 10.6 years in controls and exposed group, respectively. Numerous (29) renal outcome measures were examined including serum creatinine and β_2-microglobulin, and urinary NAG, RBP, Clara cell protein, β_2-microglobulin, 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), prostaglandin E_2 (PGE_2) and thromboxane B_2 (TXB_2). <u>Urinary RBP</u> 46 µg/g cr (exposed) 42 µg/g cr (controls) <u>Urinary β_2-microglobulin</u> 89 µg/g cr (exposed) 37 µg/g cr (controls) <u>Serum creatinine</u> 0.63 mg/dL (exposed) 0.63 mg/dL (controls)	<u>Blood lead</u> 13.3 µg/dL (exposed) 3.9 µg/dL (controls)	Significantly higher mean serum β_2-microglobulin, and urinary transferrin, 6-keto-$PGF_{1\alpha}$, thromboxane B_2, epidermal growth factor, β_2-microglobulin, PGE_2, and Clara cell protein in the exposed children. In contrast, NAG-B was lower in the exposed group. Categorical blood lead associated with prevalence of values above the upper reference limits for several biomarkers. Urinary 6-keto-$PGF_{1\alpha}$, TXB_2, β_2-microglobulin, Clara cell protein, epidermal growth factor and PGE_2 positively correlated with blood lead (r = 0.441, 0.225, 0.203, 0.261, 0.356, and 0.23, respectively; all with significant p-values) Limitations = data analysis, limited adjustment

Table AX6-4.5 (cont'd). Renal Effects of Lead – Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Price et al. (1999) Belgium, Poland, Germany and Italy Study date not provided	Urinary lead measured in 481 European children (236 controls, 245 exposed) aged 6 – 14 years. Several renal outcome measures assessed including urinary NAG and β_2-microglobulin; values not reported	Mean urinary lead Range from 3.9 to 7.2 µg/g cr (controls) Range from 5.2 to 24.6 µg/g cr (exposed)	Urinary lead generally higher in exposed children as compared to controls. Authors unexpectedly found substantial differences in renal biomarkers by study site. Authors note several renal biomarkers differed between exposed and control groups. Also questioned the use of "control" groups in ubiquitous exposures.
Scharer et al. (1991) Germany 1988-1989	22 children, age 5-14 years, with CRI 20 siblings or neighbors as lower exposed group 16 control children without known lead exposure	Mean blood lead 2.9 µg/dL in children with CRI, not tested in other groups Mean dental lead content 2.8 µg/g in children with CRI 1.7 µg/g in sibs/neighbors 1.4 µg/g in controls	Lead levels in teeth significantly higher in both the patient and sibling/neighbor control groups compared to the unexposed control group.
Sonmez et al. (2002) Turkey Study date not provided	39 adolescent auto repair workers (mean age 16.2 years) 13 adult battery workers as positive controls (mean age 32 years) 29 rural adolescents as negative controls (mean age 14.8 years) Serum creatinine 0.99 mg/dL (exposed group) 0.99 mg/dL (positive/ adult controls) 0.89 mg/dL (negative/ adolescent controls) Urinary NAG 4.7 IU/g cr (exposed group) 7.4 IU/g cr (positive/ adult controls) 3.1 IU/g cr (negative/ adolescent controls)	Blood lead 8.13 µg/dL (exposed group) 25.3 µg/dL (positive/adult controls) 3.49 µg/dL (negative/ adolescent controls)	All participants had normal blood urea, creatinine, and uric acid levels as well as normal routine urinalysis Blood lead level and urinary NAG significantly higher in adolescent auto repair workers compared to the negative control group Limitations = data analysis, lack of adjustment

Table AX6-4.5 (cont'd). Renal Effects of Lead – Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Staessen et al. (2001) Belgium 1999	100 exposed and 100 control children Mean age = 17 years Two exposed groups were recruited from industrialized suburbs while the control group was recruited from a rural area. β_2-microglobulin 5.22 µg/mmol cr (controls) 5.3 µg/mmol cr (exposed group 1) 9.09 µg/mmol cr (exposed group 2) Cystatin-C 0.65 mg/L (controls) 0.63 mg/L (exposed group 1) 0.71 mg/L (exposed group 2) Multiple linear regression adjusting for sex and smoking status	Blood lead 1.5 µg/dL (controls) 1.8 µg/dL (exposed group 1) 2.7 µg/dL (exposed group 2)	Blood lead, β_2-microglobulin, and Cystatin-C levels higher in exposed group 2 as compared to controls and exposed group 1 After adjustment for sex and smoking status, blood lead was associated with both β_2-microglobulin and cystatin-C. A two-fold increase in blood lead was associated with a 3.6 % increase in Cystatin-C ([95% CI: 1.5, 5.7]; $p < 0.0001$) and a 16% increase in β_2-microglobulin ([95% CI: 2.7, 31]; $p = 0.02$). Blood cadmium was not associated with either outcome.
Verberk et al. (1996) Romania 1991-1992	151 children who resided at different distances from a lead smelter Mean age = 4.6 years. Renal outcomes = urinary RBP, NAG, α_1-microglobulin, albumin and alanine aminopeptidase. Geometric means Urinary RBP 49.4 µg/g cr Urinary NAG 6.9 U/g cr Urinary α_1-microglobulin 2.4 mg/g cr Urinary alanine aminopeptidase 19.8 U/g cr Multiple regression analysis adjusting for age and gender	Blood lead 34.2 (22.4) µg/dL	After adjustment for age and gender, a 10 µg/dL increase in blood lead was associated with a 13.5% increase in NAG excretion (90% CI = 10.2-17%). No threshold was observed. No other significant associations noted.

Table AX6-4.5 (cont'd). Renal Effects of Lead – Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Africa			
Diouf et al. (2003) Senegal 1998	38 Senegalese children (19 exposed, 19 controls) Age range = 8 – 12 years old. Renal function assessed by measuring urinary alpha-glutathione S-transferase (αGST)	Mean (SD) blood lead 10.7 (1.7) µg/dL (exposed) 6.1 (1.8) µg/dL (controls)	Blood lead significantly higher in exposed group (urban dwellers) as compared to controls (rural dwellers). Unclear as to whether αGST was higher or lower in controls as compared to exposed group (stated to be higher in controls in the results section BUT stated to be higher in the exposed group in the discussion). Regardless, the difference was not statistically significant. Limitations = small sample size, data analysis

CHAPTER 6 ANNEX

ANNEX TABLES AX6-5

Table AX6-5.1. Cardiovascular Effects of Lead

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States			
Cheng et al. (1998) US-Boston, Normative Aging Study (VA) 1991-1995	775 males (97% white), mean age (SD) [range] 67.8 years (7.3) [48-93]. Multiple linear regression models of heart rate-corrected QT and QRS electrocardiogram intervals were adjusted by stepwise entry of covariates, retaining only those that remained significant at $p < 0.10$. Linear blood lead, tibia, and patella bone lead were apparently (not described in text) entered separately. Logistic regression models for Minnesota ECG Coding Center diagnoses of intraventricular conduction deficit (IVCD), atrioventricular conduction deficit (AVCD), and arrhythmia were adjusted by covariates the same way. Only analyses stratified by age (<65 years, n = 277; ≥65 years, n = 498) were presented	Arithmetic mean (SD) blood lead: 5.8 µg/dL (3.4) Mean (SD) tibia lead: 22.2 µg/g (13.4) Mean (SD) patella lead: 30.8 µg/g (19.2)	Multiple regression models of QT intervals, adjusted for age, alcohol intake, BMI, and diastolic blood pressure, found that only tibia and patella lead were significantly related to outcome in the under 65 group. Every 10 µg/g increase of tibia and patella lead was associated with a 5.0 ms (95% CI: 0.8, 9.2) and 3.0 ms (95% CI: 0.2, 5.8) increase in QT interval, respectively. Multiple regression models of QRS intervals, adjusted for age, fasting glucose level, and diastolic blood pressure, also found that only tibia and patella lead were significantly related to outcome in the under 65 group. Every 10 µg/g increase of tibia and patella lead was associated with a 4.8 ms (95% CI: 1.8, 7.8) and 2.2 ms (95% CI: 0.1, 4.4) increase in QRS interval, respectively. There were no significant effects of lead in the 65 and over group. Logistic regression models of IVCD, adjusted for age and serum HDL level, found that only tibia lead was significantly related to outcome in the under 65 group. Every 10 µg/g increase of tibia lead was associated with increased odds of IVCD, OR 2.23 (95% CI: 1.28, 3.90). There were no significant lead effects in the 65 and over group for IVCD. Logistic regression models of AVCD, adjusted for age and serum HDL level, found that both tibia and patella lead were significantly related to outcome in the 65 and over group. Every 10 µg/g increase of tibia lead and patella lead was associated with increased odds of AVCD, OR 1.22 (95% CI: 1.02, 1.47) and OR 1.14 (95% CI: 1.00, 1.29), respectively. Lead was not significantly related to AVCD in the under 65 group. There were no significant effects of lead on arrhythmia in either age group. Stepwise models may capitalize on chance associations. Linear blood lead specification may not be appropriate in some or all of these models. Not clear if three models were constructed for each stratified analysis for each outcome, each based on a different lead index. No statistical comparisons across strata. No model diagnostics were presented.

Table AX6-5.1 (cont'd). Cardiovascular Effects of Lead

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Cheng et al. (2001) US-Boston, Normative Aging Study (VA) 1991-1997	833 males (~97% white), average age (SD): 65-68 years, depending on hypertension group (6.8-7.8) for blood pressure study. 474 males with no history of hypertension at first measurement, returning up to 6 years later for hypertension study. Linear multiple regression models of blood pressure and Cox proportional hazard models of new cases of hypertension after up to 7 years, with one group of covariates forced into models based on biological plausibility and another group forced based on significant univariate or bivariate results or >20% effect modification of lead variable coefficient in multiple models. Linear blood lead, tibia lead, and patella lead forced in separate models.	Arithmetic mean (SD) blood lead: 5.9-6.4 µg/dL (3.7-4.2), depending on hypertension group (only data shown).	Multiple regression models of blood pressure always included age, age-squared, BMI, family history of hypertension, daily alcohol consumption, and daily calcium consumption. Increasing tibia lead concentration was associated with increased systolic blood pressure (diastolic not addressed) in baseline measurements in subjects (n = 519) free from definite hypertension (systolic > 160 mmHg, diastolic > 95 mmHg, or taking daily antihypertensive medication). Each increase of 10 µg/g tibia lead concentration was associated with an increase in systolic blood pressure of 1.0 mmHg (95% CI: 0.01, 1.99). Patella and linear blood lead were not significant. Cox proportional hazard models always included age, age-squared, BMI, and family history of hypertension. In follow up (n = 474), only increasing patella lead predicted increasing risk of definite hypertension in those classified as normotensive at baseline. For every 10 µg/g increase in patella lead risk ratio increased 1.14 (95% CI: 1.02, 1.28). Combining borderline hypertension (systolic 141-160 mmHg or diastolic 91-95 mmHg) with definite hypertension (n = 306), the relative risk ratio of becoming a combined hypertensive associated with a 10 µg/g increase in patella lead was 1.23 (95% CI: 1.03, 1.48). Linear blood lead and tibia lead were not significant. Linear blood lead is not indicated for blood pressure models due to strong likelihood of significant residual heteroscedasticity and non-normality. Relatively small sample size may have prevented tibia blood lead significance in the Cox proportional hazard models.

Table AX6-5.1 (cont'd). Cardiovascular Effects of Lead

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Den Hond et al. (2002) US-NHANES III 1988-1994	4,685 white males, 5,138 white females, 1,761 black males, 2,197 black females, from 20 years up. Log-transformed blood lead, systolic and diastolic blood pressure measured at survey time and analyzed with forward, stepwise multiple regression with covariates.	Geometric Mean (25^{th}-75^{th} percentile) blood lead: White Male Mean 3.6 µg/dL (2.3-5.3) White Female Mean 2.1 µg/dL (1.3-3.4) Black Male Mean 4.2 µg/dL (2.7-6.5) Black Female Mean 2.3 µg/dL (1.4-3.9)	After adjusting for age, age-squared, BMI, hematocrit, smoking, alcohol, and an indicator variable for use of antihypertensive medications, each model was further modified by a unique mix of other covariates, including: coffee consumption, dietary calcium, dietary sodium/calcium ration, total serum protein, total serum calcium, diabetes, and poverty index. Log lead was forced in last. In stratified analyses, only blacks had significant positive blood pressure associations with log blood lead. Each doubling of blood lead was associated with increase of black male systolic blood pressure of 0.9 mmHg (95% CI: 0.04, 1.8), black female systolic blood pressure of 1.2 mmHg (95% CI: 0.4, 2.0), and female diastolic blood pressure of 0.5 mmHg (95% CI: 0.01, 1.1). In white males only, each doubling of blood lead was significantly associated with a decrease in diastolic blood pressure of −0.6 mmHg (95% CI: −0.9, −0.3). Stepwise models can relay on chance associations due to multiple testing and usually lead to a different pattern of covariate adjustment in different models. Inclusion of likely confounding variables such as serum calcium could have affected estimated lead effects. No justification given for age and race stratification. No testing for significant lead coefficient differences between each stratum. No model diagnostic tests reported. No explanation offered for inverse relationship between lead and diastolic blood pressure in white males. No adjustment for survey design.
Gerr et al. (2002) US-Spokane WA and area around Silver Valley ID 1994	502 young people, age 19-29 years, 53% female, nearly evenly divided into the Spokane group (no unusual childhood exposure) and the Silver Valley group, where a lead smelter operated during their childhood. Multiple regression models of systolic blood pressure and diastolic blood pressure. All covariates forced into model as block with both linear blood lead and tibia bone lead in each model.	Mean (SD) blood lead only given stratified on tibia lead category: (Tibia < 1 µg/g) blood lead mean 1.9 µg/dL (1.6) (Tibia 1-5 µg/g) blood lead mean 2.3 µg/dL (2.1) (Tibia 6-10 µg/g) blood lead mean 2.4 µg/dL (2.4) (Tibia <10 µg/g) blood lead mean 3.2 µg/dL (2.3) No other descriptive tibia lead data given.	Adjusting for sex, age, height, BMI, education, income, current smoker, current alcohol use, childhood residence (the two recruitment areas), current birth control pills, hemoglobin, and serum albumin, only tibia lead, and not linear blood lead, was significantly related to systolic and diastolic blood pressure. Compared to the < 1 µg/g tibia lead category, subjects in the >10 µg/g category had 4.3 mmHg (95% CI: 1.4, 6.7) higher systolic blood pressure and 2.8 mmHg (95% CI: 0.4, 5.2) higher diastolic blood pressure. Linear blood lead is not indicated for blood lead-blood pressure models. No diagnostic testing reported. Insufficient descriptive data given for tibia lead.

Table AX6-5.1 (cont'd). Cardiovascular Effects of Lead

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Glenn et al. (2003) US-New Jersey 1994-1998	496 males, mean (SD) (range) age 55.8 (7.4, (40-71) years, working or formerly working at a plant producing tetraethyl or tetramethyl lead until 1991, were followed from 10 months to 3.5 years during which blood pressure was repeatedly tested. Blood lead was tested only at baseline. Tibia lead was tested in 1991 (at the end of organic lead production at the plant) and called "peak tibia lead" and again during 1997 (year 3). Generalized estimating equations with an exchangeable correlation structure for repeated measurements were used for systolic and diastolic blood pressure. One group of covariates was forced into the model as a block (age at baseline, race, BMI, indicator variable for technician, lead variable (linear blood lead, peak tibia lead, and tibia lead each tested separately), duration of follow up, and the interaction between the lead variable and the duration term. Potential confounding variables were entered stepwise and retained in the model if significant. Alternate models not using linear time were constructed, using quartile of follow up time to avoid assuming a linear relationship of change in blood pressure with time.	Arithmetic mean (SD, range) blood lead at baseline: (4.6, 2.6-1-20) µg/dL. Tibia lead at year 3: 14.7 (9.4, ‑1.6-52) µg/g Peak tibia lead: 24.3 (18.1, ‑2.2-118.8)	Controlling for baseline age, BMI, antihypertensive medication use, smoking, education, technician and number of years to each blood pressure measurement, each 1 µg/dL increase in linear baseline blood lead was associated with average systolic blood pressure increase of 0.25 mmHg/year (95% CI: 0.05, 0.44), each 10 µg/g increase in year 3 tibia lead with an average increase of 0.78 mmHg/year (95% CI: 0.24, 1.31), and each increase of 10 µg/g of peak tibia lead with an average increase of 0.34 mmHg/year (95% CI: 0.05, 0.62). Similar results were obtained using the follow up time quartile designation for systolic blood pressure with all subjects and with subjects not taking antihypertensive medications. This was one of the few studies using a prospective design and that used a statistical technique accounting for repeated measures. No justification given for using an exchangeable correlation structure instead of an alternate one. Only examined cortical bone lead (tibia) and not trabecular bone lead (patella or calcaneus). Linear blood lead may not be indicated for use in blood lead-blood pressure models. Stepwise modeling involves multiple testing of the same data set with no control for altered probabilities. No model diagnostics presented.

Table AX6-5.1 (cont'd). Cardiovascular Effects of Lead

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Glenn et al. (2001) US-New Jersey 1996-1997	213 males (92% white), mean (SD) age 58.0 (7.4) years, working or formerly working at a plant producing tetraethyl or tetramethyl lead until 1991, were genotyped for ATP1A2(5') and ATP1A2(3') polymorphism. ATPase is thought to play a role in regulating blood pressure and lead inhibits its activity. Blood pressure, blood lead, and tibia lead were measured. Multiple linear regression models were used for systolic and diastolic blood pressure. Logistic regression model was reported for hypertension (systolic > 160 mmHg, diastolic ≥ 96 mmHg, or taking antihypertensive medications). Covariate entry methods not specified, but were likely stepwise. Covariates for the blood pressure model were age, use of antihypertensive medications, alcohol, smoking, season of year, linear blood lead, tibia lead (the two lead measures apparently tested separately), ATP1A2(5') and ATP1A2(3') polymorphism (each tested separately), and an interaction term between polymorphism and lead. Covariates for the hypertension models were age, BMI, lifetime alcoholic drinks, linear blood lead and tibia lead, and polymorphism, each lead measure and polymorphism tested separately.	Arithmetic mean (SD, range) blood lead: 5.2 µg/dL (3.1, 1-20). Mean (SD) tibia lead: 16.3 µg/g (9.3)	None of the relationships between the ATP1A2(5') polymorphism and either blood or bone lead or blood pressure were significant. The ATP1A2(3') polymorphism was homogenous for the 10.5 kilobase allele (10.5/10.5) in 11 subjects, heterogeneous for the 10.5 and 4.3 kilobase allele (10.5/4.3) in 82 subjects, and heterogeneous (10.5/4.3) in 116 subjects. Prevalence of the 10.5 allele was significantly higher in blacks than in whites. Regression coefficients of 4.3/4.3 and 10.5/4.3 genotypes were not significantly different and all subsequent analyses compared the 10.5/10.5 genotype with the combined 4.3/4.3-10.5/4.3) genotype. The significant interaction between linear blood lead and the 10.5/10.5 genotype showed that for every 1 µg/dL of blood lead systolic blood pressure increased 5.6 mmHg (95% CI: 1.2, 9.9) more than the blood pressure of the combined genotype group. Blood lead range of the combined genotype group was twice that of the 10.5/10.5 group. When data were truncated to make blood lead of both groups cover the same range, coefficients of the genotype-linear blood lead interaction term did not change appreciably. Authors state that tibia lead interacted with genotype on blood pressure but showed no data to estimate either type or size of effect. Diastolic blood pressure was not related to genotype, to lead or to the interaction between lead and genotype. Prevalence of hypertension (30% in total sample) was significantly higher among the 10.5/10.5 group (63.4 %) than among the combined group (28.3 %). Adjusting for age, BMI, and lifetime alcohol, the odds of hypertension in the 10.5/10.5 group were OR 7.7 (95% CI: 1.9, 31.4) compared to the 4.3/4.3 group. The heterogeneous group was not significantly different from the 4.3/4.3 group. Linear blood lead specification not indicated for blood lead-blood pressure modeling. Examination of partial residual plot for systolic blood pressure and linear blood lead shows typical heterogeneity of residuals as a function of predicted values. Thus, presented coefficients may be inefficient and biased. Only 9 subjects were homogenous for 10.5/10.5 in the multiple regression model. Only cortical bone lead was tested, not trabecular bone lead. Cortical bone lead models not shown or quantitatively described. Blood lead rounded to nearest unit µg/dL. Mixed organic-inorganic lead exposure. Relatively small sample size may have prevented detection of other significant effects. No model diagnostics described.

Table AX6-5.1 (cont'd). Cardiovascular Effects of Lead

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Hu et al. (1996) US-Boston-Normative Aging Study-VA 1991-1994	590 males (over 98% white), mean age around 67 years, divided into 146 hypertensives (systolic > 160 mmHg, diastolic >95 mmHg, or daily antihypertensive medication) and 444 non-hypertensives. Linear blood lead, tibia and patella bone lead added separately to logistic regression model containing forced covariates of age, race, BMI, family history of hypertension, pack-years smoking, alcohol ingestion dietary sodium and calcium. Then, a backward elimination procedure starting with all covariates, including all lead variables, resulted in a model in which only significant covariates were retained.	Hypertensives: Arithmetic mean (SD) blood lead:6.9 µg/dL (4.3) Mean tibia lead: 23.7 µg/g (14.0) Mean patella lead: 35.1 µg/g (19.5) Non-hypertensives: Arithmetic mean (SD) blood lead: 6.1 µg/dL (4.0) Mean tibia lead: 20.9 µg/g (11.4) Mean patella lead: 31.1 µg/g (18.3)	Logistic regression model with all forced covariates revealed no significant lead effects when the three lead variables were forced into the model separately. After backward elimination, the only significant covariates left were BMI and family history of hypertension. Of all the lead variables, only tibia lead remained in the model. With each increase of 10 µg/g of tibia lead, odds of being classified hypertensive rose (OR 1.21; 95% CI: 1.04, 1.43). Stepwise regression, backward or forward, involves multiple testing with the same data set, capitalizes on chance occurrence in the data set, and gives over-optimistic probability values. No model diagnostic testing.
Korrick et al. (1999) US-Boston-Nurse Health Study 1993-1995	284 women, from 47-74 years, mean age (SD) 58.7 (7.2), were divided into 97 cases (systolic ≥140 mmHg, diastolic ≥90 mmHg, or physician-diagnosed hypertension) and 195 controls. Controls were further classified as low normal (<121/75 mmHg) and high normal >121/75 mmHg). Three ordinal regression models were constructed, each containing either blood lead, tibia lead or patella lead with forced entry of all other covariates. A final backwards elimination ordinal regression model started with all covariates, including all lead variables, excluding each until only significant variables were left. Interactions were tested in the final model between patella lead and alcohol use, age, and menopausal status.	Mean blood lead (SD, range): 3.1 µg/dL (2.3 , <1-14) Mean tibia lead (SD): 13.3 µg/g (9.0, −5-69) Mean patella lead (SD): 17.3 µg/g (11.1, −5-87)	Only patella lead was significantly related to increased odds of hypertension in the preliminary models, adjusted for age, BMI, alcohol, dietary calcium and sodium, ever smoke, and family hypertension. Each 10 µg/g increase in patella lead was associated with increased odds of hypertension OR 1.28 (95% CI: 1.03, 1.60). In the backward elimination model adjusted for age, BMI dietary sodium and family hypertension, only natural log transformed patella lead remained in the model. One natural log increase in patella lead was associated with increased odds of hypertension OR 1.03 (95% CI: 1.00, 1.05). None of the interaction tests were significant. Small study size may have limited power to detect significant interactions. The proportional odds assumption of the ordinal regression model was verified. Note that the odds ratios above are for movement from one of the two lower categories, low normal and high normal, to the next higher category as patella lead increased. No other model diagnostic tests reported. It was not clear why the original models appeared to use all linear lead terms and the final model appeared to use natural log transformed lead terms, at least for the bone lead.

Table AX6-5.1(cont'd). Cardiovascular Effects of Lead

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Lustberg and Silbergeld (2002) US-NHANES II 1976-1980, follow up to 1992	4190 persons, 30 to 74 years, 929 of whom died during follow up, had baseline blood lead measurements during the NHANES II period. Proportional hazard models for circulatory disease-related death (ICD-9 codes 390-459) were based on the complex survey design, but not weighted. Presented models were unadjusted, adjusted for age and sex, and adjusted for age, sex, location, education, race, income, smoking, BMI, and exercise. Blood lead was entered as an ordinal three-category variable.	Blood lead <10 µg/dL, n = 818 Blood lead 10-19 µg/dL, n = 2735 Blood lead 20-29 µg/dL, n = 637 Blood lead ≥30 µg/dL, n = 102, excluded from analysis	Crude, sex and age adjusted, and multivariate adjusted circulatory disease mortality were all significantly increased in the 20-29 µg/dL group compared to the <10 µg/dL reference group. Risk ratio for the highest lead group for crude circulatory mortality was 1.74 (95% CI: 1.25, 2.40), for age and sex adjusted circulatory mortality was 1.48 (95% CI: 1.10, 2.01), and for multivariate circulatory mortality was 1.39 (95% CI: 1.01, 1.91). Stratified analyses were performed by race, sex, age, smoking, education, etc., but only for all-cause mortality. No model diagnostics reported.

Table AX6-5.1 (cont'd). Cardiovascular Effects of Lead

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Nash et al. (2003) US-NHANES III 1988-1994	1084 premenopausal and 633 postmenopausal women, from 40 to 59 years. Multiple linear regression models with covariates, including linear blood lead, entered as a block for systolic and diastolic blood pressure. Logistic regression models with same covariates and lead quartile added last for hypertension.	Mean (range) blood lead by lead quartile: 1st quartile mean 1.0 µg/dL Range: 0.5, 1.6 2nd quartile mean 2.1 µg/dL Range 1.7, 2.5 3rd quartile mean 3.2 µg/dL Range 2.6, 3.9 4th quartile mean 6.4 µg/dL Range 4.0, 31.1	Linear blood lead was entered last after forcing in age, race/ethnicity, alcohol use, cigarette smoking, BMI, and kidney function (serum creatinine) in multiple regression models for all women and women stratified by menopause status for systolic and diastolic blood pressure. Lead quartile was added to logistic regression models of hypertension (systolic ≥140 mmHg, diastolic ≥90 mmHg or taking antihypertensive medication with the same covariates as the blood pressure models, in all women and stratified by menopausal status. Tested additional models in which women treated for hypertension were excluded from models. All models were adjusted for sample design and weighting. Each increase of 1 µg/dL of blood lead was significantly associated with a 0.32 mmHg (95% CI: 0.01, 0.63) increase of systolic blood pressure and a 0.25 mmHg (95% CI: 0.07, 0.43) increase of diastolic blood pressure in all women without respect to menopausal status. In analyses stratified by menopausal status, only postmenopausal women showed a significant blood lead effect. For each 1 µg/dL increase of blood lead was associated with significantly increased diastolic blood pressure of 0.14 (95% CI: −0.11, 0.39 *sic.*) only in postmenopausal women. Referenced to the first blood lead quartile, no other quartile showed significantly increased odds for hypertension in all subjects or in subjects stratified by menopausal status. With further analyses stratified by systolic and diastolic hypertension without women taking antihypertensive medications, in the combined group of pre and postmenopausal women the odds of diastolic hypertension were significant when the 4th lead quartile was compared to the 1st quartile (OR 3.4 [95% CI: 1.3, 8.7]). In a model of only postmenopausal women untreated for hypertension, odds of diastolic hypertension were significantly increased in the higher three quartiles of blood lead (OR 4.6 [95% CI: 1.1, 19.2]); OR 5.9 [95% CI: 1.5, 23.1]); OR 8.1 [95% CI: 2.6, 24.7]), respectively) and odds of systolic hypertension were significant only for the two middle lead quartiles (OR 3.0 [95% CI: 1.3, 6.9]; OR 2.7 [95% CI: 1.2, 6.2]), respectively. Linear blood lead is suspect in linear regression models of blood pressure as it is usually associated with biased and inefficient estimation of lead coefficients due to probable heteroscedasticity and non-normal distribution of residuals. No model diagnostics were reported. No statistical testing for differences in lead coefficients according to strata. Nine stratified models overall. Not all stated significance levels and standard errors in the blood pressure model table corresponded for certain variables.

Table AX6-5.1 (cont'd). Cardiovascular Effects of Lead

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Nawrot et al. (2002) 31 US and European studies, community and occupationally exposed, published between 1981 and 2001.	48 different groups, 32 of which were only of men, 15 of which were only of women, and one studying both sexes. Total meta-analysis N > 58,490. Age ranged from 15 to 93 years, depending on the study. Two methods of meta-analysis were used, subject-weighted and non-weighted, using study-reported effect sizes and standard errors, transformed from the original study specification of blood lead (linear, logarithmic, or blood lead group) to a single effect size for doubling of blood lead. Also did analyses stratified by race and sex.	Mean blood lead concentration across studies ranged from 2.3 to 63.8 μg/dL. Total range of blood lead across studies was 0 to 97.9 μg/dL.	Each doubling of blood lead was associated with a significant 1.0 mmHg (95% CI: 0.5, 1.4) increase in systolic blood pressure and a significant 0.6 mmHg (95% CI: 0.4, 0.8) increase in diastolic blood pressure. Stated that differences in lead effect were not statistically different between sexes, but did not describe test nor give statistics other than p-values. Presented black and white differences as a trend for blacks to be "more susceptible than whites," but presented no tests. Statistically examined assumptions of homogeneity of effect and found no significant heterogeneity. Tested for publication bias (statistically significant results tend to be published more than non-significant results) and found no evidence. Found no significant effects of removing one study at a time in sensitivity analysis. It appears that the presented results of effect sizes and confidence intervals were calculated by the subject-weighted method, but this was not made explicit. Included some studies that presented no lead coefficients or standard errors, assuming effect size of zero, though the reported effect sizes without these studies did not appear to be different from overall effect sizes. For studies using a linear lead measure, effect sizes were calculated by doubling the arithmetic mean blood lead. If the concentration-response curve for the lead-blood pressure relationship was really better characterized by a log-linear function, the authors' use of studies with a linear blood lead term with high average blood lead led to over-estimation of the slope of the relationship and those studies with low blood lead averages produced an under-estimation of the slope of the relationship.

Table AX6-5.1 (cont'd). Cardiovascular Effects of Lead

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Procter et al. (1996) US-Boston-Normative Aging Study (VA) 1992-1993	798 men from 17 to 44 years. Multiple linear regression models of natural log blood lead on systolic and diastolic blood pressure. All covariates forced into model.	Arithmetic mean (SD, Range) blood lead: 6.5 µg/dL (4.0, 0.5 – 35)	Natural log blood lead, age, age-squared, BMI, adjusted dietary calcium, exercise, indicator variables for current and former smoker, daily alcohol consumption, sitting heart rate, and hematocrit were entered into multiple regression models without regard for significance. Increased diastolic, but not systolic, blood pressure was significantly associated with increased blood lead. Each natural log increase in blood lead was associated with a 1.2 mmHg (95% CI: 0.1, 2.2) increase in diastolic blood pressure. Interactions between dietary calcium and blood lead on blood pressure were not significant. Further analyses stratified on use of antihypertensive medication and those older than or equal to 74 years still revealed significant blood lead-diastolic blood pressure relationships. Blood lead in over half the study group (n = 410) was determined by analyzing previously frozen erythrocytes collected several years prior to the blood pressure measurements used in the study and corrected by using hematocrit values also measured when blood was originally collected. Combining both groups means that nearly half the group was tested for the effects of blood lead on blood pressure measured at the same time, the other half measured several years apart. There was no correction in models for this potential effect. The effect of taking antihypertensive medication could have been assessed in a single model by using an indicator variable. No statistical testing for the effects of stratification on the blood lead-blood pressure relationship. No model diagnostics.

Table AX6-5.1 (cont'd). Cardiovascular Effects of Lead

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Rothenberg et al. (1999) U.S.-Los Angeles 1995-1998	1188 immigrants and 439 immigrants, from 15 to 43 years, all women in third trimester of pregnancy. Multiple regression models of natural log blood lead on systolic and diastolic blood pressure with all covariates forced into models. Covariates selected from larger set based on significant univariate or bivariate tests.	Geometric Mean (SD) blood lead: Immigrants: 2.3 µg/dL (1.4, 1.5) Non-immigrants: 1.9 µg/dL (1.2,1.4)	Natural log blood lead, age, BMI, coffee drinking, iron supplementation, and job stress were entered as a block without regard to significance in linear multiple regression models of systolic and diastolic blood pressure stratified by immigration status. Increased blood lead was significantly associated with increased blood pressure only in immigrants. Each natural log unit increase in blood lead was associated with a 1.7 mmHg (95% CI: 0.7, 2.8) increase in systolic blood pressure and a 1.5 mmHg (95% CI: 0.5, 1.9) increase in diastolic blood pressure in immigrants. Used and reported model diagnostic tests, as evidenced by the use of standard error calculations robust to residual heteroscedasticity. Stated reasons for stratification on immigrant status were significant differences between the two groups in blood lead, blood pressure, age, BMI, and education. Did not statistically test difference in lead coefficients between the immigration strata. Did not correct for potential non-linearity in age effects on blood pressure.
Rothenberg et al. (2002) U.S.-Los Angeles 1995-2001	668 women, 15 to 44 years, studied in third trimester pregnancy and again a mean of 10 weeks postpartum. Exclusion criteria were diabetes, renal or cardiovascular disease, extreme postnatal obesity (BMI > 40), and subjects using stimulant drugs. Multiple linear regression models of natural log blood lead, tibia and calcaneus lead on systolic and diastolic blood pressure with all covariates and all lead variables forced into model. Separate models for third trimester and postpartum, excluding all women with hypertension (see below) during each specific period. Logistic regression for hypertension (systolic ≥140 mmHg or diastolic ≥90), specific to third trimester and postpartum periods with the same covariates and lead variables.	Geometric mean blood lead (SD): 3rd trimester: 1.9 µg/dL (3.6, 1.0) postpartum: 2.3 µg/dL (4.3, 1.2) Tibia mean lead (SD): 8.0 µg/g (11.4) Calcaneus mean lead (SD): 10.7 µg/g (11.9)	Multiple linear regression models for normotensives adjusted for postnatal hypertension (3rd trimester model only), BMI, age, parity, smoking, alcohol, immigrant status, and educational level plus all three lead indices. Only calcaneus lead was associated with blood pressure in 3rd trimester models. Every 10 µg/g increase in calcaneus lead was associated with 0.70 mmHg (95% CI: 0.04, 1.36) increase in systolic blood pressure and a 0.54 mmHg (95% CI: 0.01, 1.08) increase in diastolic blood pressure. In postpartum models, natural log blood lead was the only variable statistically associated with blood pressure. Every natural log unit increase in blood lead was associated with −1.52 mmHg (95% CI: −2.83, −0.20) decrease in systolic blood pressure and a −1.67 mmHg (95% CI: −2.85, −0.50) decrease in diastolic blood pressure. In logistic models, only calcaneus lead was significantly associated with increased odds for hypertension. Each 10 µg/g increase in calcaneus lead was associated with an OR 1.86 (95% CI: 1.04, 3.32) of 3rd trimester hypertension. None of the lead variables was associated with postpartum hypertension. Models did not use age-squared covariate. Models did not use repeated measures statistics. No statistical comparisons between 3rd trimester and postpartum models. Model diagnostic tests reported.

Table AX6-5.1 (cont'd). Cardiovascular Effects of Lead

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Schwartz et al. (2000) US-Eastern 1996-1997	543 mostly former organolead workers, predominantly white (92.8%), at a tetraethyl/tetramethyl plant, mean (SD) [range] age 7.6 (7.6) [41.7-73.7] years had blood lead, DMSA-chelatable lead (4-hr. urinary lead excretion after a single 10 mg/kg dose of DMSA) measured for modeling systolic and diastolic blood pressure and hypertension (systolic >160 mmHg or diastolic ≥96 mmHg or taking antihypertensive medications. Tibia lead ~2 years later was also used as a lead index. For blood pressure, linear multiple regression with backward elimination of non-significant covariates or covariates that "had important influence on the coefficients for the lead-dose terms." Each lead variable was tested in a separate model. Potential covariates for these models were age, BMI, current tobacco use, and current use of antihypertensive medications. Other models were constructed taking out those subjects using antihypertensive medications. Both linear and linear + quadratic blood and tibia lead terms were tested. Logistic regression analyses were used to test the effect of the lead variables on hypertension, controlling for age, diabetes, lifetime alcohol consumption, and BMI. Logistic models also tested each lead measure in interaction with age.	Blood lead arithmetic mean (SD, range) 4.6 µg/dL (2.6, 1-20) DMSA-chelatable lead mean (SD, range) 19.0 µg (16.6, 1.2-136) Tibia lead mean (SD, range) 14.4 (9.3, −1.6-52)	Adjusting for age, BMI, current smoking, and current use of antihypertensive medications, each 1 µg/dL increase in blood lead-squared was significantly associated with 0.189 mmHg (95% CI: 0.087, 0.330) increase in systolic blood pressure with three outliers removed. With the same covariates, each 1 µg/dL increase in linear blood lead was significantly associated with 0.310 mmHg (95% CI: 0.028, 0.592) in diastolic blood pressure taken over a 2-year period (n = 525). No other lead variables were significant. For the hypertension models, only the interaction of linear blood lead by age was significant, with subjects showing significant decrease in odds ratio of hypertension with every joint increase of 1 µg/dL blood lead and 1 year increase in age (linear blood lead × age OR 0.98; [95% CI: 0.97, 0.99]). The interaction suggested a concentration-response relationship between linear blood lead and hypertension only up to ~58 years of age. Authors note that blood pressure findings "were not affected by exclusion or inclusion of subjects using antihypertensive medications," but do not present either the data or the statistical tests to evaluate that conclusion. No other model diagnostics were reported. Although blood lead was also modeled as a quadratic lead term for systolic blood pressure, no analysis was shown for non-linear blood lead terms for diastolic blood pressure. Trabecular bone lead was not tested, though other studies indicate that it is a better lead index than cortical lead for cross-sectional blood pressure and hypertension study. Although the backward procedures described could have resulted in less than the full set of considered covariates entering the models, all model presentations were limited to showing the lead coefficients and all models indicated in a footnote that the lead coefficients were adjusted for each possible covariate for that model. While this is possible with the short list of covariates, given the 14 models presented one might expect to see at least one model where one of the covariates did not remain.

Table AX6-5.1 (cont'd). Cardiovascular Effects of Lead

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Schwartz (1991) NHANES II US 1976-1980	Under 10,000 subjects (exact number not reported), males and females, aged 25 to 74 years for left ventricular hypertrophy results with logistic regression. Linear blood lead used for LVH. For blood pressure results, multiple linear regressions stratified by sex, with one block of variables forced, another block of variables entered with stepwise regression, aged 6 months to 74 years, exact number not given. Natural log blood lead used for linear regression. Both logistic and linear regressions adjusted for survey design.	No blood lead descriptive data given.	Used logistic regression to study lead effect on left ventricular hypertrophy (LVH) determined by a combination of electrocardiogram parameters and body habitus, controlling for age, race, and sex. Every 10 µg/dL blood lead increase was associated with increased odds of LVH of 1.33 (95% CI: 1.20, 1.47). Interaction terms for race by blood lead and sex by blood lead were not significant. Blood pressure models stratified by sex always included BMI, age and age-squared, race, and natural log blood lead. Male blood pressure model also included family history of hypertension, cholesterol, height, cigarette use, serum zinc, and tricep skin fold. Female model also included serum zinc, family history of hypertension, tricep skin fold, and cholesterol. Every 1 natural log unit of blood lead increase was associated with an increase in diastolic blood pressure of 2.93 mmHg (95% CI: 0.93, 4.98) in males and 1.64 mmHg (95% CI: 0.27, 3.01). Used interaction terms for race-blood lead and sex-blood lead in a non-stratified model and found no significant effect of race or sex on the blood lead-blood pressure coefficient. Incomplete reporting of subject size for models and for descriptive statistics for all variables in models. Tested both linear and log transformed lead in preliminary testing. Found log lead had lower probability values than linear lead for blood pressure, and linear lead had lower probability values than log lead for LVH. No testing of significant difference between the two blood lead specifications. No model diagnostics reported. Only reported diastolic blood pressure results.
Schwartz (1995) 15 prior US and European studies published between 1985 and 1993	Total subjects not specified, men and women ages 18 to 74 years. Random effects meta-analysis with inverse variance weighting of lead-blood pressure coefficients from each study. Sensitivity analysis performed by dropping study with largest or smallest effect.	Blood lead levels not stated.	Each doubling of natural log blood lead level was associated with an increase of 1.25 mmHg (95% CI: 0.87, 1.63) systolic blood pressure. Sensitivity analysis showed negligible change in meta-analysis coefficient. Concluded that adding newer studies would not change calculated coefficient. Noted lead-blood pressure slope was larger at lower lead levels than at higher lead levels. The study only analyzed systolic, not diastolic, blood pressure. Superseded by Nawrot et al. (2002).

Table AX6-5.1 (cont'd). Cardiovascular Effects of Lead

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Vupputuri et al. (2003) US-NHANES III 1988-1994	5188 white women, 2300 black women, 5360 white men, and 2104 black men, aged 18 years and older. Survey adjusted multiple linear and logistic regression were used to assess linear blood lead effect on systolic and diastolic blood pressure and hypertension in race and sex stratified models.	Arithmetic mean (SD) blood lead: White women 3.0 µg/dL (7.2) Black women 3.4 µg/dL (4.8) White men 4.4 µg/dL (7.3) Black men 5.4 µg/dL (9.3)	Multiple linear regression models were all adjusted for age, education, BMI, alcohol consumption, leisure time physical activity, dietary sodium and potassium, and total calories. Only black women and men showed significant linear lead effects. Every 1 µg/dL increase in blood lead was associated with an increase of 0.47 mmHg (95% CI: 0.14, 0.80) in systolic and 0.32 mmHg (95% CI: 0.11, 0.54) diastolic blood pressure in black women, and 0.25 mmHg (95% CI: 0.06, 0.44) systolic and 0.19 mmHg (95% CI: 0.02, 0.36) diastolic blood pressure in black men. Odds of hypertension (systolic ≥140 mmHg, diastolic ≥90 mmHg, or taking antihypertensive medication) significantly increased for every SD (3.3 µg/dL) of blood lead level in black women (OR 1.39 [95% CI: 1.21, 1.61]), in white women (OR 1.32 [95% CI: 1.14, 1.52]), in black men (OR 1.26 [95% CI: 0.99, 1.19]), but not in white men. Linear blood lead terms are usually not appropriate in multiple linear regression models of blood pressure. Furthermore, they reported their results in terms of change in 1 SD unit of lead. Linear SD of lead is incorrect for log-normal distributions of blood lead. No model diagnostic tests reported. Discrepancy between Methods report of race-lead and sex-lead interactions in simple, not multiple, analyses, but Results reports significant interactions for race-lead and sex-lead in multiple regression models for both linear regression and logistic regression models, without showing the results of the interaction analyses. The probability of the stated interactions ($p < 0.001$) appears extremely low, given the degree of 95% CI overlap in lead coefficients among the stratified models. No model diagnostics reported.

Table AX6-5.1 (cont'd). Cardiovascular Effects of Lead

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Michaels et al. (1991) US-New York City 1961-1984	1261 males, average age (range) at the beginning of study 49.6 years (19-83), representing 24,473 person-years were followed. 498 died in the interval. Subjects belonged to the International Typographical Union and worked at two large city newspapers. Hot lead linotyping was discontinued at the newspapers during 1974-1978, providing the primary source of occupational exposure. Last exposure for all subjects still employed was at the end of 1976. Standardized mortality ratios (SMR) were calculated using the LTAS program developed by NIOSH, calculating the expected number of deaths of the cohort referenced to a comparison population, in this case disease-specific mortality rates from New York City. Cohort was stratified based on years of employment. Causes of death were based on ICD-8 codes.	Exposure was estimated based on years of linotype employment before the end of 1976. Authors note that, based on measurements at other print shops using hot lead linotype, air lead levels probably did not exceed 20 $\mu g/m^3$.	Standardized mortality ratio was significant (SMR = 1.68 [95% CI: 1.18, 2.31]) only for cerebrovascular disease in those working, and thus exposed, for 30 years or more. Neither arteriosclerotic heart disease (ICD-8 410-414) nor vascular lesions of the central nervous system (ICD-8 430-438) had significant SMR in the total cohort not stratified by years of exposure. No direct measurement of lead exposure. Many groupings of ICD codes were explored in stratified and unstratified analyses, with the only significantly elevated SMR found for cerebrovascular disease. No *a priori* hypotheses. General weakness of all studies relying on a comparison population is that the cohort belongs to the comparison population and can influence the comparison mortality rates in direct proportion to the ratio between cohort and comparison population size.
Morris et al. (1990) US-sampled from general population around Portland, OR responding to ads to participate in clinical trials of non-pharmacological management of blood pressure. 1984-1989?	145 males and 106 females, 73% with arterial pressures > 105 mmHg, provided blood pressure measurements once a week over four consecutive weeks. Blood for lead analysis was collected during this period. Stepwise multiple regression was used to construct separate models of systolic and diastolic blood pressure stratified by sex. Covariates available to be entered were age, BMI, dietary calcium and "other nutrient intakes," ionized serum calcium, erythrocyte protoporphyrin and natural log transformed blood lead	Arithmetic mean (SD) blood lead: Males: 8.0 µg/dL (4.4) Females: 6.9 µg/dL (3.6)	Natural log blood lead was only a significant predictor of blood pressure in males. Adjusting for age and ionized serum calcium, every one natural unit increase in blood lead was significantly associated with a 4.58 mmHg (neither SE nor CI stated) in systolic blood pressure and, adjusting for hemoglobin, age, and current smoking, a 1.90 mmHg (neither SE nor CI stated) in diastolic blood pressure. The usual precautions regarding multiple testing and different covariate patterns in stratified models constructed with stepwise regression apply. Reporting of effects not complete. Small sample size limits conclusions about non-significant effects. High prevalence of hypertensives in sample due to study recruitment design. Blood lead technique, as represented by presented graph, had a detection limit of 5 µg/dL. No model diagnostics.

Table AX6-5.1 (cont'd). Cardiovascular Effects of Lead

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Navas-Acien (2004) US-NHANES IV-Phase 1 1999-2000	2125 subjects (1070 males, 1055 females), age 40-> 70 years were tested for peripheral arterial disease (PAD; n = 139) by taking the ratio of the ankle mean systolic blood pressure to the arm mean systolic blood pressure. Any subject with the ratio <0.90 was classified as PAD. Logistic regression analysis was weighted and adjusted by sample design. Covariates forced into the models were age, sex, race, education, and lead quartile (Model 1); Model 1 covariates plus BMI, alcohol intake, hypertension, diabetes, hypercholesterolemia, glomerular filtration rate, and C-reactive protein (Model 2); Model 2 covariates plus self-reported smoking status and serum cotinine (Model 3); and Model 3 covariates plus cadmium quartile (Model 4). Tested interactions between lead and cadmium on PAD, and between lead and sex, race, smoking status, renal function, and c-reactive protein on PAD. Tested for trend of OR as a function of lead quartile.	Geometric mean blood lead (25%-75% percentile): 2.1 µg/dL (1.4, 2.9) Lead quartile 1: <1.4 µg/dL Lead quartile 2: 1.4-2.1 µg/dL Lead quartile 3: 2.1-2.9 µg/dL Lead quartile 4: >2.9 µg/dL	Odds for PAD significantly increased with lead quartile (1st quartile used as comparison) for all four models. Only models 1 and 2, however, showed a significant increase in odds of PAD for the 4th lead quartile compared to the 1st lead quartile, OR 3.78 (95% CI: 1.08, 13.19) and OR 4.07 (95% CI: 1.21, 13.73). None of the tested interactions with blood lead quartile were significant. Well-designed study with sound statistical analysis. Including two variables for smoking in Models 3 and 4 (smoking status and cotinine) may have over-controlled for smoking). There was a trend toward increased blood lead level with increased smoking status and with increased cotinine levels, though no statistical tests of trend were reported. Thus the two smoking variables and lead may have been confounded with PAD. No model diagnostic tests reported.
Sokas et al. (1997) US-Maryland 1989-1990	264 active or retired construction workers, over 99% men, who were not involved in lead work at time of testing, mean age (range) 43 years (18-79). Multiple regression modeling of systolic and diastolic blood pressure adjusted for covariates of BMI, age, hematocrit, erythrocyte protoporphyrin, race, linear blood lead and a race-linear blood lead interaction. Method of covariate entry not made explicit, though it appeared to be forced.	Mean blood lead (range): 8.0 µg/dL (1-30).	Linear blood lead was not significantly related to either systolic or diastolic blood pressure, though the race by linear blood lead interaction was marginally significant (p = 0.09). Each 1 µg/dL increase in blood lead increased black systolic blood pressure 0.86 mmHg (no SE or 95% CI reported) more than white systolic blood pressure. Linear blood lead term may not be appropriate. Small sample compromises interpretation of non-significant results. By using erythrocyte protoporphyrin and blood lead in the same model, these two measures of lead exposure may have been confounded. Incomplete reporting of procedures and results. No model diagnostic tests reported.

Table AX6-5.1 (cont'd). Cardiovascular Effects of Lead

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Sorel et al. (1991) US-NHANES II 1976-1980	2056 females, 2044 males, 473 blacks and 3627 whites, from 18-74 years, were used in survey design and weight adjusted multiple linear regressions stratified by sex, with separate models for systolic and diastolic blood pressure. Covariates included age, BMI, race, and poverty income ratio and linear blood lead. Method of covariate entry not specified but may have been forced. Different covariate groups were used for different models. Primary test for the effect of race on the lead-blood pressure relationship was to note the change in the race coefficient in models with and without the linear blood lead variable.	Age-adjusted arithmetic mean blood lead: Black female: 13.2 µg/dL (no variance information for any blood lead) White female: 12.1 µg/dL Black male: 20.1 µg/dL White male: 16.8 µg/dL	Linear blood lead was significantly related only to diastolic blood pressure in males, adjusting for age and BMI. For every 1 µg/dL blood lead increase diastolic blood pressure increased 0.13 mmHg (95% CI: 0.04, 0.21). Adding race to the model with and without linear blood lead terms did not appear to change the race coefficient. Adding poverty index to the models with and without blood lead produced the same small change in poverty index coefficient. Linear blood lead may not be appropriate. Only confidence intervals were used to assess the significance of changes in race and poverty index coefficients across models with and without lead, instead of using interaction terms of these two variables with lead. Incomplete reporting of procedures and results. No model diagnostic tests reported.
Sharp et al. (1990) US-San Francisco, CA 1986	After exclusion of subjects under treatment for hypertension, 249 male bus drivers, 132 of whom were black, age from 31 to 65 years, were used in race stratified multiple regression models of systolic and diastolic blood pressure with covariate forced entry of age, age-squared, BMI, caffeine use, tobacco use, and natural log blood lead. Alcohol use was added in other models. Other models stratified by caffeine use.	Geometric mean (range) blood lead: Black males: 6.5 µg/dL (3-21) Non-black males: 6.2 µg/dL (2-15)	Significant log blood lead effects were noted in blacks. In models excluding alcohol use, for every one natural log unit increase of blood lead, systolic blood pressure rose 7.53 mmHg (95% CI: 0.86, 14.2) and diastolic blood pressure rose 4.72 mmHg (95% CI: 0.15, 9.29). Stratified by infrequent/frequent caffeine users, only black infrequent caffeine users showed a significant response to blood lead. For every one natural log unit increase of blood lead, systolic blood pressure rose 16.69 mmHg (95% CI: 3.83, 29.5) and diastolic blood pressure rose 10.43 mmHg (95% CI: 1.26, 19.6). Non-black blood pressure was decreased with increasing natural log lead but was marginally significant. In all non-black subjects, for every unit increase in natural log blood lead, systolic blood pressure decreased −5.71 mmHg (95% CI: −12.0, 0.6). Addition of alcohol to the models decreased all coefficients a small amount. Progressive addition of age, BMI, caffeine, and tobacco, in that order, progressively increased the coefficient of natural log blood lead in models of systolic and diastolic blood pressure in blacks. Removal of two black outliers did not materially change the results for blacks. No statistical tests for comparing stratified models, models with and without caffeine use, effect of progressive addition of covariates, or addition of alcohol. Influence diagnostics reported for detecting the two outlying subjects. No other diagnostic tests reported. Small differences in text and table reports of the same coefficients. Small sample size limits interpretation of non-significant results.

Table AX6-5.1 (cont'd). Cardiovascular Effects of Lead

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe			
Menditto et al. (1994) Europe-Rome-New Risk Factors Survey 1989-1990	1319 males, mean (range) age 63 (55-75) years, not treated for hypertension, were used in forward stepwise multiple linear regression models of systolic and diastolic blood pressure with available covariates of age, BMI, heart rate, serum high density lipoprotein, non-high density lipoprotein, triglycerides, glucose, cigarette use, alcohol use, sum of five skinfold thicknesses (triceps, biceps, subscapular, suprascapular, and suprailiac), and natural log transformed blood lead.	Median (2.5^{th}-97.5^{th} percentiles, range) blood lead 11.3 µg/dL (6.2-24.7, 4-44.2)	Only BMI, heart rate, and serum glucose were not simultaneously and significantly correlated with both natural log blood lead and blood pressure. In a systolic blood pressure model adjusted for BMI, age, heart rate, high and non-high density lipoprotein, triglycerides, glucose, and cigarettes, each unit increase in natural log blood lead was significantly associated with a 5.6 mmHg (95% CI: neither SE nor CI stated) increase in blood pressure. In a diastolic blood pressure model adjusted for BMI, heart rate, age, cigarettes, triglycerides, and high density lipoprotein, each unit increase in natural log blood lead was significantly associated with a 1.7 mmHg (95% CI: neither SE nor CI stated) increase in blood pressure. In stratified models for alcohol drinkers (n = 1068) and non-drinkers (n = 251) only alcohol drinkers showed significant natural log blood lead associated blood pressure increase, with lead coefficients similar to those of the entire group. Authors observed change in natural log blood lead coefficient produced by successive addition of covariates to models. In no case did the coefficients change by more than 30% after addition of a covariate. Authors noted that wine was the predominant drink in alcohol users and that the correlation between alcohol consumption and natural log blood lead level was the highest among all correlations reported ($p < 0.001$; correlation coefficient not stated). No statistical tests were made to determine if the change in lead coefficients with addition of covariates was significant, nor were statistical tests made to determine if the lead coefficients in the alcohol use stratified models were significantly different. Small size of the non-alcohol drinking group in stratified analysis precludes interpretation of non-significant effects. Incomplete reporting of results. Paper published in a supplement issue reporting meeting papers may indicate that it received less that the normal peer-review scrutiny for published research articles. No model diagnostic tests were reported.

Table AX6-5.1 (cont'd). Cardiovascular Effects of Lead

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Møller et al. (1992) Europe-Denmark-Copenhagen County-Glostrup Population Studies 1976-1990	A cohort born in 1936 was followed at age 40 (women n = 546, men n = 504), age 45 (women n = 430, men n = 463) and again at age 51 (men only n = 439). Reported no difference in results if subjects taking antihypertensive medications were excluded. Reported results included these subjects. Linear multiple regression models of systolic and diastolic blood pressure of follow up, stratified by sex and by year, used a sequence of forced entry of covariates: natural log blood lead was tested alone (unadjusted), then adjusted for tobacco, cholesterol, physical activity, and sex (Model 1), then adjusted for the above covariates plus systolic blood pressure (Model 2), and then adjusted for the above covariates plus alcohol (Model 3). Another group of linear multiple regression models of change of systolic and diastolic blood pressure from age 40 to 51 years in men only, following the same covariate entry scheme as above, but used change in covariates instead of the original covariates. All subjects were followed until 54 years of age (from 1976 to 1990) to assess lead association with total mortality and with coronary heart disease (CHD; ICD-8 410-414) and cardiovascular disease (CVD; ICD-8 430-435) combined morbidity and mortality using Cox proportional hazards models (n = 1050). Cox models were adjusted as above.	Arithmetic mean (SD, range) blood lead by age and sex: Women 40 years: 9.6 µg/dL (3.8) [4-39] Women 45 years: 6.8 µg/dL (3.5) [2-41] Men 40 years: 13.6 µg/dL (5.7) [5-60] Men 45 years: 9.6 µg/dL (4.3) [3-39] Men 51 years: 8.3 µg/dL (4.1) [2-62]	In women, each one unit increase in natural log blood lead was associated with a significant increase in systolic blood pressure of 4.93 mmHg (p = 0.002; neither SE nor CI stated) at age 40 and an increase of 2.64 mmHg (p = 0.06; neither SE nor CI stated) at age 45, in models adjusted for tobacco, BMI, and physical activity (Model 1). When alcohol (Model 2) or alcohol plus hemoglobin (Model 3) were added to the models lead-blood pressure relationships were not significant at either age. With each one unit change in natural log blood lead, diastolic pressure increased 4.26 mmHg (p = 0.002; neither SE nor CI stated) at 40 years and 3.26 mmHg (p = 0.002; neither SE nor CI stated) at 45 years in Model 1. In Model 2, the increase in diastolic blood pressure was 3.21 mmHg (p = 0.02; neither SE nor CI stated) at 40 years and 2.86 mmHg (p = 0.01; neither SE nor CI stated) at 45 years. In Model 3, the increase in diastolic blood pressure was 2.65 mmHg (p = 0.07; neither SE nor CI stated) at 40 years and 2.78 mmHg (p = 0.01; neither SE nor CI stated) at 45 years. In men, the only significant association between natural log blood lead and blood pressure was at 45 years. For every increase of one unit of natural log blood lead the increase in systolic blood pressure was 2.73 mmHg (p = 0.05; neither SE nor CI stated). The change in blood lead between 40 and 51 years was not significantly associated with change in systolic or diastolic blood over the same period in any of the models. None of the relative hazard ratios for CHD and DVD combined morbidity and mortality between 40 and 54 years were significantly related to blood lead concentration. Total mortality, however, was significantly increased with increased blood lead. In Model 1, every increase of one natural log unit of blood lead was associated with an increased relative hazard of mortality of 1.96 (p = 0.009; neither SE nor CI stated). For Model 2, every increase of one natural log unit of blood lead was associated with an increased relative hazard of mortality of 1.82 (p = 0.03; neither SE nor CI stated). There were 40 cases of CHD recorded, of which 13 were fatal. There were 54 cases of CVD recorded, of which 19 were fatal. Of the total of 46 subjects who died during the period, 32 (70%) died of cardiovascular problems. It was not clear if blood lead at a particular age or a mean blood lead across ages was used in the Cox proportional hazards models.

Table AX6-5.1 (cont'd). Cardiovascular Effects of Lead

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Møller et al. (1992) (cont'd)			Though this study was one of the few to use a longitudinal design, it did not take advantage of that design feature in blood pressure modeling. Cross-sectional multiple regression modeling at each age loses valuable information available in repeated measures modeling. Power to detect significant effects is much higher in repeated measurement modeling than in cross-sectional modeling. Analyzing only change in blood pressure loses information regarding starting and ending blood pressure. Including change in blood lead is problematical due to the unknown history of lead exposure prior to the start of the study, the resultant bone lead load as a result of past exposure, the unknown lead contribution of bone to blood, and the unknown relative contributions of past exposure and present exposure to alteration in blood pressure. Modeling other covariates as change is also questionable. BMI, to pick a covariate with known and strong effects on blood pressure, may be high and relatively constant over the study period or low and relatively constant over the study. In both cases, the change in BMI will be small, but the high BMI will be associated with higher blood pressure than will the low BMI. Thus, both cases modeled as change in BMI should have the same effect on blood pressure when the high BMI subject has expected higher blood pressure than the low BMI subject. Using difference scores for the dependent and the exposure variables also risks confounding secular trends in either or both of these variables, for whatever reasons, with independent difference variable effect on dependent difference variable effect. The Cox proportional hazards model, however, is longitudinal in nature. Failure to detect significant associations between lead and cardiovascular morbidity/mortality could have been due to the small sample size used for this type of analysis. The blood pressure part of the study did not take mortality into account during the study, which could have produced a progressively increasing "healthy subject" effect. Since subjects taking antihypertensive medications were included in analyses, an indicator variable should have been used to account for them, whether or not their exclusion in preliminary testing produced no apparent change in results. This paper contained a good discussion of confounding variables. Incomplete reporting of results and procedures. No model diagnostic tests were reported.

Table AX6-5.1 (cont'd). Cardiovascular Effects of Lead

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Staessen et al. (1996) Europe-Belgium-PheeCad study. 1985-1995	359 men and 369 women participated at baseline (between 1985 and 1989) and again about 5 years later (median 5.2 years) at follow up (between 1991 and 1995), mean age (range) at baseline 46 years (20-82), about half of whom were recruited from towns surrounding a non-ferrous smelter (targeted to produce high cadmium exposure) and half from towns without heavy metal production. Over half the men had occupational exposure (59.0% from the near smelter towns, 17.4% from the other towns). Four different outcomes were explored: time-integrated conventional blood pressure (average of 10 baseline and 5 follow up blood pressure measurements), 24-hour ambulatory blood pressure only during the follow up period (average of readings every 20 minutes from 8 AM to 10 PM and every 45 minutes from 10 PM to 8 AM, weighted by interval between measurements), difference in conventional blood pressure over the five year follow up period, and incidence of developing hypertension during follow up.	Geometric mean (5%-95% percentile) by sex and time period: Baseline women: 6.6 µg/dL (3.3-14.5) Follow up women: 4.8 µg/dL (1.7-11.8) Baseline men: 11.4 µg/dL (5.6-28.8) Follow up men: 7.7 µg/dL (3.7-20.1)	The study was one of the few prospective longitudinal studies reported and was innovative in its use of 24-hour ambulatory blood pressure as one of its outcome variables. Time-integrated conventional blood pressure models: In 187 peri- and post-menopausal women, after adjusting for age, BMI, gamma-glutamyltransferase activity, and hematocrit, each increase of one unit of natural log blood lead was associated with an increase in diastolic blood pressure of 7.49 mmHg (95% CI: 1.48, 13.50). No other time-integrated conventional blood pressure measurements were significantly associated with time-integrated natural log blood lead in either men or women, nor in stratified groups within sex. Ambulatory 24-hour blood pressure models: In all 345 women, after adjusting for age, hematocrit, gamma-glutamyltransferase activity, and oral contraceptive use, each one unit increase in natural log blood lead was associated with an increase of diastolic blood pressure of 3.49 mmHg (95% CI: 0.02, 6.96). When the group was limited to the 174 premenopausal women each unit increase in natural log blood lead was associated with an increase of diastolic blood pressure of 5.48 mmHg (95% CI: 0.56, 10.40). Difference in blood pressure between baseline and follow up: After adjustment for change in BMI, beginning use of antihypertensive medication and contraceptive medication during the follow up period, and starting smoking there was no significant relationship between difference in either systolic or diastolic blood pressure and blood lead in women. After adjustment for change in BMI, change in exposure at work, change in smoking, beginning use of antihypertensive medication in men there was no significant relationship between difference in either systolic or diastolic blood pressure and blood lead in men. Incidence of hypertension: At baseline 107 (14.7%) and 120 (16.5%) subjects had borderline and definite hypertension, respectively. At follow up 98 (13.5%) and 186 (25.5%) had borderline and definite hypertension, respectively. 51 of 501 initially normotensive subjects became borderline hypertensive and 47 of the 501 became border line hypertensive during the follow up period. After adjusting for sex, age, and BMI, natural log baseline blood lead was not related to significant risk ratios of becoming hypertensive (not stated, but presumably combined definite and borderline hypertension) or becoming a definite hypertensive.

Table AX6-5.1 (cont'd). Cardiovascular Effects of Lead

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Staessen et al. (1996) (cont'd)	Multiple regression models were used to test the association between natural log transformed blood lead (mean of baseline and follow up lead) and blood pressure (systolic and diastolic), stratified by sex, then further stratified by use of antihypertensive medications in men and menopausal status in women. Age and age-squared (calculated in quintiles) were forced into the models, then remaining covariates were stepwise added to the model. Though not explicitly stated, natural log blood lead (mean of baseline and follow up) was likely forced in last. Other candidate covariates were BMI, hemoglobin or hematocrit, serum gamma-glutamyltransferase activity (an index of alcohol use) and serum calcium, 24 hour urinary sodium and potassium excretion, energy expenditure, exposure to heavy metals (at the workplace), social class, smoking and drinking habits, menstrual status in women, and use of antihypertensive medications, oral contraceptives, and hormone replacement therapy. In ambulatory blood pressure models, differences between baseline and follow up blood pressure models were constructed in the same way. For the difference models "concurrent variations in blood lead concentrations" were used, presumably difference in baseline and blood lead. For the hypertension incidence model two definitions of hypertension were used: definite hypertension (systolic >160 mmHg, diastolic >95 mmHg or taking antihypertensive medications) and borderline hypertension (systolic between 141 to 159 mmHg and diastolic between 91 to 94 mmHg). Method of covariate entry into hypertension incidence models not stated. Baseline natural log blood lead was used as the exposure index.		The study does not use the full power of repeated measurements in the analyses. For problems encountered when collapsing repeated measurements to difference measures, see Møller (1992) above. Stepwise regressions are prone to capitalizing on chance results due to multiple testing of the same data and almost always produce a different mix of covariates when they are stratified. Thus, it was puzzling to find that where information on the effects of stepwise covariate addition to models was available in this article, that the same covariates were listed for both models based on the stratification variable. There is excessive reliance on fractionation of the data set due to multiple stratification, sometimes reducing the number of subjects in a model to as few as 171. Even the models using the most subjects had only 359 subjects. Low power to detect significant effects cautions against any interpretation of non-significant results. The time-integrated model used 10 baseline blood pressure measurements and 5 follow up blood pressure measurements, thus weighting the average toward baseline blood pressure. The entry of the biochemical correlate of alcohol use in most of the models suggests that lead effects and lead-containing alcohol effects on blood pressure were confused, especially given the European setting and the time period during which the study was conducted. Control for use of hypertensive medication rarely entered models and partial control for this variable was achieved only by stratified analyses, further reducing power to detect significant effects in the remaining subgroup. No justification was given for stratified analyses. Incomplete information in statistical methods and results complicates interpretation. It was uncertain if stepwise regression was used for logistic models. No comparisons were performed to assess possible bias due to subject attrition over the course of the study. The over six decades of age represented in the sample was modeled by linear and quadratic terms based on age quintiles rather than continuous age, making it likely that adequate control for age effects on blood pressure was not achieved and that the "healthy subject" effect seen in older groups was not controlled. If stepwise addition of significant covariates was used in the blood pressure difference models, were covariates in those models that were marked in the coefficient column as non-significant not included in the models, and if that were so, it is unclear from where the probability values that substitute for the coefficients of those variables were derived. There were no model diagnostic tests reported.

Table AX6-5.1 (cont'd). Cardiovascular Effects of Lead

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Staessen et al. (1993) Belgium-Cadmibel Study 1985-1989	827 males and 821 females recruited from two areas in Belgium, one of them surrounding a non-ferrous smelter, mean age (SD) 46 (15) and 44 (15) years, in men and women respectively. Subjects taking antihypertensive medication were excluded from the analyses. Stepwise multiple regression models of systolic and diastolic blood pressure were stratified by sex. Covariates available for entry were age and age-squared, BMI, pulse rate, log protoporphyrin, log gamma-glutamyltranspeptidase, serum calcium, log serum ferritin, log serum creatinine, log serum zinc, urinary calcium, urinary sodium, and urinary potassium. Natural log blood lead was the only variable forced into the models. Additional models tested the interaction of serum calcium and blood lead on blood pressure.	Geometric mean blood lead (range), stratified by sex: Male blood lead 10.4 µg/dL (2.7, 84.9) Female blood lead 6.2 µg/dL (1.3, 42.4)	In men, adjusting for age and age-squared, BMI, pulse rate, log gamma-glutamyltranspeptidase, serum calcium, and log serum creatinine, every unit natural log blood lead increase was significantly associated with a −5.2 mmHg (95% CI: −0.5, −9.9) decrease in systolic blood pressure. Natural log blood lead was not significant in the model for diastolic blood pressure for men nor the systolic or diastolic blood pressure for women. Adjusting for age and age-squared, BMI, pulse rate, and log gamma-glutamyltranspeptidase, the interaction term between natural log blood lead and serum calcium was only significant for systolic blood pressure in women. Every doubling of blood lead was associated with a 1.0 mmHg decrease in systolic blood pressure at serum calcium concentration of 2.31 mmol/L (25^{th} percentile) and an increase in systolic blood pressure of 1.5 mmHg at serum calcium concentration of 2.42 mmol/L (75^{th} percentile). Stepwise multiple regression analyses run risks of accepting chance associations due to multiple analyses of the same data set. The role of alcohol use or alcohol use markers in confounding lead effect on blood pressure in this setting has already been noted. The unexplained interaction between serum calcium and blood lead highlights the potential confounding role of serum calcium with lead in blood pressure studies. The study shows graphs indicating distinct differences in the age-serum calcium and age-blood lead relationships for men and women. From 50-70 years of age serum calcium is higher than from ≤ 29-49 years in women and exceeds serum calcium of men at those older ages. The steepest rise in women's blood lead with age occurs between the 40-49 and 50-59 year decades. The timing of these changes in women suggests that menopause may be a factor, which was accounted for only in the model for diastolic blood pressure. It also suggests that serum calcium level and age were also confounded in the blood pressure models. As serum creatinine clearance and blood lead are inversely related, and serum creatinine is a significant covariate in the systolic blood pressure model for men with a significant negative blood lead coefficient, it is possible that serum creatinine and blood lead are confounded with blood pressure in the men's systolic blood pressure model. There were no assessments of subject selection bias due to exclusions. The authors note examining quintile blood pressure relationships with all covariates to determine the acceptability of the linear relationship implied by the linear modeling technique. No other model diagnostic tests were reported.

Table AX6-5.1 (cont'd). Cardiovascular Effects of Lead

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Bost et al. (1999) Europe-England-Health Survey for England 1995	2763 women and 2563 men from a multi-stage stratified probability survey representative of the English population living in private residences, mean (SE) age for men 47.5 years (0.34) and for women 47.7 years (0.33) (all subjects 16 years and older) were used in an analysis of blood lead association with systolic and diastolic blood pressure. Stepwise multiple regression analysis were used testing natural log blood lead against common log systolic blood pressure and non-transformed diastolic blood pressure, with the following potential covariates: age, BMI, smoking status, region of residence, social class, and alcohol consumption. Models were stratified by sex, with and without adjustment for alcohol, including or excluding those taking antihypertensive medications.	Geometric mean blood lead: Men: 3.7 µg/dL (no stated measure of variance) Women: 2.6 µg/dL (no stated measure of variance	Model tables presented only standardized variable coefficients. The most consistent results were reported on common log lead association with men's diastolic blood pressure. Every doubling of blood lead was significantly associated with an increase of 0.78 mmHg (95% CI: 0.01, 1.55) diastolic blood pressure, adjusted for age, log BMI, and alcohol, but excluding men on antihypertensive medication. Every doubling of blood lead was significantly associated with an increase of 0.88 mmHg (95% CI: 0.13, 1.63) in the same model with men on antihypertensive medication. Every doubling of blood lead was significantly associated with an increase of 0.96 mmHg (95% CI: 0.23, 1.70) in the same model excluding men on antihypertensive medication and not adjusting for alcohol. Every doubling of blood lead was significantly associated with an increase of 1.07 mmHg (95% CI: 0.37, 1.78) including men taking antihypertensive medication and not accounting for alcohol. None of the multiple regression models had significant lead terms for women. This report was not sufficiently detailed. Stepwise regression modeling is prone to the usual pitfalls. Survey design adjusted analysis not used. Lead was not entered in models in which criterion probability was exceeded ($p > 0.05$). No rationale given for stratifying. No testing of differences among lead coefficients for the different models was made, which would have been especially valuable to compare models adjusted and not adjusted for alcohol use. No explanation for using log systolic blood pressure as dependent variable. No model diagnostics reported.
Fewtrell et al. (2004) Global 1988-2002	Using available global figures on categorized blood lead ranges by age group, authors calculated relative risk ratios relating increased blood pressure to ischemic heart disease, cerebrovascular disease, hypertensive disease, and other cardiac diseases. They used a calculation of "impact fraction," based on the proportion of the population within the particular lead exposure category and the relative risk at that exposure category compared to the risk at the reference level. They used the meta-analysis of Schwartz (1995) to derive an accumulating 1.25 mmHg increase in blood pressure in men for 5-10, 10-15, and 15-20 µg/dL, and an increase of 3.75 mmHg for blood lead levels above 20 µg/dL. Comparable blood pressure increases in women for each lead category was 0.8 mmHg for each of the first three categories and 2.4 mmHg for blood lead >20 µg/dl.	See left for blood lead categories used.	The largest risk ratios were for hypertensive disease populations at ages 15-44, calculated at 1.12, 1.41, 1.78, and 2.00 for each of the four lead categories for men, and 1.08, 1.25, 1.45, and 1.56 for women. Risk ratios for all disease categories increased with increasing lead category and decreased for populations older than 44 years. The authors assumed a linear relationship between blood pressure and blood lead, whereas available evidence suggests it may be non-linear. If blood lead-blood pressure concentration-response function is log-linear, as implicitly accepted by over half the reviewed studies, the calculated global risk ratios for all cardiovascular disease will be overestimated at higher blood lead levels and underestimated at lower blood lead levels.

Table AX6-5.1 (cont'd). Cardiovascular Effects of Lead

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Gerhardsson et al. (1995) Europe-southern Sweden 1969-1989	664 male workers at a secondary lead smelter had blood lead tested every 2-3 months since 1969. The past blood lead level of 201 workers who had been working at the plant from before 1969 was estimated from their 1969 results. Median (10^{th} percentile, 90^{th} percentile) year of birth was 1943 (1918, 1960). Median (10^{th} percentile, 90^{th} percentile) duration of employment was 2.8 years (0.3, 25.7) and median (10^{th} percentile, 90^{th} percentile) duration of follow up was 13.8 years (2.8, 20.9). A total of 8706 person-years were represented in the study. Standardized mortality ratios based on county mortality tables by calendar year, cause, sex and five-year age group were calculated. Cardiovascular diseases were coded by ICD-8 from death certificates.	Arithmetic mean blood lead levels dropped from approximately 62 µg/dL in 1969 to approximately 33 µg/dL in 1985. 95% confidence intervals were difficult to extract from the presented graph, but appeared to be no more than 5-6 µg/dL about the mean.	All cardiovascular disease mortality (ICD-8 390-458) was significantly elevated above that expected from the county mortality tables (SMR = 1.46 [95% CI: 1.05, 2.02]), with 39 of the 85 deaths observed in the cohort. For just ischemic heart disease (ICD-8 410-414), SMR = 1.72 (95% CI: 1.20, 2.42) in the plant workers with 34 of the 85 deaths observed in the cohort. There were no deaths recorded for cerebrovascular diseases (ICD-9 430-438). There was no apparent concentration-response relationship, using peak blood lead and time-integrated blood lead. Problems inherent in using standardized mortality ratios in such mortality studies have been discussed above. The sample size was too small (85 all cause deaths among 664 workers) to interpret non-significant results.

Table AX6-5.1 (cont'd). Cardiovascular Effects of Lead

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Hense et al. (1994) Denmark-Augsburg-MONICA study 1987-1988	1703 men and 1661 women, age 28-67 years, used multiple regression models stratified by sex, and in men, stratified by rural/urban residence to examine the effect of recent alcohol consumption (over last weekend) on the relationship of linear blood lead on systolic and diastolic blood pressure. Alcohol consumption was stratified into 0 g/day, <40 g/day, and ≥40 g/day groups. Women's results were adjusted by age, hematocrit, BMI, place of residence, and smoking. Covariates of men's blood pressure not stated.	Total range of blood lead for women (mean and variance not given): 3-14 µg/dL Total range of blood lead for men (mean and variance not given): 5-14.5 µg/dL Women's blood lead stratified by alcohol: 0 g/day: 3-8 µg/dL <40 g/day: 4-10 µg/dL ≥40 g/day: 5-14 µg/dL Rural men's blood lead stratified by alcohol: 0 g/day: 5-11 µg/dL <40 g/day: 6-12 µg/dL ≥40 g/day: 7-14.5 µg/dL Urban men's blood lead range not shown. Blood lead ranges read from graphs and are only approximate.	The effect of linear blood lead on systolic and diastolic blood pressure varied directly by alcohol consumption. In women, every 1 µg/dL increase in blood lead was significantly associated with an increase of 1.30 mmHg (95% CI: 0.45, 2.15) systolic blood pressure only for women drinking ≥40 g/day of alcohol (n = 83). Every 1 µg/dL increase in blood lead was significantly associated with an increase of 0.27 mmHg (95% CI: 0.02, 0.52) and 0.86 mmHg (95% CI: 0.33, 1.39) diastolic blood pressure in those drinking <40 g/day (n = 877) and ≥40 g/day, respectively. In urban men, there were no significant effects of linear blood lead on blood pressure. In rural men drinkers consuming <40 g/day (n = 463) and ≥40 g/day (n = 356) each 1 µg/dL increase in blood lead was significantly associated with an increase of systolic blood pressure of 0.65 mmHg (95% CI: 0.21, 1.09) and 0.45 mmHg (95% CI: 0.05, 0.99), respectively. In these same two groups of men, each 1 µg/dL increase in blood lead was associated with an increase of 0.39 mmHg (95% CI: 0.09, 0.69) and 0.30 mmHg (95% CI: 0.03, 0.57), respectively. The report lacked important details. Method of covariate entry not mentioned. Men's covariates not mentioned. Complete blood lead description not given. No comparison of selected group with non-selected group (10.4% of total sample not used due to missing data). Uncontrolled confounding between range of blood lead and alcohol consumption was especially notable in women (blood lead range and mid-points increased with increasing alcohol consumption. Linear blood lead may not be the appropriate metric to use for blood pressure studies. Age-square covariate should also have been used, given the range of ages. No rationale given for stratification. Authors could not explain why rural men and not urban men showed the direct association between alcohol consumption and strength of lead effect. No statistical comparison of lead coefficients within and across strata. No model diagnostic tests presented.

Table AX6-5.1 (cont'd). Cardiovascular Effects of Lead

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Maheswaran, et al. (1993) Europe-England-Birmingham 1981	809 out of 870 workers, mean (SD) age 43.3 (10.4) years, at an lead acid battery plant were used in the study. Women and workers taking antihypertensive medications were excluded. Used multiple linear regression analyses of systolic and diastolic blood pressure, forcing age, BMI, alcohol use, linear blood lead, zinc protoporphyrin, years of work exposure, cigarette smoking as covariates.	Geometric mean (SD) blood lead was: 31.6 µg/dL (5.5 *sic.*)	Linear blood lead was not significant for either systolic or diastolic blood pressure. Authors used two indices of lead exposure in the same models. Over much of the studied blood lead range, zinc protoporphyrin was likely collinear with blood lead. Linear blood lead may not be the appropriate metric to use in blood pressure models. Did not use age-squared to adjust for non-linear relationship of blood pressure with age. Did not report model diagnostics.
Telišman et al. (2004) Europe-Croatia-Zagreb Date of data collection not given.	100 workers from factories producing lead-based products, mean (range) age 30 (20-43) years. Exclusion criteria were absence of psychological stress (e.g., death in family) over last 4 months, absence of verified diabetes, coronary heart disease, cerebrovascular and peripheral vascular disease, renal disease, hyperthyroidism, androgenital syndrome, primary aldosteronism, and "other diseases that could influence blood pressure or metal metabolism." Linear or natural log blood lead were considered for stepwise entry in models of systolic and diastolic blood pressure, forcing in all other covariates: blood cadmium, BMI, age, serum zinc, serum copper, hematocrit, smoking, and alcohol.	Arithmetic mean (range) blood lead: 36.7 µg/dL (9.9-65.9)	Neither linear nor natural log blood lead entered as significant in multiple regression models of systolic and diastolic blood pressure. Very small sample size limited power to detect significant effects; non-significant effects should not be interpreted as lack of effect. Too many covariates for a small study. Almost no subjects below 10 µg/dL. Taking hypertensive medications not controlled, likely a problem with top systolic and diastolic blood pressure in the group 170 mmHg and 110 mmHg, respectively. No model diagnostic testing reported.

Table AX6-5.1 (cont'd). Cardiovascular Effects of Lead

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia			
Lee et al. (2001) Korea-Chonan 1997-1999	798 workers from various lead-using or producing factories, mean (SD, range) age 40.5 years (10.1) [17.8-64.8], 79.4% male, were classified as to Vitamin D receptor genotype (VDR: bb or Bb/BB) and delta-aminolevulinic acid dehydratase (ALAD: 1-1 or 1-2) genotype, as VDR polymorphism has been implicated in modifications of lead absorption and lead uptake and release from bone as well as risk for elevated blood pressure and hypertension, and ALAD polymorphism affects lead binding to it in the erythrocyte, the major storage depot of lead in blood. The hypothesis was that polymorphism type could influence the effect of lead on blood pressure and hypertension. Multiple linear regression models of linear blood lead, DMSA-chelatable lead, and tibia lead effect on systolic and diastolic blood pressure with potential covariates of age and age-squared, sex, creatinine clearance, hemoglobin, weight, height, BMI, job duration, tobacco and alcohol consumption, pack-years of tobacco, and cumulative life time alcoholic drinks. Stepwise procedure allowed retention of covariates only if they were significant or "there were substantive changes in the coefficients of predictor variables after" their inclusion. In the models shown, Appearance of multiple lead variables and the interaction between lead variables and genotype for each gene depended upon the specific model. Both ALAD and VDR receptor polymorphism were sometimes tested simultaneously in each model containing polymorphism terms and sometimes VDR appeared without ALAD.	Arithmetic mean (SD, range) blood lead 32.0 µg/dL (15.0, 4-86) Mean (SD, range) DMSA-chelatable lead 186 µg (208.4, 4.8-2103) Mean (SD, range) tibia lead 37.2 µg/g (40.4, −7 to 338)	With simple t-tests, subjects with VDR Bb/BB allele were significantly older, had more DMSA-chelatable lead, and had higher systolic and diastolic blood pressure than subjects with VDR bb allele. In multiple regression models of systolic blood pressure, controlling for age and age-squared, sex, BMI, antihypertensive medication use, and cumulative life-time alcoholic drinks, adding tibia lead, VDR type, and ALAD type, each increase of 10 µg/g of tibia lead was associated with an increase of 0.24 mmHg (95% CI: −0.01, 0.49) and VDR BB/Bb type was associated with an increase of 3.24 mmHg (95% CI: 0.18, 6.30) blood pressure compared to the VDR bb type. ALAD genotype was not significant. In the same model, but substituting linear blood lead for tibia lead, each increase in 1 µg/dL of linear blood lead was associated with an increase of 0.07 mmHg (95% CI: 0.00, 0.14) and VDR BB/Bb type was associated with an increase of 2.86 mmHg (95% CI: −0.22, 5.94) blood pressure compared to the VDR bb type. ALAD genotype had no significant effects on blood pressure. When both tibia and linear blood lead were entered simultaneously along with VDR genotype, adjusting for the same covariates, only VDR Bb/BB was significant; compared to VDR bb, blood pressure was 3.51 mmHg (95% CI: 2.41, 8.61) higher. ALAD genotype had no significant effects on blood pressure. In a model without any lead terms, VDR genotype was interacted with the age and the age-squared terms. The VDR Bb/BB genotype interaction with the linear age term was significant for systolic blood pressure. Compared with the bb genotype the VDR Bb/BB genotypes' blood pressure increased 0.36 mmHg (95% CI: 0.06, 0.66) per year faster with increasing age. There were no significant effects of any lead variable with diastolic blood pressure, though the VDR Bb/BB genotype had significantly higher blood pressure (1.9 mmHg; not enough information given to calculate CI) than the bb genotypes. There were no significant interactions of the lead measures with the genotypes for either ALAD or VDR.

Table AX6-5.1 (cont'd). Cardiovascular Effects of Lead

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Lee et al. (2001) (cont'd)	Logistic regression analysis was used to test the effect of the lead indices on hypertension (systolic >160 mmHg or diastolic >96 mmHg or taking antihypertensive medications) using the same group of potential covariates, testing the lead terms and the lead-genotype interaction terms separately. The hypertension models tested both gene polymorphisms separately.		Subjects with the Bb/BB genotypes had a significantly higher odds hypertension prevalence (OR 2.1 [95% CI: 1.0, 4.4]) than subjects with the bb genotype, adjusting for age, sex, BMI, tibia lead, and current alcohol use. There were no significant effects of any lead variable nor of ALAD on hypertension status. Linear blood lead may not give efficient and unbiased estimates of blood lead effect on blood pressure. The descriptive data shows highly skewed distributions for blood lead, DMSA-chelatable lead, and tibia lead in this group, suggesting that coefficients of all lead effect on blood pressure may not have been efficient and unbiased. Stepwise models usually produce different covariate patterns for different models, though the tables indicate that the covariates used for all the models discussed above were the same. No model diagnostic tests were reported.
Lustberg et al. (2004) Korea-Chonan 1997-1999 (period of enrollment; no statement on dates of data collection)	793 (number given for genotype analysis; numbers in models not given) current and former lead workers, mean (SD) age 40 (10) years and 80% male, were genotyped for the three polymorphisms of endothelial nitric oxide synthase (eNOS) (GG, GT, TT), an enzyme that is a modulator of vascular resistance. The effect of genotype and the interaction of genotype with blood lead and tibia lead on systolic and diastolic blood pressure were evaluated by multiple linear regression analyses, forcing covariates of age (modeled as a 2 degree of freedom spline with knot at 45 years), sex, natural log BMI, smoking and alcohol consumption, high school education, and job duration. Both blood lead and tibia lead were entered as percentiles and entered together. Logistic models of hypertension (systolic ≥140 mmHg or diastolic ≥90 mmHg or reported use of antihypertensive medication) used the same covariates. Interaction terms between each of the lead measures (plus a lead-squared term) and genotype was used to determine differential effect of lead according to genotype.	Lead according to genotype: Arithmetic mean (SD) blood lead, GG: 32 (15) µg/dL Arithmetic mean (SD) blood lead, TG/TT: 32 (15) µg/dL Mean (SD) tibia lead, GG: 37 (42) µg/g Mean (SD) tibia lead, TC/TT: 36 (34) µg/g	85% (673/793) of the group were typed GG, 14% (114/793) were TG, and 1% (6/793) were TT. TG and TT groups were combined for analysis (TG/TT). Mean systolic and diastolic blood pressures, adjusted for all covariates, were not significantly different between GG and TG/TT groups. In multiple regression models for systolic and diastolic blood pressure, neither percentile blood lead nor percentile tibia lead, entered together, were significant predictors. Interaction terms between the lead variables and genotype were not significant. In the logistic regression model for hypertension, neither percentile blood lead nor percentile tibia lead, entered together, were significant predictors. Reporting was incomplete: number of subjects entering the models were not stated; no comparisons between recruited subjects and subjects not used in models. Despite reporting non-significant interactions, the paper showed both loess plots and tables of analyses stratified by genotype, reporting significant associations between both tibia and blood lead in the GG genotype, insignificant in the other. Inspection of the loess plots revealed striking non-linearity for both adjusted blood lead-systolic blood pressure and adjusted tibia lead-systolic blood pressure relationships. Small group size of the TG/TT genotypes and highly unbalanced terms of the interaction may have contributed to the non-significant interactions. Although the interaction lead term was also probed as a quadratic function, the tibia lead interaction was not, suggesting that poor concentration-response specification in the model may also have contributed to the lack of significant main effects and interactions.

Table AX6-5.1 (cont'd). Cardiovascular Effects of Lead

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Nomiyama et al. (2002) China, Beijing No statement on dates of data collection	123 female lead-exposed leaded crystal toy workers, mean age (range) 27.3 (17-44) years, and 70 female sewing workers (reference group), mean age (range) 24.2 (16-58) years were tested. Forward stepwise multiple regression models of systolic and diastolic blood pressure of the combined groups were used with linear blood lead and a set of covariates. Variables with $p < 0.2$ were allowed to enter. The covariate set was selected from a larger set of potential covariates by factor analysis, and a representative variable from each factor was selected for possible entry in the regressions. Alternate models were constructed using four ordered categories of blood lead, instead of the linear continuous blood lead variable. Logistic regressions were used to determine the odds of elevated systolic (≥125 mmHg) and elevated diastolic (≥80 mmHg) blood pressure as a function of blood lead category.	Blood lead mean (SD, range) in lead workers: 55.4 (13.5, 22.5-99.4 µg/dL Blood lead mean (SD, range) in non-lead workers: 6.4 (1.6, 3.8-11.4) µg/dL	Adjusted for age, urine protein, and plasma triglyceride, each 1 µg/dL increase in linear blood lead significantly associated with a 0.13 mmHg increase in systolic blood pressure (no SE or CI given; $p = 0.0003$). Adjusted for plasma triglyceride, age, urine protein, plasma low density lipoprotein, and hypertension heredity, each 1 µg/dL increase in linear blood lead was associated with a 0.10 mmHg increase in diastolic blood pressure (no SE or CI given; $p = 0.0001$). Using the ordered categories of blood lead and the same covariates for systolic and diastolic blood pressure, the 40-60 µg/dL group had 4.2 mmHg (95% CI: 0.0, 8.5) higher systolic blood pressure and 4.1 mmHg (95% CI: 1.3, 6.8) higher diastolic blood pressure than the reference group (blood lead (<11.4 µg/dL). The group with ≥60 µg/dL blood lead had 7.5 mmHg (95% CI: 3.0, 12.0) systolic blood pressure and 6.3 mmHg (95% CI: 3.4, 9.1) diastolic blood pressure higher than the reference group. Logistic regression models for "elevated" blood pressure, modeled using the same covariates were similar. In the 40-60 µg/dL group odds of systolic blood pressure ≥125 mmHg and diastolic blood pressure ≥80 mmHg were 4.26 (95% CI: 1.07, 17.04) and 2.43 (95% CI: 0.97, 6.04), respectively, higher than the reference group. The odds of "elevated" systolic and diastolic blood pressure in the group with blood lead ≥60 µg/dl were 7.48 (95% CI: 1.86, 30.12) and 3.31 (95% CI: 1.29, 8.50), respectively. Incomplete reporting in paper: no model N, no SEs for linear blood lead regressions, no description of type of factor analysis used or dates of data collection. Innovative use of factor analysis to select covariates that, depending on how the factor analysis was run, could have produced a set of orthogonal variables for model entry. However, BMI was not included in the original set of covariates or in the models. Small sample size limits conclusions based on nonsignificant results. Stepwise regression produced a different covariate pattern for each component of blood pressure. The linear blood lead variable may be inappropriate given the marked skewness of blood lead in descriptive analysis. The 11 µg/dL gap in blood lead between lead workers and non-lead workers could have introduced problems in analyses with continuous blood lead. Larger age spread in non-exposed group than in exposed group could have caused misspecification of age variable. No control for antihypertensive medication use. No model diagnostics reported.

Table AX6-5.1 (cont'd). Cardiovascular Effects of Lead

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Wu et al. (1996) Central Taiwan No statement on dates of data collection	222 workers in two lead battery plants, 112 men, mean (range) age 36.2 (18-67) years, and 110 women, mean (range) age 36.2 (18-71) years were tested for blood lead relationships with systolic and diastolic blood pressure in multiple regression models, using a fixed, forced set of covariates: age, sex, BMI, working history, years of work, noise exposure, natural log ambient air lead concentration, and ordered categorical blood lead concentration.	Arithmetic mean (SD, range) blood lead: Women: 44.6 (18.4) [8.3-103.1] µg/dL Men: 60.2 (26.8) ![17.0-150.4] µg/dL	Using four ordered blood lead categories (<25µg/dL [n = 16/222; 6.8%], 25-40 µg/dL [58/222; 26.1%], 41-60 µg/dL [63/222; 27.9%], and >60 µg/dL [85/222; 38.3%]) adjusted systolic and diastolic blood pressure were not significantly related to the top three blood lead categories compared to the lowest, natural log ambient lead. Years in work environment was a significant predictor of both systolic and diastolic blood pressure, but age was only marginally significant for systolic blood pressure and not significant for diastolic blood pressure. Small study size limits any conclusions drawn from non-significant results. Three measures, all related to lead exposure, were simultaneously tested in the models. While blood lead may only be weakly correlated with years of work, ambient air lead would be expected to be much better correlated with blood lead. There is a clear possibility of collinearity among those three variables, which would inflate standard errors and reduce coefficients. Authors selected ordered categories of lead to "avoid unnecessary assumption of linearity." The use of natural log air lead concentration suggests that some diagnostics were run, but no model diagnostic tests were reported. No control for antihypertensive medication use.

CHAPTER 6 ANNEX

ANNEX TABLES AX6-7

Table AX6-7.1. Recent Studies of Lead Exposure and Genotoxicity

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings and Interpretation
Europe			
Fracasso et al. (2002) Italy	Case-control design. 37 workers employed at a battery plant. 29 student and office worker volunteers with no known occupational exposure to genotoxins. Peripheral lymphocytes isolated from whole blood. Reactive Oxygen Species (ROS) production, cellular GSH level, PKC isoforms, and DNA breaks (via comet) assayed. ANOVA and logistic regression used to compare workers vs. healthy volunteers. Adjusted for age, alcohol use, and smoking.	Battery plant workers. Blood lead categories used for some comparisons, with <25, 25-35, and >35 µg/100mL as cutpoints. Mean blood Pb 39.6 µg/100mL for workers, 4.4 µg/100mL for volunteers.	OR (95% CI) *Workers vs. Volunteers:* ROS: 1.43 (0.79-2.60) DNA Breaks (Tail Moment): 1.07 (1.02-1.12) GSH: 0.64 (0.49-0.82) PKC α reduced in workers, atypical PKC unchanged vs. volunteers (no statistics provided). *Means (SE) via blood lead category for ROS and GSH:* <25 ug/ug/100 mL 4.9 (0.4) and 12.8 (0.8) 25-35 ug/100 mL 5.4 (0.7) and 7.7 (1.7) >35 ug/100 mL 5.4 (0.5) and 9.2 (1.2) Major analyses controlled for age, smoking, and alcohol intake. Analyses by blood lead category not controlled for age, smoking, or alcohol intake but these factors said not to influence endpoint and/or results "significantly." No control for potential coexposures.
Paulus et al. (2003) Poland	Cross-sectional design. Battery plant workers: 34 acid battery, 22 alkaline battery, and 52 plant personnel from departments with no known exposure to Pb or Cd. Lymphocytes isolated from whole blood. SCE, MN, DNA damage (via comet) assayed. Means compared via ANOVA.	Workers considered Pb-exposed if from acid battery department, Cd-exposed if from alkaline, unexposed if from other department. Mean blood Pb 504 µg/L for Pb-exposed workers, 57 µg/L for Cd-exposed, and 56 µg/L for other workers.	Mean (SD) *Pb exposed workers (all combined):* SCEs 7.48 (0.88) MN 18.63 (5.01) NDI 1.89 (no SD given) *Cd exposed workers (all combined):* SCEs 6.95 (0.79) MN 15.86 (4.92) NDI 1.96 (no SD given) *Other workers (all combined):* SCEs 6.28 (1.04) MN 6.55 (3.88) NDI 1.86 (no SD given) Elevation of SCEs and MN vs. controls at $p < 0.05$ and $p < 0.01$, respectively. Both SCEs and MN elevated among Pb exposed workers as well as Cd-exposed workers compared to controls. Differences greatest for Pb-exposed workers. Higher SCE and MN also occurred among Pb-exposed workers after stratification by smoking status. No direct control for potential coexposures, but mean blood Cd no higher in Pb-exposed than in other worker group.

Table AX6-7.1 (cont'd). Recent Studies of Lead Exposure and Genotoxicity

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings and Interpretation
Europe (cont'd)			
Van Larebeke et al. (2004) Belgium	Cross-sectional design. 99 female nonsmokers, ages 50-65, drawn from rural and industrial areas. Peripheral lymphocytes isolated from blood. HPRT variant frequency determined.	Lead concentration measured in blood (serum). Women also classified as above vs. below median for blood Pb	HPRT variant frequency *Above median serum Pb: 9.45 x 10^{-6}* *Below median serum Pb: 5.21 x 10^{-6}* P-value for difference =0.08 adjusted for age, education, smoking, BMI, and serum Se. (Significant inverse association noted between variant frequency and serum Se.) Uncontrolled for potential exposure to other genotoxins.
Latin America			
Minozzo et al. (2004) Brazil	Cross-sectional design. 26 workers employed at a battery recyclery for 0.5 to 30 years. 29 healthy volunteers of similar age range and SES. Peripheral lymphocytes isolated from whole blood. Fixed blood slides stained with Giemsa visually evaluated to determine micronuclear frequency (MN) and cellualr proliferation as nuclear division index (NDI). ANOVA and logistic regression used to compare workers vs. healthy volunteers. Adjusted for age, alcohol use, and smoking.	Battery recyclery workers were considered exposed. Blood lead also determined. Mean blood Pb 35.4 µg/dL for workers, 2.0 µg/dL for volunteers.	Mean (S.D.) *Means (SD) for workers and volunteers* MN 3.85 (2.36) and 1.45 (1.43) NDI 1.77 (0.22) and 1.89 (0.18) Kendal correlation coefficient *All workers* {assuming recyclery workers only, not total population, but no population number given in Table.} Blood Pb × MN: 0.061 (p = 0.33) Blood Pb × NDI: 0.385 (p = 0.003) Not controlled for age or SES, although worker and volunteer populations said to be of similar age and SES. Uncontrolled for potential coexposures. Correlations appear uncontrolled for smoking, age, or other factors. Differences in MN and NDI minor for smokers vs. nonsmoker, however. Diet "type" "similar" for workers and controls, although no definition of similarity provided.

Table AX6-7.2. Key Occupational Studies of Lead Exposure and Cancer

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings and Interpretation
United States			
Steenland et al. (1992) (follow-up of Selevan et al. (1985) U.S. 1940-1988	Cohort design. 1,990 male workers employed for at least 1 year in a lead-exposed department at a U.S. lead smelter in Idaho during 1940-1965. Mortality traced through 1988 to determine cause of death. SMR computed for workers vs. national rates for age-comparable counterparts.	Exposure categorizations based on airborne lead measurements from 1975 survey. High-lead-exposure subgroup consisted of 1,436 workers from departments with an average of least 0.2 mg/m^3 airborne lead or ≥50% of jobs showing 0.40 mg/m^3 or greater. Mean blood lead 56 µg/dL in 1976.	SMR (95% CI); no. of deaths *Total cohort:* Nonsignificantly elevated RRs: kidney, bladder, stomach, and lung cancer. *High-lead-exposure subgroup:* Kidney 2.39 (1.03, 4.71); 8 Bladder 1.33 (0.48, 2.90); 6 Stomach 1.28 (0.61, 2.34); 10 Lung 1.11 (0.82, 1.47); 49. No control for smoking or exposure to other metals.
Wong and Harris (2000) (follow-up of Cooper et al. (1985) U.S. 1947-1995	Cohort design. Lead battery plant (4,518) and smelter (2,300) workers. Worker mortality was followed up through 1995. Cause of death was identified from death certificates. Mortality was compared with U.S. national age-, calendar-year-, and gender-specific rates to compute the SMR. (See additional entry for nested study of stomach cancer.)	Workers were evaluated as a whole, and also as separate battery plant and smelter worker populations. Job histories were also used to stratify workers by cumulative years of employment (1-9, 10-19, 20+), date of hire (pre-1946 vs. 1946 on), and lag between exposure and cancer (<20, 20-34, >34 years). Mean blood lead 80 µg/dL during 1947-72 among smelter workers, 63 µg/dL among battery workers.	SMR (95% CI) *Battery plant workers:* All cancer 1.05 (0.97, 1.13) All respiratory 1.13 (0.98, 1.29) Stomach 1.53 (1.12, 2.05), significant Lung, trachea, bronchus 1.14 (0.99, 1.30), marginal significance Thyroid, Hodgkin's: nonsignificant Bladder 0.49 (0.23, 0.90), significant depression *Smelter workers:* Digestive, respiratory, thyroid: nonsignificant Lung 1.22 (1.00, 1.47), nonsignificant *Battery plant and smelter workers combined:* All cancer 1.04 (0.97, 1.11) All respiratory 1.15 (1.03, 1.28), significant Stomach 1.47 (1.13, 1.90), significant Lung, trachea, bronchus 1.16 (1.04, 1.30), significant Thyroid/endocrine 3.08 (1.33, 6.07), significant Lung and stomach risks lower for pre-1946 hires; higher for workers employed 10-19 years than <10, but lower for >19 years; SMRs peaked with 20- to 34-year latency for lung, but <20 years for stomach. No control for smoking or exposure to other agents. No assessment of employment history after 1981.

Table AX6-7.2 (cont'd). Key Occupational Studies of Lead Exposure and Cancer

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings and Interpretation
United States (cont'd)			
Wong and Harris (2000) U.S. 1947-1995. (Nested in Wong and Harris 200 cohort.)	Case-control design. *Cases*: the 30 stomach cancer cases occurring in a Philadelphia lead battery plant. *Controls*: 120 age-matched cohort members. Mean exposure was compared for cases vs. controls. Odds of exposure were also computed for increasing quartiles of cumulative exposure.	Job titles were used to classify lead exposure as low, intermediate, or high; total months of any exposure, of intermediate or high exposure only, and of cumulative exposure, with months weighted by 1, 2, or 3 if spent in low-, intermediate-, or high-exposure job.	Mean months of employment, of intermediate or high exposure, or of weighted exposure to lead were all nonsignificantly lower among cases. OR for cumulative weighted exposure in the 10 years prior to death: First quartile 1.00 Second quartile 0.62 Third quartile 0.82 Fourth quartile 0.61 P for trend = 0.47; ORs showed no positive association with any index of exposure. Analyses appear uncontrolled for smoking, other occupational exposures, or other risk factors.
Europe			
Fanning (1988) (Cases overlap those occurring in Dingwall-Fordyce and Lane, 1963; and Malcolm and Barnett, 1982). U.K. 1926-1985	Proportional mortality/cohort design. *Subjects:* 2,073 deceased males identified through pension records of lead battery and other factory workers in the U.K. Workers dying from a specific cancer were compared with workers dying from all other causes	Workers were classified as High or moderate lead exposure vs. little or no exposure based on job titles.	OR (95% CI) [Number of deaths] Lung cancer: 0.93 (0.8, 1.1) [76 deaths] Stomach cancer 1.34 [31 deaths] No associations for other cancer types; elevations in stomach and total digestive cancers limited to the period before 1966.

Table AX6-7.2 (cont'd). Key Occupational Studies of Lead Exposure and Cancer

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings and Interpretation
Europe (cont'd)			
Anttila et al. (1995) Finland 1973-1988	Cohort plus case-referent design. 20,700 workers with at least one blood lead measurement between 1973 and 1983. Workers were linked to the Finnish Cancer Registry for follow-up through 1988. Fro deceased workers, cause of death was identified from death certificate. Mortality and incidence were compared with gender-, 5-year age, and 4-year calendar-year matched national rates.	Blood lead concentration. Exposure was categorized according to the highest peak blood level measured: Low: 0-0.9 µmol/L [0 to 18.6 µg/dL] Moderate: 1-1.9 µmol/L [20.7 to 39.4 µg/dL] High: 2-7.8 µmol/L [41.4 to 161.6 µg/dL] Mean blood lead 26 µg/dL.	*Total cohort:* No elevation in total or site-specific cancer mortality *Moderately exposed:* Total respiratory and lung cancer: SIR = 1.4 (95% CI: 1.0, 1.9) for both Total digestive, stomach, bladder, and nervous system: nonsignificant elevations *Highly exposed:* No increase in risks *All cancer:* RR = 1.4 (95% CI: 1.1, 1.8) *Lung or tracheal:* RR = 2.0 (95% CI: 1.2, 3.2) No increase in high-exposure group No RRs reported for other cancers *Case-referent substudies:* Lung cancer ORs increased with increasing cumulative exposure to lead Highly exposed: squamous-cell lung cancer OR = 4.1 (95% CI: 1.1, 15) after adjustment for smoking. Short follow-up period limits statistical power, offset to a large degree by the substantial sample size. No control for exposure to other potential carcinogens.

Table AX6-7.2 (cont'd). Key Occupational Studies of Lead Exposure and Cancer

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings and Interpretation
Europe (cont'd)			
Anttila et al. (1996) Finland 1973-1988 (Nested analysis based on Antilla et al. 1995 cohort)	Case-control design. (See Anttila et al. 1995 for basic information on the source population.) *Cases*: 26 Finnish men with CNS cancer. *Controls:* 200 Finnish men without CNS cancer. Nested case-control analysis.	Peak blood lead levels used to categorize exposure as 0.1-0.7, 0.8-1.3, and 1.4-4.3 µg/L. Cumulative exposure estimated by using mean annual blood lead level to categorize exposure as 0, 1-6, 7-14, or 15-49 µg/L. Interviews were used to obtain occupational history and other risk-factor data from patients or next of kin.	OR (no. of cases or deaths) CNS cancer incidence *(26 cases):* Rose with increasing peak lifetime blood lead measurements; not significant Glioma mortality *(16 deaths):* Rose consistently and significantly with peak and mean blood lead level, duration of exposure, and cumulative exposure. Mortality by cumulative exposure, controlled for cadmium, gasoline, and year monitoring began: Low (13 subjects) 2.0 (2) Medium (14 subjects) 6.2 (2) High (16 subjects) 12.0 (5) 1 death among 26 subjects with no exposure: test for trend significant at $p = 0.02$. Controlled for smoking as well as exposure to cadmium and gasoline. Complete follow-up with minimal disease misclassification.
Gerhardsson et al. (1995a) Sweden 1969-1989	Cohort design. 684 male Swedish secondary lead smelter workers with lead exposure. Cancer incidence among workers was traced through 1989. Incidence was compared with county rates.	Blood lead level: any worker with a detectable blood lead level was classified as exposed.	SIR (95% CI); no. of cases *All malignancies:* 1.27 (0.91, 1.74); 40 *Respiratory:* 1.32 (0.49, 2.88); 6 *All gastrointestinal:* cohort 1.84 (0.92, 3.29; 11 highest quartile 2.34 (1.07, 4.45); 9 *Stomach:* 1.88 (0.39, 5.50); 3 *Colon:* 1.46 (0.30, 4.28); 3 SIRs for all other sites except brain were nonsignificantly elevated; too few cases. No control for smoking. Small numbers, so meaningful dose-response analyses not possible for most cancer sites.

Table AX6-7.2 (cont'd). Key Occupational Studies of Lead Exposure and Cancer

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings and Interpretation
Europe (cont'd)			
Lundström et al. (1997) (follow-up of Gerhardsson et al. (1986) (see also subcohort analyses of Englyst et al., 2001). Sweden 1928-1987	Cohort design. 3,979 copper and lead smelter workers. Standardized mortality and incidence ratios were computed for workers compared with age-, year-, gender-, and county-specific rates for the general population.	Fro some analyses, the entire cohort was treated as exposed. For others, job histories were used to single out 1,992 workers belonging to departments thought to be exposed to "lead only." Mean blood lead monitoring test results across time were used to single out a "highly exposed" group of 1,026 workers with blood lead levels ≥10 µmol/L [≥207 µg/dL]. Mean blood lead 60 µg/dL in 1959.	SMR (95% CI); no. of deaths *Lung:* Total cohort 2.8 (2.0, 3.8); 39 Highly exposed 2.8 (1.8, 4.5); 19 SIR (95% CI); no. of cases *Lung with 15-year lag:* Total cohort 2.9 (2.1, 4.0); 42 Highly exposed 3.4 (2.2, 5.2); 23 Lead-only 3.1 (1.7, 5.2); 14 Lead-only highly exposed 5.1 (2.0, 10.5); 7 *Other highly exposed (total cohort), with 15-year lag:* Brain 1.6 (0.4, 4.2); 4 Renal pelvis, ureter, bladder 1.8 (0.8, 3.4); 9 Kidney 0.9 (0.2, 2.5); 3 All cancer 1.1 (0.9, 1.4); 83. No control for smoking.
Englyst et al. (2001) (follow-up and sub-analysis of Lundström et al., 1997). Sweden 1928-1987	Nested cohort analysis. Limited to 1,093 workers in the smelter's lead department, followed through 1997. Incidence was compared with county rates; age-specific SIRs with 15-year lag.	Workers were divided into Subcohorts I and II for ever and never worked in areas generally associated with exposure to arsenic or other known carcinogens (701 and 383 workers, respectively). Detailed individual assessment of arsenic exposure was made for all lung-cancer cases.	SIR (95% CI); no. of cases Subcohort I (coexposed): Lung 2.4 (1.2, 4.5); 10 Subcohort II (not coexposed): Lung 3.6 (1.2, 8.3); 5 Subjects with lung cancer found to have history of "considerable" exposure to arsenic: 9/10 among Subcohort I, 4/5 among Subcohort II. No control for smoking.

Table AX6-7.2 (cont'd). Key Occupational Studies of Lead Exposure and Cancer

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings and Interpretation
Europe (cont'd)			
Carta et al. (2003) Sardinia 1972-2001	Cohort design. 918 lead smelter workers. Mortality traced from 1972 through 2001. Standardized mortality ratios computed.	Smelter workers considered exposed. Job histories also used to categorize degree of exposure based on environmental and blood lead measurements for specific departments and tasks during 1985-2001.	SMR; number of cases *Smelter workers as a whole* All cancer 1.01 ; 108 Gastric cancer 1.22 ; 4 Lymphoma/leukemia 1.82 ; 6 Lung cancer 1.21 ; 18 *Highly exposed workers* Lung cancer 1.96 (95% C.I. 1.02, 3.68) for highest exposure group, with statistically significant upward trend. Analyses for worker population as a whole supported by presence of dose-response pattern for lung cancer based on estimated exposure. Modest population size, inability to assess dose-response for cancers of interest other than lung. No control for smoking or other occupational exposures.

Table AX6-7.3. Key Studies of Lead Exposure and Cancer in the General Population

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings and Interpretation
United States			
Jemal et al. (2002) (same cohort as in Lustberg and Silbergeld, 2002 except for inclusion criteria) U.S. 1976-1992.	Cohort design. 3,592 white participants from the 1976-1980 NHANES II survey who had blood lead measured at entry. Mortality was followed through 1992 via NDI and SSADMF. RRs were calculated for the various exposure groups compared to survey participants with the lowest exposure, adjusted for age and smoking.	Blood lead (µg/dL) was measured by atomic absorption and used to classify subjects into exposure quartiles or groups above vs. below median exposure. Median blood lead 12 µg/dL.	RR (95% CI); no. of deaths Lung (above vs. below median): Total cohort 1.5 (0.7, 2.9); 71 M 1.2 (0.6, 2.5); 52 F 2.5 (0.7, 8.4); 19 Stomach (above vs. below median): Total cohort 2.4 (0.3, 19.1); 5 M 3.1 (0.3, 37.4); 4 F no deaths in referent group All cancer: total cohort by quartile (age-adjusted) 1.0, 1.2, 1.3, 1.5 (P for trend 0.16). Smoking was controlled for. Lead levels occurring in the general population were examined, not just those in workers with high occupational exposure potential. Exposure to other carcinogens were not examined. Potential for residual confounding by degree and duration of smoking exists (only controlled for never, former, current <1, current 1+ pack/day). Limited case numbers yield low statistical power for stomach or other cancers.
Lustberg and Silbergeld (2002) (same cohort as Jemal et al., 2002 except for inclusion criteria) U.S. 1976-1992.	Cohort design. 4,190 U.S. participants from the 1976-1980 NHANES II health and nutrition survey who had blood lead measured at entry and whose levels fell below 30 µg/dL. Mortality was followed through 1992 via NDI and SSADMF. RRs were calculated for the various exposure groups compared to survey participants with the lowest exposure, adjusted for age, smoking and other factors.	Blood lead (µg/dL) measured by atomic absorption was used to classify subjects into exposure groups: Low: <10 Medium: 10-19 High: 20-19 Mean blood lead 14 µg/dL.	RR (95% CI) *All cancer, vs. low exposure:* Medium 1.5 (0.9, 2.5) High 1.7 (1.0, 2.8) *Lung, vs. low exposure:* Medium 1.7 (0.6, 4.8) High 2.2 (0.8, 6.1) *Non-lung, vs. low exposure:* Medium 1.5 (0.8, 2.8) High 1.5 (0.8, 2.8). Significant upward trends noted for all-cause and for cardiovascular mortality with increasing lead category. Smoking was controlled for. Lead levels occurring in the general population were examined , with individuals showing levels consistent with intense occupational exposure excluded, thus allowing exploration of potential effects outside of groups experiencing intense occupational exposure. Exposure to other carcinogens were not examined. Potential for residual confounding by degree and duration of smoking exists (only controlled for never, former, current < 1, current 1+ pack/day). Limited case numbers yield low statistical power for stomach or other cancers.

Table AX6-7.4. Other Studies of Lead Exposure and Cancer

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings and Interpretation
United States			
Mallin et al. (1989) Illinois 1979-1984	Case-control design. Cases: random sample of 10,013 deaths from 7 specific cancers, identified from death certificates for Illinois males between 1979 and 1984. Controls: 3,198 randomly selected deaths from other causes. Odds of exposure computed for glass workers vs. other occupations.	Exposure was based on occupations abstracted from death certificates. No specific measure of lead exposure; glass workers can be considered potentially exposed.	Brain cancer, white male glass workers: OR = 3.0, P < 0.05 (significant) No significant associations for other cancer sites. No control for smoking or other risk factors. Poor specificity for lead exposure.
Cocco et al. (1998a) U.S. 1984-1992	Case-control design. Cases: all 27,060 brain cancer deaths occurring among persons aged 35 or older during 1984-1992, from U.S. 24-state death certificate registry. Controls: 4 gender-, race-, age-, and region-matched controls per case selected from deaths due to nonmalignant causes. Subjects were subdivided into 4 groups by gender and race (white or African-American) for all analyses.	A job-exposure matrix was applied to death certificate-listed occupations to categorize persons as having low, medium, or high probability and intensity of exposure.	Risk of brain cancer mortality increased consistently with intensity of exposure among African-American males, but not other race-gender groups. Probability of exposure alone was not consistently associated with risk. In the high-probability group, risk increased with exposure intensity for all groups except African-American women (only 1 death in the high-probability group). Exposure estimate was based solely on occupation listed on death certificate, hence there was substantial opportunity for misclassification.
Cocco et al. (1998b) U.S. 1984-1992	Case-control design. Cases: all 28,416 CNS cancer deaths occurring among persons aged 35 or older during 1984-1992, from U.S. 4-state death certificate registry. Controls: 4 gender-, race-, age-, and region-matched controls per case selected from deaths due to nonmalignant causes. Subjects were subdivided into 4 groups by gender and race (white or African-American) for all analyses.	Death certificate listed industry and occupation was used to categorize decedents. No estimates of lead exposure specifically.	OR (95% CI) All occupations or industries with ORs above 1.0 and P-value <0.05 in at least one race-gender group were reported Newspaper printing and publishing industry: white M 1.4 (1.1-1.8) black M 3.1 (0.9-10.9) Typesetting and compositing: white M 2.0 (1.1-3.8) white F 1.3 (0.4-3.8) black F 4.2 (0.6-30.7) No deaths among black males. Only two lead exposure associated occupations or industries showed a statistically significant elevation of mortality. No specific measures of lead exposure. Occupation based solely on death certificate, hence there was substantial opportunity for misclassification.

Table AX6-7.4 (cont'd). Other Studies of Lead Exposure and Cancer

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings and Interpretation
United States (cont'd)			
Cocco et al. (1999) U.S. 1984-1996	Case-control design. Cases: all 41,957 stomach cancer deaths occurring among persons aged 35 or older during 1984-1996, from U.S. 24-state death certificate registry. Controls: 2 gender-, race-, age-, and region-matched controls per case selected from deaths due to nonmalignant causes. Subjects were subdivided into 4 groups by gender and race (white or African-American) for all analyses.	A job-exposure matrix was applied to death certificate-listed occupations to categorize persons as having low, medium, or high probability and intensity of exposure.	OR (95% CI) Adjusted for age, ethnicity, marital status, urban residence, and socioeconomic status. Elevated ORs: white F, high prob. 1.53 (1.10-2.12) black M, high prob. 1.15 (1.01-1.32) black F, high prob. 1.76 (0.74-4.16) Highly exposed group included 1,503 white and 453 black men and 65 white and 10 black women; no pattern of increase across exposure levels. Intensity of exposure showed no association with stomach cancer except for black women: Low 1.82 (1.04-3.18) (significant) Moderate 1.39 High 1.25. No control for other occupational exposures. Exposure estimate based on occupation listed on death certificate and hence subject to misclassification due to missing longest-held job.
Canada			
Risch et al. (1988) Canada 1979-1982	Case-control design. *Cases:* 826 Canadian men with histologically confirmed bladder cancer during 1979-1982. *Controls:* 792 controls from Canadian population, matched on age, gender, and area. Odds of exposure to lead for cases vs. controls were computed, adjusted for smoking and other risk factors.	Subjects were interviewed regarding length of occupational exposure to lead compounds, as well as 17 other substances.	OR (95% CI) *61 men ever exposed to lead (smoking-adjusted):* 2.0 (1.2-3.5) *Trend per 10 years' duration of exposure:* 1.45 (1.09-2.02) (significant). No other substances showed significant associations with bladder cancer. Controlled for smoking, marital status, socioeconomic status, education, ethnicity, and urban vs. rural residence. No control for other occupational exposures. Low control interview rate (53%), which could result in biased control sample.

Table AX6-7.4 (cont'd). Other Studies of Lead Exposure and Cancer

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings and Interpretation
Canada (cont'd)			
Siemiatycki et al. (1991) Canada	Case-control design. *Cases:* 3,730 various histologically confirmed cancers. *Controls:* specific cancer types were compared with other cancers as a control group, excluding lung cancer. Separate subgroup analysis was restricted to French Canadians.	Occupational exposure to 293 substances, including lead, was estimated from interviews. Exposure was classified as "any"; a subgroup with "substantial" exposure also was identified.	OR (90% CI); no. of cases *Any exposure to lead:* Lung 1.1 (0.9-1.4); 326 (French Canadians only) Stomach 1.2 (1.0-1.6); 126 Bladder 1.3 (1.0-1.6); 155 (French Canadians only) Kidney 1.2 (1.0-1.6); 88 ORs rose in the "substantial" exposure subgroup for stomach and lung, but not for bladder or kidney cancer. Controlled for smoking but not for other occupational exposures.
Europe			
Sankila et al. (1990) Finland 1941-1977	Cohort design. 1,803 male and 1,946 female glass workers employed for at least 3 months at one of 2 Finnish glass factories in 1953-1971 or 1941-1977. Cancer incidence was compared with age-, gender-, and calendar-year-specific national rates. Stomach, lung, and skin cancer rates also were compared separately for 201 male and 34 female glassblowers and non-glassblowers.	No specific lead exposure indices were computed. Analyses did examine glass workers as a whole and then glassblowers specifically, which comprised the group at highest risk for lead exposure.	SIR (95% CI); no. of cases *Lung cancer, all glass workers:* male 1.3 (1.0-1.7); 62 female 1.1 (0.5-2.3); 7 Lung cancer risk showed no specificity for glassblowers. *Skin cancer, M & F combined:* All workers 1.5 (0.8-2.7); 11 little difference between genders Glassblowers 6.2 (1.3-18.3); 3 *Stomach cancer, M & F combined:* Glassblowers 2.3 (0.9-5.0); 6 No increase in other glass workers No increase in cancers of other sites. No control for smoking or occupational coexposures.

Table AX6-7.4 (cont'd). Other Studies of Lead Exposure and Cancer

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings and Interpretation
Europe (cont'd)			
Kauppinen et al. (1992) Finland 1976-1981	Case-control design. *Cases:* 344 primary liver cancer deaths reported to the Finnish Cancer Registry in 1976-1978 or 1981. *Controls:* registry-reported stomach cancer (476) or myocardial infarction (385) deaths in the same hospitals, frequency matched by age and gender.	Questionnaires regarding job history and personal habits were sent to the closest available relative. U.K. based job-exposure matrix was used to rate potential exposure to 50 substances, including lead compounds Industrial hygienists also inspected histories to identify those with highly probable exposure and rate it as high, low, or moderate (< 10 years high or 10+ years low exposure)	OR (95% CI) *52 workers with potential lead exposure*: 0.91 (0.65-1.29) *11 women with potential lead exposure*: 1.84 (0.83-4.06) *5 men with probable moderate exposure:* 2.28 (0.68-7.67) None had high exposure and only 1 had low exposure, whereas 4 controls had high exposure. Female controls appeared to underreport their job history. Most controls had stomach cancer, which if caused by lead would bias results toward the null. Few subjects were rated as having a high probability of exposure.
Wesseling et al. (2002) Finland 1971-1995	Cohort design, but at ecologic level. 413,877 Finnish women with occupation reported in 1970 linked to Finnish Cancer Registry to identify new cases of brain or nervous system cancer arising form 1971 to 1995. Poisson regression was used to calculate SIRs for exposed vs. unexposed groups.	Reported occupation in 1970 was used to classify women into job titles. Potential exposure for each job title was estimated using a job matrix after excluding women in the highest social classes or in farming. Lead and 23 other workplace agents examined. Rates for each job title were calculated, and SIRs for low and medium/high exposure calculated (average estimated blood lead of 0.3 µmol/L served as cutpoint between low and medium/high exposure).	SIR (95% CI), unadjusted for other metals Low exposure: 1.25 (0.68-1.81) Medium/High exposure: 1.33 (0.90-1.96) SIR (95% CI), adjusted for Cd and Ni exposure Low exposure: 1.18 (0.88-1.59) Medium/High exposure: 1.24 (0.77-1.98) All results were adjusted for birth cohort, period of diagnosis, and job turnover rate. Incidence, exposure to lead, and potential confounding factors were calculated at the level of job title rather than at the individual level. Exposure and other estimates were based on data for all workers pooled, not for women specifically. Job classification was based on a single year, not lifetime job history.

Table AX6-7.4 (cont'd). Other Studies of Lead Exposure and Cancer

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings and Interpretation
Europe (cont'd)			
Pesch et al. (2000) Germany 1991-1995	Case-control design. *Cases:* 935 renal-cell cancer patients in five German areas. *Controls:* 4,298 region, age, and gender-matched controls from the surrounding population. ORs were adjusted for age, center, and smoking.	Job histories were used to categorize exposure to cadmium, lead, and other potential as low vs. medium, high, or substantial. Separate exposure estimates were obtained from British and from German-derived job-exposure matrices.	OR (95% CI); no. of cases *Substantial lead exposure based on British matrix:* M 1.5 (1.0-2.3); 29 F 2.6 (1.2-5.5; 11 *Substantial lead exposure based on British matrix:* M 1.3 (0.9-2.0); 30 F not reported. Analyses controlled for smoking. No control for exposure to other occupational agents.
Kandiloris et al. (1997) Greece	Case-control design. *Cases:* 26 patients with histologically confirmed laryngeal carcinoma and no history of lead exposure or toxicity. *Controls:* 53 patients with similar demographic profiles and no history of cancer from the same hospital.	Blood lead levels and ALAD activity were measured.	Blood lead levels were similar, but ALAD activity was significantly lower in cases than controls (Mean 50.79 U/L vs. 59.76 U/L, $p < 0.01$). No control for other risk factors. Potential distortion by effects of disease on Pb and/or ALAD parameters.)
Cordioli et al. (1987) Italy 1953-1967	Cohort design. 468 Italian glass workers employed for at least one year between 1953 and 1967. Mortality among workers was tracked and cause of death was determined for deceased workers. Standardized mortality ratios were computed for workers vs. national population counterparts.	Workers producing low-quality glass containers were classified as lead-exposed.	SMR (95% CI); no. of deaths All cancer 1.3 (0.8-1.8); 28 Lung 2.1 (1.1-3.6); 13 Laryngeal 4.5 (1.2-11.4); 4

Table AX6-7.4 (cont'd). Other Studies of Lead Exposure and Cancer

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings and Interpretation
Europe (cont'd)			
Cocco et al. (1994a) (expansion of Carta et al. 1994). Sardinia 1931-1992	Cohort design. 1,741 male Sardinian lead and zinc miners from two mines employed at least one year between 1931 and 1971. Mortality traced through 1992 to determine cause of death. Mortality among miners was compared with age- and calendar-year-specific regional rates to compute an SMR.	All miners were considered to be exposed to lead.	SMR (95% CI); no. of deaths All cancer 0.94 (0.83-1.05); 293 Prostate 1.21; 16 Bladder 1.15; 17 Kidney 1.28; 7 Nervous system 1.17; 8 Oral 0.61; 8 Lymphohemopoietic 0.91; 21 Digestive 0.83; 86 Peritoneum 3.67 (1.35-7.98); 6 (significant) No other *P*-values <0.05. No control for smoking or exposure to silica, radon, or other exposures.
Cocco et al. (1994b) Sardinia 1951-1988	Cohort design. 526 female Sardinian lead and zinc miners from the same mines as in Cocco et al. (1994a). Mortality traced through 1992 to determine cause of death. Mortality among miners was compared with age- and calendar-year-specific regional rates to compute an SMR.	All miners were considered to be exposed to lead.	SMR (95% CI) Liver 5.02 (1.62-11.70) (significant) Lung 2.32 (0.85-5.05) (nonsignificant) Other cancers showed nonsignificantly reduced rates. No control for smoking or exposure to silica, radon, or other exposures. Low statistical power due to small population and paucity of cancers during follow-up.
Cocco et al. (1996) Sardinia 1973-1992	Cohort design. 1,222 male Sardinian lead and zinc smelter workers whose G6PD phenotypes had been determined, employed any time from 1973-1990. Mortality traced through 1992 to determine cause of death. Mortality was compared with regional rates.	All workers were considered to be exposed to lead. Workers were subdivided into 6PD-normal and -deficient groups.	SMR (no. of deaths) All cancer and lung cancer: lower than expected. Stomach cancer: higher (2 observed vs. 0.6 expected). G6PD-normal 0.26 (10) G6PD-deficient 0.18 (2) G6PD deficiency had little apparent effect on mortality: cancer and all-cause mortality was slightly lower among G6PD-deficient workers than among G6PD-normal workers. No control for smoking or exposure to other agents in the smelter. Healthy worker bias-evident (all-cause mortality 31 observed vs. 44 expected), brief follow-up, low proportion of older ages (mean age at entry 30, average follow-up less than 11 years), no cumulative exposure data.

Table AX6-7.4 (cont'd). Other Studies of Lead Exposure and Cancer

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings and Interpretation
Europe (cont'd)			
Cocco et al. (1997) Sardinia 1931-1992	Cohort design. 1,388 male production and maintenance workers employed for at least 1 year at a Sardinian lead and zinc smelter between June of 1932 and July of 1971. Mortality was followed up through 1992. Mortality was compared with age- and calendar-year-specific regional rates. Since regional rates were only available for 1965 and later, analyses were limited to this period.	All workers were considered to be exposed to lead.	SMRs vs. regional rates (95% CI); no. of deaths Lung 0.82 (95% CI 0.56-1.16); 31 Stomach 0.97 (0.53-1.62); 14 All cancers 0.93 (0.78-1.10); 132 Kidney 1.75 (0.48-4.49); 4 Bladder 1.45 (0.75-2.53); 12 Brain 2.17 (0.57-5.57); 4 Kidney cancer showed a significant trend toward increasing risk with increasing duration of exposure No significant trends were noted for lung or other cancers Brain cancer excess was limited to workers employed for 10 years or less. No control for smoking or exposure to arsenic or other smelter-related exposures. No data on intensity of exposure. Strong association of smelter work with pneumoconiosis and other respiratory disease (SMR = 4.47, 95% CI = 3.37 to 5.80); since this outcome includes silicosis, which is thought to predispose individuals to lung cancer, some lung cancer deaths may have been missed due to misclassification of cause of death based on death certificates.
Wingren and Axelson (1987, 1993) (update of Wingren and Axelson, 1985, same basic cohort as in Wingren and Englander (1990) Sweden 1950-1982	Case-control design. *Source population:* 5,498 men aged 45 or older in 11 Swedish parishes, including 887 glass workers. Cancer-specific nested case-control analysis: *Cases:* deaths due to stomach, colon, and lung cancer from 1950-1982 *Controls:* deaths due to causes other than cancer or cardiovascular disease	Glass workers were considered exposed. Glassblowers also singled out as workers with higher exposure potential. Job history applied to job matrix to categorize occupations as low, moderate, or high lead exposure.	OR (90% CI); no. of deaths *All glass workers:* Lung 1.7 (1.1-2.5); 86 Stomach 1.5 (1.1-2.0); 206 Colon 1.6 (1.0-2.5); 79 *Glassblowers:* Lung 2.3 Stomach 2.6 Colon 3.1 Glassblowers singled out from glass workers as a whole thus showed higher estimated risk. ORs for high or moderate vs. low exposure showed no consistent increase for lung or stomach cancer, however, although they did show mild upward trend for colon cancer. No control for smoking or other occupational exposures.

Table AX6-7.4 (cont'd). Other Studies of Lead Exposure and Cancer

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings and Interpretation
Europe (cont'd)			
Wingren and Englander (1990) Sweden 1964-1985 (same population as in case-control analyses of Wingren and Axelson 1985, 1987, 1993)	Cohort design. 625 Swedish glass workers employed for at least 1 month between 1964 and 1985. Mortality was compared with national rates.	Workers from areas with airborne lead levels up to 0.110 mg Pb/m^3 were classified as exposed.	SMR (95% CI) *Pharyngeal:* 9.9 (1.2-36.1) (significant) *Lung:* 1.4 (0.5-3.1) (nonsignificant) *Colon*: (nonsignificant)
Dingwall-Fordyce and Lane (1963) U.K. 1925-1962	Cohort design. 425 male employees drawing pensions from U.K. battery plants. Standardized mortality for employees vs. national population counterparts.	Battery plant workers were assumed to be exposed, and their mortality compared to that of like age and gender in the U.K. population as a whole. Urinary lead excretion was also used to categorize workers by estimated exposure (none, light, or heavy): 80 lightly and 187 heavily (at least 100 μg/L) exposed.	SMR (95% CI); no. observed deaths All cancer: 1.2 (0.8-1.7); 267 No consistent increase in SMRs across categories of increasing lead exposure. Limitations: No cancer site-specific analyses. No control for potential confounders including smoking and exposure to arsenic or other metals.
Malcolm and Barnett (1982) (follow-up of Dingwall-Fordyce and Lane, 1963) U.K. 1925-1976	Cohort design. 1,898 lead-acid battery workers. Mortality was traced for the lead-acid battery workers to determine cause of death. The proportion of deaths due to cancer (all types and major subcategories) among the worker population was compared to that seen in corresponding members of the general population, yielding a PMR.	Job histories were reviewed to classify workers' lead exposure as high, medium, or none.	Proportionate mortality ratio (PMR) *All cancers:* 1.15 (136 deaths), $p > 0.05$ By exposure: None 1.02 Medium 1.06 High 1.30 No significant excesses for individual cancer sites except for digestive cancer PMR of 1.67, $p < 0.01$, among nonexposed workers. The difference in exposure for the high and medium exposure groups narrowed greatly over the follow-up, thus complicating interpretation of dose-response patterns. No control for smoking or occupational exposure to other carcinogens.

Table AX6-7.4 (cont'd). Other Studies of Lead Exposure and Cancer

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings and Interpretation
Europe (cont'd)			
Ades and Kazantzis (1988) U.K. 1943-1982	Cohort design. 4,393 male zinc, lead, and cadmium smelter workers. (Workers born after 1939 or who had worked less than one year at the facility were excluded.) Workers followed up for mortality. Nested case-control analysis also conducted to quantitatively assessed cadmium and, secondarily, arsenic, lead, and other metal exposures among 174 cases.	Job histories were used to quantify cadmium exposure and assign ordinal ranks for exposure to lead and other metals. Standardized lung cancer mortality ratio computed for workers vs. national rates.	SMR (95% CI); no. of deaths *Cohort:* Lung 1.25 (1.07-1.44) (174) Increased significantly with duration of employment. Nested case-control analyses did not implicate any department or process, nor did cadmium, zinc, sulfur dioxide, or dust exposure account for the observed increase. Cumulative exposure to lead and to arsenic both showed positive associations with lung cancer, but the relative importance of these two exposures could not be determined. Cadmium exposure did not account for the elevated SMR, but analyses could not control for exposure, and were not adjusted for smoking.
Asia			
Hu et al. (1998) China 1989-1996	Case-control design. *Cases:* 218 patients with histologically-confirmed primary gliomas occurring during 1989-1996 at 6 Chinese hospitals. *Controls:* 436 patients with non-neurological, nonmalignant disease, matched by age, gender, and residence from the same hospitals (excluding one cancer-only center).	Patients were interviewed, and those with factory or farm occupations were further interviewed to identify exposure to lead (or other potentially toxic substances).	*Occupational exposure to lead* Not reported for any glioma patients, but was reported for 4 controls. No control for exposure to other occupational or environmental agents.
Hu et al. (1999) China 1989-1996	Case-control design. *Cases:* 383 patients with histologically confirmed primary meningiomas occurring during 1989-1996 at 6 Chinese hospitals. *Controls:* 366 patients with non-neurological, nonmalignant disease matched by age, gender, and residence from the same hospitals (excluding one cancer-only center).	Patients were interviewed, and those with factory or farm occupations were further interviewed to identify exposure to lead (or other potentially toxic substances).	OR (95% CI); no. of cases *Occupational exposure to lead:* M 7.20 (1.00-51.72); 6 F 5.69 (1.39-23.39); 10 Results were adjusted for income, education, and fruit and vegetable intake, plus cigarette pack-years for the women. No control for exposure to additional metals or other occupational exposures.

Table AX6-7.4 (cont'd). Other Studies of Lead Exposure and Cancer

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings and Interpretation
Asia (cont'd)			
Shukla et al. (1998) India 1995-1996	Case-control design. *Cases:* 38 patients with newly diagnosed, histologically confirmed gall bladder cancer cases assembled from a surgical unit. *Controls:* 58 patients with gall stones diagnosed at the same surgical unit, matched on geographic area. Mean bile lead content was compared between cases and controls.	Heavy metal content was measured in bile drawn from the gall bladder at time of surgery.	*Bile lead content: mean (SE) (mg/L):* Gall bladder cancer: 58.38 (1.76) Gallstones: 3.99 (0.43) Cadmium and chromium levels also were elevated in cancer patients, but less than lead. No control for smoking or any other risk factors.

CHAPTER 6 ANNEX

ANNEX TABLES AX6-8

Table AX6-8.1. Effects of Lead on Immune Function in Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States			
Sarasua et al. (2000) ATSDR Multi-site Study: Granite City, IL, Galena, KA; Joplin, MO; Palmerton, PA 1991	Design: cross-sectional Subjects: children and adults (n = 2036) Outcome measures: total lymphocyte count, lymphocyte phenotype abundance, serum IgA, IgG, and IgM. Analysis: multivariate linear regression	Blood lead (µg/dL) mean (SD, 5th–95th %tile): 6–35 mo: 7.0 (16, 1.1–16.1) 36–71 mo: 6.0 (4.3, 1.6–14.1) 6–15 yr: 4.0 (2.8,1.1–9.2) 16–75 yr: 4.3 (2.9, 1.0–9.9)	Significant association ($p < 0.05$) between increasing blood lead and increasing serum IgA, IgG, IgM, and B-cell abundance (%, no.), and decreasing T-cell abundance (%) in 6–35 mo age category; adjusted for age, sex, and study site. Comparison of outcome means across blood lead quartiles (1st quartile as reference, [+], higher, [-] lower): [+] lymphocyte count (4th quartile, $p = 0.02$), T-cell count (4th quartile, $p = 0.09$), B-cell count (4th quartile, $p < 0.01$), B-cell % (4th quartile, $p = 0.09$).
Rabinbowtiz et al. (1990) Boston, MA 1979–1987	Design: cross-sectional Subjects: infants/children (n = 1768) Outcome measures: incidence of illness in children was solicited from parents by questionnaire Analysis: relative risk of illness estimated from incidence ratios, highest: combined lower blood lead deciles, without adjustment for covariates or confounders.	Cord blood lead (µg/dL) ~90th %tile: 10 Shed tooth lead (µg/g) ~90th %tile: 5	Relative risk (unadjusted) was elevated for the following illness categories: severe incidence of ear infection, 1.2 (95% CI: 1.0–1.4), other respiratory illness, 1.5 (96% CI: 1.0–2.3), school absence for illness other than cold or flu, 1.3 (95% CI: 1.0–1.5)
Lutz et al. (1999) Springfield-Green Co, MO NR	Design: cross-sectional Subjects: children (n = 279; age range: 9 mo–6 yr) Outcome measures: differential blood cell counts; lymphocyte phenotype abundance (%); and serum IL-4, soluble CD25, CD27, IgE and IgG (Rubella). Analysis: nonparametric comparison of outcome measures (adjusted for age) for blood lead categories, correlation	Blood lead (µg/dL) range: 1–45 Blood lead categories: <10 µg/dL, 10–14 µg/dL, 15–19 µg/dL, 20–45 µg/dL	Significant association ($p < 0.05$) between increasing blood lead (categorical) and increasing serum IgE levels, after adjusting for age.

Table AX6-8.1 (cont'd). Effects of Lead on Immune Function in Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe			
Annesi-Maesano et al. (2003) France 1985, 1992	Design: cross-sectional Subjects: mother/newborn pairs (n = 374), mean age 30 yr Outcome measures: maternal venous and newborn cord serum IgE levels Analysis: multivariate linear regression, ANOVA	Blood lead (µg/dL) mean (SD) infant cord: 67.3 (47.8) maternal: 96.4 (57.7) Hair lead (ppm) mean (SD): infant: 1.38 (1.26) maternal: 5.16 (6.08)	Significant ($p < 0.0001$) association between increasing infant hair lead and infant cord serum IgE levels. Although medical histories were taken to identify potential IgE risk factors (asthma, allergies) and "confounders" (e.g., smoking), these do not appear to have been quantitatively integrated into the regression models. Allergy status and blood levels were reportedly unrelated to load biomarkers or serum IgE (basis for conclusion not reported).
Karmaus et al. (2005) Germany 1994–1997	Design: cross-sectional Subjects: children (n = 331, 57% male), age 7–8 yrs (96%), 9–10 yrs (4%) Outcome measures: differential blood cell count; lymphocyte phenotype abundance; and serum IgA, IgE, IgG, IgM Analysis: multivariate linear regression	Blood lead (µg/dL) mean (95% CI): males: 2.5 (1.1–4.4) females (2.8 (1.5–4.8) Blood lead quartile ranges: <2.2 (n = 82) 2.2–2.8 (n = 81) 2.8–3.4 (n = 86) >3.4 (n = 82)	Significant association between blood lead ($p < 0.05$) and serum IgE (not monotonic with quartile range). Comparison of adjusted mean outcomes ($p \leq 0.05$) across blood lead quartiles (1st quartile as reference, [+], higher, [-] lower): [-] $CD3^+$ T-cells (2nd quartile), [-] $C3^+CD8^+$ T-cells (2nd quartile), [+] $C3^+CD5^+CD19^+$ B-cells (2nd quartile). Covariates retained: age, sex, environmental exposure to tobacco smoke, infections (in last 12 mo), serum cholesterol, and triglycerides.
Reigart and Graber (1976) NR NR	Design: clinical Subjects: children (n = 19), ages 4–6 years Outcome measures: serum IgA, IgG, IgM, total complement and C-3, before and after immunization with tetanus toxoid Analysis: none; presentation of prevalence of clinically low, normal, and high values of outcome measures	Blood lead (µg/dL) mean (range): high: >40 (n = 12): 45.3 (41–51) low: ≤30 (n = 7): 22.6 (14–30)	No apparent difference in prevalence of abnormal values for serum immunoglobulin or complement (no statistical analysis applied).

Table AX6-8.1 (cont'd). Effects of Lead on Immune Function in Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Wagernova et al. (1986) Czech NR	Design: longitudinal cohort (repeated measures for 2-years) Subjects: children (n = 92, 38 females) ages 11–13 yrs residing near a smelter; reference group (n = 67, 36 females), ages 11–13 years Outcome measures: serum IgA, IgE, IgG, IgM Analysis: comparison of outcome measures and between exposed and reference groups, stratified sex and season of sampling	Blood lead (µg/dL) mean: lead: ~23–42 reference: ~5–22	Significant (p NR, statistic NR) lower serum IgE and IgM levels in exposed group compared to reference group.
Latin America			
Pineda-Zavaleta et al. (2004) Mexico NR	Design: cross-sectional Subjects: children (n = 30 female, 35 male) ages 6–11 years, residing near smelter Outcome measures: mitogen- (PHA) and cytokine- (IFN-γ) induced nitric oxide and superoxide production in lymphocytes Analysis: multivariate linear regression	Blood lead (µg/dL) mean (range) for 3 schools: 1 (n = 21): 7.0 (3.5–25.3) 2 (n = 21): 20.6 (10.8–49.2) 3 (n = 23): 30.4 (10.3–47.5)	Significant (p = 0.036) association between increasing blood lead concentration and covariate adjusted decreasing nitric oxide production in PHA-activated lymphocytes (β = -0.00089, 95% CI: -0.0017 to -0.00005). Significant (p = 0.034) association between increasing blood lead concentration and covariate adjusted increasing super oxide production in IFN-γ-activated lymphocytes (β = −0.00389, 95% CI: 0.00031 to 0.00748). Covariates considered included age, sex, allergies, and blood arsenic (age, sex, and blood arsenic were retained). Significant effect of sex on associations, significant blood lead-arsenic interaction. Covariates considered included age, sex, allergies, urinary arsenic (age, sex, and urinary arsenic were retained).

Table AX6-8.1 (cont'd). Effects of Lead on Immune Function in Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia			
Sun et al. (2003); Zhao et al. (2004) China NR	Design: cross-sectional Subjects: children (n = 73) age 3–6 yrs Outcome measures: serum IgE, IgG, IgM; lymphocyte phenotype abundance Analysis: Nonparametric comparisons of outcome measures stratified by blood lead	Blood lead (µg/dL) mean (SD, range) (n = 217): 9.5 (5.6, 2.6–43.7)	Females: significantly higher ($p < 0.05$) IgE levels in high blood lead category (≥10 µg/dL, n = 16) compared to low category (<10 µg/dL, n = 17), and significantly lower IgG and IgM levels. A multivariate analysis of association between blood lead and IgE was noted but not described in sufficient detail to evaluate. All children: significantly lower ($p < 0.05$) $CD3^+CD4^+$ (%), $CD3^+CD8^+$ (%), $CD4^+CD8^+$ (%) in high blood lead (≥10 µg/dL, n = 38) compared to low blood lead (10 µg/dL, n = 35) group.

ANOVA, analysis of variance; CI, confidence interval; Ig, immunoglobulin; IFn-γ interferon- γ; IgG, immunoglobulin G; IgM, immunoglobulin M; IL-4, interleukin-4; NR, not reported; PHA, phytohemagglutinin; SD, standard deviation

Table AX6-8.2. Effects of Lead on Immune Function in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States			
Pinkerton et al. (1998) U.S. NR	Design: cross-sectional cohort Subjects: adult male smelter workers (n = 145, mean age 32.9±8.6); reference group, male hardware workers (n = 84, mean age 30.1±9.3) Outcome measures: differential blood cell counts; lymphocyte phenotype abundance; serum IgA, IgG, IgM; salivary IgA; lymphocyte proliferation (tetanus toxoid) Analysis: multivariate logistic regression with comparison of adjusted outcome measures between exposed and nonexposed groups	Blood lead (µg/dL) median (range) lead: 39 (15–55) reference: <2 (<2–12)	Covariate-adjusted outcomes in lead workers that were significantly ($p < 0.05$) different from nonexposed ([+], higher, [-] lower): [-] % monocytes, [-] % $CD4^+CD8^+$ cells, [-] % $CD8^+CD56^+$ cells. Significant ($p < 0.05$) adjusted regression coefficients in exposed group for independent variable: blood lead: [+] $CD19^+$ B-cells (%, no) time-integrated blood lead: [-] serum IgG, [+] $CD4^+CD45RA^+$ cells (%, no.) Covariates considered in the analysis included age, race, smoking habits, alcohol consumption, marijuana use, work shift, and various factors that might stimulate or suppress the immune system (e.g., exposure to direct sunlight, sleep hours, allergy, flu or cold symptoms). Covariates retained in the final model were age, age, race, work shift, smoking habits.
Sarasua et al. (2000) ATSDR Multi-site Study: Granite City, IL, Galena, KA; Joplin, MO; Palmerton, PA 1991	Design: cross-sectional cohort Subjects: children and adults (n = 2036) Outcome measures: total lymphocyte count, lymphocyte phenotype abundance, serum IgA, IgG, and IgM Analysis: multivariate linear regression	Blood lead (µg/dL) mean (SD, 5^{th}–95^{th} %tile): 6–35 mo: 7.0 (16, 1.1–16.1) 36–71mo: 6.0 (4.3, 1.6–14.1) 6–15 yr: 4.0 (2.8,1.1–9.2) 16–75 yr: 4.3 (2.9, 1.0–9.9)	No significant association (<0.05) between blood lead and outcomes in adults (age ≥16 yr). Covariates retained: age, sex, cigarette smoking, and study site.

Table AX6-8.2 (cont'd). Effects of Lead on Immune Function in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Fischbein et al. (1993) New York NR	Design: cross-sectional cohort Subjects: adult firearms instructors (n = 51), mean age 48 yr; age-matched reference subjects (n = 36). Outcome measures: lymphocyte phenotype abundance, lymphocyte proliferation (PHA, PMW, *Staph. aureus*) Analysis: comparison of outcome measures between reference and blood lead categories; multivariate linear regression	Blood lead (µg/dL) mean (SD) lead high (≥25): 31.4 (4.3) lead low (<25): 14.6 (4.6) reference: <10	Outcomes in lead workers that were significantly ($p < 0.05$) different from reference group ([+], higher, [-] lower): [-] CD^{+}_{3} cells (%, no.), [-] $CD4^{+}$ cells (%, no.), [-] $CD4^{+}CD8^{+}$ cells (no.), [-] HLA-DR cells (no.), [+] $CD20^{+}$ cells (%, no.), [-] mitogen (PHA)-induced lymphocyte proliferation, [-] mitogen (PWM)-induced lymphocyte proliferation; [-] lymphocyte response in mixed-lymphocyte culture. No effect on antigen (*Staph. aureus*)-induced lymphocyte proliferation. Significant ($p < 0.05$) association between increasing blood lead and decreasing abundance of $CD4^{+}$ phenotypes (%), and decreasing lymphocyte proliferative response in mixed lymphocyte cultures. Covariates retained: age, sex, smoking habits, and duration of exposure.
Europe			
Bergeret et al. (1990) France NR	Design: cross-sectional cohort Subjects: adult battery smelting workers (n = 34), mean age 40 yr; reference subjects (n = 34), matched for age, sex, ethnic origin, smoking and alcohol consumption habits, intake of antibiotics, and NSAIDs Outcome measures: PMN chemotaxis (FMLP); PMN phagocytosis (opsonized zymosan) Analysis: comparison of outcome measures between worker and reference groups	Blood lead (µg/dL) mean (SD): lead: 70.6 (18.) reference: 9.0 (4.3)	Significantly ($p < 0.05$) lower PMN chemotactic response (index) and phagocytic response in lead workers.

Table AX6-8.2 (cont'd). Effects of Lead on Immune Function in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Ewers et al. (1982) Germany NR	Design: cross-sectional cohort Subjects: adult male battery manufacture or smelter workers (n = 72), mean age 36.4 yr (16–58); reference workers (n = 53), mean age 34.8 yr (21–54) Outcome measures: serum IgA, IgG, IgM, C3; saliva IgA Analysis: parametric and nonparametric comparison of outcome measures between lead workers and reference subjects; linear regression	Blood lead (µg/dL) mean (range): lead: 55.4.0 (18.6–85.2) reference: 12.0 (6.6–20.8)	Significantly ($p < 0.05$) lower serum IgM, lower salivary IgA in lead workers compared to reference group.
Coscia et al. (1987) Italy NR	Design: cross-sectional cohort Subjects: adult lead workers (n = 32, 2 female), mean age 42.8 yr (SD 11.5); reference subjects (n = 25), mean age 38.6 yr (SD 13.3) Outcome measures: serum IgA, IgG, IgM, C3-C4; lymphocyte phenotype abundance Analysis: parametric comparison of outcome measures between worker and reference groups	Blood lead (µg/dL) mean (SD): lead: 62.3 (21.6) reference: NR	Outcomes in lead workers that were significantly ($p < 0.05$) different from reference group ([+], higher, [-] lower): [-] serum IgM, [+] serum C4, [+] lymphocyte abundance (%), [-] T-cell abundance (%, no., E-rosette forming cells), [+] B-cell abundance (%,no., immunoglobulin-bearing cells), [+] $CD8^+$ cell abundance (no.).
Governa et al. (1987) Italy NR	Design: cross-sectional cohort Subjects: adult male battery manufacture workers (n = 9), mean age 38.4 yr (SD 13.7); age-matched reference subjects (n = 18) Outcome measures: PMN chemotaxis (zymosan-activated serum) Analysis: parametric comparison of outcome measures between worker and reference groups, correlation	Blood lead (µg/dL) mean (SD): lead: 63.2 (8.2) reference: 19.2 (6.4)	Significantly ($p < 0.05$) lower PMN chemotactic response to zymosan activated serum. Effect magnitude was not correlated with blood lead.

Table AX6-8.2 (cont'd). Effects of Lead on Immune Function in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Valentino et al. (1991) Italy NR	Design: cross-sectional cohort Subjects: adult male lead scrap refining workers (n = 10), mean age 41.1 yr (SD 7.3, range: 28–54); age-matched reference subjects (n = 10) Outcome measures: PMN chemotaxis (C5 or FMLP) and phagocytosis (FMLP) Analysis: comparison of outcome measures between worker and reference groups, correlation	Blood lead (µg/dL) mean (SD, range): lead: 33.2 (5.6, 25–42) reference: 12.6 (2.5, 8.9–18)	Significantly ($p < 0.002$) lower PMN chemotactic response to C5 or FMLP and higher stimulated production of LT (leukotriene)B4 in lead workers compared to reference group. Effect magnitude correlated with blood lead. No effect on phagocytic activity.
Kimber et al. (1986) UK NR	Design: cross-sectional cohort Subjects: adult male TEL manufacture workers (n = 39) mean age: 45.1 yr; and age-matched reference subjects (n = 21); mean age 32.2 yr Outcome measures: serum IgA, IgG, IgM; mitogen (PHA)-induced lymphoblastogenesis; and NK cell cytotoxicity Analysis: comparison of outcome measures for exposed and reference groups	Blood lead (µg/dL) mean (SD, range): lead: 38.4 (5.6, 25–53) reference: 11.8 (2.2, 8–17)	No significant ($p < 0.05$) differences in outcomes between exposed and reference groups.
Latin America			
Queiroz et al. (1993) Brazil NR	Design: cross-sectional cohort Subjects: adult male battery manufacture workers (n = 39), mean age 33.9 yr (SD 12.1, range: 18–56); reference subjects (n = 39) matched by age and race Outcome measures: PMN chemotaxis (endotoxin LPS); phagocytic (endotoxin LPS) respiratory burst activity (NBT reduction) Analysis: nonparametric comparison of outcome measures between worker and reference groups	Blood lead (µg/dL) range: lead: 14.8–91.4 (>30, n = 52) reference: <10	Significantly ($p < 0.001$) lower chemotactic activity of PMNs, and lower phagocytic respiratory burst, in lead workers relative to reference group.

Table AX6-8.2 (cont'd). Effects of Lead on Immune Function in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Latin America (cont'd)			
Queiroz et al. (1994a) Brazil NR	Design: cross-sectional cohort Subjects: adult male battery manufacture workers (n = 60), mean age 33.9 yr (range: 18–56); reference subjects (n = 49) matched by age and race Outcome measures: PMN phagocytic/lytic activity (opsonized yeast) Analysis: nonparametric comparison of outcome measures between worker and reference groups	Blood lead (µg/dL) range: lead: 14.8–91.4 (>30, n = 27) reference: <10	Significantly ($p < 0.001$) lower lytic activity of PMNs in lead workers relative to reference group.
Queiroz et al. (1994b) Brazil NR	Design: cross-sectional cohort Subjects: adult male battery manufacture workers (n = 33), mean age 32.4 yr (range: 18–56); reference subjects (n = 20) matched by age and race Outcome measures: serum IgA, IgG, IgM; mitogen (PHA)-induced lymphocyte proliferation Analysis: parametric comparison of outcome measures between worker and reference groups	Blood lead (µg/dL) range: lead: 12.0–80.0 (>30, n = 27) reference: <10	No significant difference in outcomes ($p <$ NR; SD of lead worker and reference groups overlap) between lead workers and reference group.
Asia			
Kuo et al. (2001) China NR	Design: cross-sectional cohort Subjects: adult battery manufacture workers (n = 64, 21 female), ages: <40 yr 14, >50yr, 14); nonexposed reference subjects (n = 34, 17 female). Outcome measures: differential blood cell counts, lymphocyte phenotype abundance Analysis: comparison of outcome measures in exposed and reference groups, multivariate linear regression	Blood lead (µg/dL) mean: lead workers: 30	Significantly ($p < 0.05$) adjusted mean higher monocytes (%, no.), lower B cells (%), lower lymphocytes (no.), and lower granulocytes (no.) in lead workers compared to controls. Covariates retained: age, gender, and disease status (definition not reported).

Table AX6-8.2 (cont'd). Effects of Lead on Immune Function in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Mishra et al. (2003) India NR	Design: cross-sectional cohort Subjects: adult males occupationally exposed to lead (n = 84), mean age 30 yr; reference subjects (n = 30), mean age 29 yr Outcome measures: serum IFN-γ level, mitogen (PHA)-induced lymphocyte proliferation, NK cell cytotoxicity Analysis: comparison of outcome measures between lead-exposed and reference groups, correlation	Blood lead (μg/dL) mean (SD, range): 3-wheel drivers (n = 30): 6.5 (4.7, 0.0–17.5) battery workers (n = 34): 128.1 (13.2–400.8) jewelry makers: 17.8 (18.5, 3.1–76.8) reference: 4.5 (NR, 1.6–9.8)	Significantly ($p < 0.001$) lower lymphocyte proliferative response to PHA in lead-exposed groups compared to reference groups, higher IFN-γ production by blood monocytes.
Alomran and Shleamoon (1988) Iraq NR	Design: cross-sectional cohort Subjects: adult lead (oxide) workers (n = 39), mean age 35.6 yr (9,2, SD); age-matched reference subjects (n = 19) Outcome measures: serum IgA, IgG; mitogen (PHA, Con-A)-induced lymphocyte proliferation Analysis: comparison of outcome measures between lead workers and reference group	Blood lead (μg/dL) mean: lead: 54–64 reference: NR	Significantly ($p < 0.05$) lower lymphocyte proliferative response to PHA or Con A in lead workers, compared to reference group.
Cohen et al. (1989) Israel NR	Design: cross-sectional cohort Subjects: adult male occupationally lead exposed (n = 10), age range 22–70; age-matched reference subjects (n = 10) Outcome measures: mitogen (Con A, PHA)-induced-lymphocyte proliferation and T-suppressor cell proliferation; lymphocyte phenotype abundance Analysis: parametric comparison of outcome means between lead-exposed and reference groups	Blood lead (μg/dL) range: exposed: 40–51 reference: <19	Significantly ($p < 0.02$) higher mitogen (Con-A)-induced suppressor cell activity. No significant (p not reported) effects on abundance of T-cells (E-rosette-forming cells), OKT^{+}_{4}, OKT^{+}_{8}, or $OKT4^{+}/T8^{+}$ ratio; mitogen (Con A or PHA)-induced lymphocyte proliferation.

Table AX6-8.2 (cont'd). Effects of Lead on Immune Function in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Sata et al. (1998) Japan NR	Design: cross-sectional cohort Subjects: adult male lead stearate manufacture workers (n = 71), mean age 48 yr (range: 24–74); reference subjects (n = 28), mean age 55 yr (range: 33–67). Outcome measures: lymphocyte phenotype abundance Analysis: comparison of outcome measures in exposed and reference groups (ANCOVA), multivariate linear regression	Blood lead (µg/dL) mean (range): lead: 19 (7–50) reference: NR	Lead workers vs. reference: significantly ($p < 0.05$) covariate-adjusted lower $CD3^+CD45RO^+$ (no.) and higher $CD8^+$ cells (%). Significant ($p < 0.05$) association between exposure (categorical: yes/no) and lower $CD3^+CD45RO^+$cells (no.). Covariates retained: age and cigarette smoking habits.
Sata et al. (1997) Japan NR	Design: clinical Subjects: adult male lead smelter workers (n = 2) who underwent CaEDTA therapy Outcome measures: serum IgA, IgG, IgD, IgM; lymphocyte phenotype abundance Analysis: Parametric comparison of outcome measures before and after treatment, correlation of outcome means with blood lead	Blood lead (µg/dL): subject 1: 81 µg/dL at referral; mean before EDTA: 45.1 (SD 16.0); after chelation: 31.0 (9.8) subject 2: 68 µg/dL at referral; mean before EDTA: 43.3 (SD 14.1); after chelation: 33.7 (7.2)	Blood lead and outcome measures were sampled prior to and 24 hours after 3 CaEDTA treatments (on consecutive days) per week for 10 weeks. Comparison of mean outcome measures assessed before and after treatments showed significantly ($p < 0.05$) higher IgA, IgG, and IgM; and significantly higher $CD8^+$ T-cells and $CD57^+$ NK cells after treatment in subject 1. Serum IgG levels in subject 1 were significantly correlated (r-0.72) with blood lead concentration.
Heo et al. (2004) Korea NR	Design: cross-sectional cohort Subjects: adults, battery manufacture workers (n = 606; 52 females); ages: <30 yr, n = 184; >40 yr, n = 123. Outcome measures: serum IgE, IL-4, IFNγ Analysis: comparison of outcomes measures (ANOVA), stratified by age and blood lead	Blood lead (µg/dL) mean (SD): <30 yr: 22.0 (10.4) 30–39 yr: 23.0 (11.3) ≥40 yr: 24.1 (9.3)	Significantly higher ($p < 0.05$) serum IgE levels in blood lead category (≥30 µg/dL) compared to low categories (<10 or 10–29 µg/dL).

Table AX6-8.2 (cont'd). Effects of Lead on Immune Function in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Ündeger et al. (1996); Basaran and Ündeger (2000) Turkey NR	Design: cross-sectional cohort Subjects: adult male battery manufacture workers (n = 25), mean age, 33 yr (22–55); reference subjects (n = 25) mean age 33 yr (22–56). Outcome measures: differential blood cell counts; lymphocyte phenotype abundance; serum IgA, IgG, IgM, C3, and C4; neutrophil chemotaxis (zymosan-activated serum); latex particle-induced neutrophil phagocytic (latex particles) respiratory burst (NBT reduction) Analysis: nonparametric and parametric comparisons of outcome measures for exposed and reference groups	Blood lead (µg/dL) mean (SD): lead: 74.8 (17.8) reference: 16.7 (5.0)	Workers relative to reference: significantly ($p < 0.05$) lower serum IgG, IgM, C3, and C4 levels; lower $CD4^+$ ("T-helper") abundance, lower neutrophil chemotactic response; no significant difference in $CD20^+$ (B-cell) , $CD8^+$ ("T-suppressor") cell, $CD56^+$ (NK) cell abundance, or particle-induced NK cell respiratory burst.
Yücesoy et al. (1997a) Turkey NR	Design: cross-sectional cohort Subjects: adult male battery manufacture workers (n = 20), ages 39–48 yr; age-matched reference subjects (n = 12) Outcome measures: serum cytokines IL-1β, IL-2, TNFα, IFN-γ Analysis: parametric and nonparametric comparison of outcome measures in exposed and reference groups	Blood lead (µg/dL) mean (SE, range): lead: 59.4 (3.2, 42–94) reference: 4.8 (1.0, 2–15)	Significantly ($p < 0.05$) lower serum IL-1β and IFN-γ levels in lead workers compared to controls.
Yücesoy et al. (1997b) Turkey NR	Design: cross-sectional cohort Subjects: adult male battery manufacture workers (n = 50), ages 39–48 yr; age-matched reference subjects (n = 10) Outcome measures: lymphocyte phenotype abundance, NK cell cytotoxicity Analysis: comparison of outcome measures in exposed and reference groups	Blood lead (µg/dL) mean (SE, range): lead 1 (n = 20): 59.4 (3.2, 42–94) lead 2 (n = 30): 58.4 (2.5,26–81) reference: 4.0 (0.4, 2–6)	Significantly ($p < 0.05$) lower $CD20^+$ B-cell (%) abundance in lead workers compared to controls, no difference in % $CD4^+$ T-cell abundance.

Table AX6-8.2 (cont'd). Effects of Lead on Immune Function in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Africa			
Anetor and Adeniyi (1998) Nigeria NR	Design: cross-sectional cohort Subjects: adult male "lead workers" (n = 80), mean age, 36 yr (21–66) and reference subjects (n = 50), mean age 37 yr (22–58). Outcome measures: serum IgA, IgG, and IgM; lymphocyte count Analysis: comparison of outcomes measures in workers and reference group, linear regression, principal component analysis	Blood lead (μg/dL) mean (SE): lead: 53.6 (0.95) reference: 30.4 (1.4)	Significantly lower ($p < 0.05$) serum IgA, IgG, and total blood lymphocyte levels; significant associations and interactions between blood lead and serum total globulins (note high blood lead levels in reference).

ANOVA, analysis of variance; EDTA, ethylenediaminetetraacetic acid; FMLP, N-formyl-L-methionyl-L-leucyl-L-phenyl-alanine; IFn-γ interferon-γ; Ig, immunoglobulin A; LPS, lipopolysaccharide; LT, leukotriene; NBT, nitroblue tetrazolium; NK, natural killer; NR, not reported; NSAIDS, non-steroidal anti-inflammatory agents; PHA, phytohemagglutinin; PMW pokeweed mitogen; SD, standard deviation; SE, standard error; TEL, tetraethyl lead

CHAPTER 6 ANNEX

ANNEX TABLES AX6-9

Table AX6-9.1. Effects of Lead on Biochemical Effects in Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States			
Piomelli et al. (1982) New York 1976	Design: cross-sectional Subjects: children (n = 2002), ages 2–12 yr Outcome measures: EP Analysis: linear regression	Blood lead (μg/dL) range: 2–98	Regression equation relating blood lead concentration to EP (log-transformed): $\alpha = 1.099$, $\beta = 0.016$, r: 0.509, $p < 0.001$ Threshold for increase in EP estimated to be: 15.4 μg/dL (95% CI: 12.9–18.2)
Soldin et al. (2003) Washington DC 2001–2002	Design: cross-sectional Subjects: children (n = 4908, 1812 females), age range 0–17 yr Outcome measures: EP Analysis: locally weighted scatter plot smoother (LOWESS)	Blood lead (μg/dL): mean (range 1–17 yr): 2.2–3.3 median (1–17 yr): 3 range: <1–103	EP increases as blood lead concentration increased above 15 mg/dL. A doubling of EP occurred with an increase in blood lead concentration of approximately 20 μg/dL (a polynomial expression for EP as a function of blood lead (PbB) is: $EP = -0.0015(PbB)^3 + 0.1854(PbB)^2 - 2.7554(PbB) + 30.911$ ($r^2 = 0.9986$) (derived from data in Table 2 of Soldin et al. (2003)
Europe			
Roels and Lauwerys (1987) Belgium 1974–1980	Design: cross-sectional Subjects: children (n = 143), age range 10–13 yr Outcome measures: ALAD, urinary ALA, EP Analysis: linear regression, correlation	Blood lead (μg/dL) range: children: 15–41	Linear regression for EP (log-transformed) and blood lead concentration: $\alpha = 1.321$, $\beta = 0.025$, r = 0.73 (n = 51) Linear regression for ALA (log-transformed) and blood lead concentration: $\alpha = 0.94$, $\beta = 0.11$, r = 0.54 (n = 37) Linear regression for ALAD (log-transformed) and blood lead concentration: $\alpha = 1.864$, $\beta = -0.015$, r = -0.87 (n = 143)

Table AX6-9.1 (cont'd). Effects of Lead on Biochemical Effects in Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Latin America			
Perez-Brava et al. (2004) Chile NR	Design: cross-sectional; Subjects: children (n = 93, 43 males), age range: 5-12 yrs who attended school near a powdered lead storage facility Outcome measures: blood Hb and Hct, ALAD genotype Analysis: comparison of outcome measures between ALAD genotype strata	Blood lead (µg/dL) mean (SE): ALAD 1 (n = 84): 13.5 (8.7) ALAD 2 (n = 9): 19.2 (9.5)	Mean blood lead, blood Hct, and Hct not different between ALAD genotypes (p = 0.13)

ALA, δ-aminolevulinic acid; ALAD, δ-aminolevulinic acid dehydratase; EP, erythrocyte protoporphyrin

Table AX6-9.2. Effects of Lead on Biochemical Effects in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe			
Gennart et al. (1992) Belgium NR	Design: cross-sectional cohort Subjects: adult battery manufacture workers (n = 98), mean age, 37.7 yr (range 22–55); reference group (n = 85), mean age 38.8 yr (24–55) Outcome measures: blood Hct, blood EP, urine ALA Analysis: linear regression	Blood lead (μg/dL) mean (SD, range): lead: 51.0 (8.0, 40–70) reference: 20.9 (11.1, 4.4–30.0	Significant association between increasing blood lead concentration and increasing (log) blood EP ($\alpha = 0.06$, $\beta = 0.019$, $r = 0.87$, $p = 0.0001$) or (log) urine ALA ($\alpha = 0.37$, $\beta = 0.008$, $r = 0.64$, $p < 0.0001$) (No apparent analysis of covariables)
Mohammed-Brahim et al. (1985) Belgium NR	Design: cross-sectional cohort Subjects: adult smelter and ceramics manufacture workers (n = 38, 13 females); reference subjects (n = 100) matched with worker group by age, sex, and socioeconomic status. Outcome measures: blood P5N, EP, ALAD, R/ALAD (ratio of ALAD before and after reactivation). Analysis: comparison of outcome measures (ANOVA) between lead workers and reference group; correlation	Blood lead (μg/dL) mean (SD, range): lead: 48.5 (9.1, 27.8–66.6) reference: 14.3 (6.7, 5.6–33.6) Urine lead (μg/g creatinine) mean (SD, range): lead: 84.0 (95.9, 21.8–587) reference: 10.5 (8.2, 1.7–36.9)	Significantly lower (p = NR) P5N in lead workers (males or females, or combined) compared to corresponding reference groups. Correlations with blood lead: log P5N $r = -0.79$ ($p < 0.001$) log ALAD $r = -0.97$ (p = NR) R/ALAD $r = -0.94$ ($p < 0.001$) log EP $r = 0.86$ (p = NR) Correlations with urine lead: log P5N $r = -0.74$ (p = NR) log ALAD $r = -0.79$ (p = NR) R/ALAD $r = -0.84$ ($p < 0.001$) log EP $r = 0.80$ (p = NR)
Roels and Lauwerys (1987) Belgium 1974–1980	Design: cross-sectional Subjects: adults (n = 75, 36 females) Outcome measures: ALAD, urinary ALA, EP Analysis: linear regression, correlation	Blood lead (μg/dL) range: adult males: 10–60 adult females: 7–53	Linear regression for EP (log-transformed) and blood lead concentration: adult male (n = 39): $\alpha = 1.41$, $\beta = 0.014$, $r = 0.74$, $p < 0.001$ adult female (n = 36): $\alpha = 1.23$, $\beta = 0.027$, $r = 0.81$, $p < 0.001$ Linear regression for ALA (log-transformed) and blood lead concentration: adult male (n = 39): $\alpha = 0.37$, $\beta = 0.006$, $r = 0.41$, $p < 0.01$ adult female (n = 36): $\alpha = 0.15$, $\beta = 0.015$, $r = 0.72$, $p < 0.001$

Table 6-9.2 (cont'd). Effects of Lead on Biochemical Effects in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Grandjean (1979) Denmark NR	Design: longitudinal Subjects: male battery manufacture workers (n = 19), mean age 32 yr (range 22–49) Outcome measures: EP Analysis: EP and blood lead for serial measurements displayed graphically	Blood lead (µg/dL) median (range): Group 1(n = 5): 47.7 (22.8–53.9) group 2 (n = 5): 37.3 (35.2–53.9)	Five subjects (group 1) showed declines in EP with declining blood lead (33–58 µg/dL) over a 10-month period; 5 subjects (group 2) showed no change in EP with a change in blood lead concentration (25–54 µg/dL) over the same period.
Alessio et al. (1976) Italy NR	Design: cross-sectional Subjects: adult male lead worker (n = 316), age range NR Outcome measures: blood ALAD, EP, urine ALA, CP Analysis: linear regression, correlation	Blood lead (µg/dL) range: 10–150	Regression relating outcomes to blood lead concentration: ALAD (ln-transformed) (n = 169): α3.73, $\beta = -0.031$, r = 0.871 ALAU (ln-transformed) (n = 316): $\alpha = 1.25$, $\beta = 0.014$, r = 0.622 UCP (ln-transformed) (n = 252): $\alpha = 2.18$, $\beta = 0.34$, r = 0.670 EP (log-transformed (males, n = 95): $\alpha = 0.94$, $\beta = 0.0117$ EP (log-transformed (females, n = 93): $\alpha = 1.60$, $\beta = 0.0143$
Cocco et al. (1995) Italy 1990	Design: longitudinal Subjects: adult male foundry workers (n = 40), mean age 25.1 yr (SD 2.1, range 21–28) Outcome measures: serum total-, HDL- and LDL-cholesterol, blood Hb, urine ALA, erythrocyte G6PD Analysis: comparison of outcomes between pre-exposure (at start of employment, sample 1) and after 172 (range 138–217, sample 2) days	Blood lead (µg/dL) mean (range): sample 1: 10.0 (7–15) sample 2: 32.7 (20–51)	G6PD levels were unrelated to starting blood lead; however, they increased in subjects whose blood lead concentration increased from <30 µg/dL to >30 µg/dL or decreased from >30 µg/dL to <30 µg/dL. Increasing exposure duration was significantly associated with decreasing magnitude of change in G6PD (sample 1 <30 µg/dL: $\beta = -0.3980$, SE 0.1761, $p < 0.05$; sample 1 >30 µg/dL: $\beta = -1.3148$, SE 0.3472, $p < 0.05$) and, in the >30 µg/dL subgroup, increasing blood lead was associated with decreasing magnitude of change of G6PD ($\beta = -2.0797$, SE 0.7173, $p < 0.05$). Serum cholesterol levels were unrelated to blood lead concentration.

Table 6-9.2 (cont'd). Effects of Lead on Biochemical Effects in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Francaso et al. (2002) Italy NR	Design: cross-sectional cohort Subjects: adult battery manufacture workers (n = 37, 6 females), mean age 41 yr (SD 7); reference office workers (n = 29, 8 females), mean age 38 yr (SD 21) Outcome measures: lymphocyte DNA strand breaks, ROS, GSH Analysis: comparison of outcome measures between lead workers and reference group (ANOVA), logistic regression	Blood lead (μg/dL) mean (SD): lead: 39.6 (7.6) 4.4 (8.6)	Covariate-adjusted DNA strand breaks were significantly higher in lead workers compared to the reference group and significantly associated with increased blood lead ($p = 0.011$). Covariate-adjusted lymphocyte ROS was significantly higher and GSH significantly lower in the lead workers compared to the reference group. Lower GSH levels were significantly associated with increasing blood lead concentration ($p = 0.006$). Odds ratios (OR) for DNA strand breaks and lower GSH levels were significant (lead workers vs. reference): DNA strand breaks: OR = 1.069 (95% CI: 1.020–1.120, $p = 0.005$) GSH: OR = 0.634 (95% CI: 0.488–0.824, $p = 0.001$) ROS: OR = 1.430 (95% CI: 0.787–2.596, $p = 0.855$) Covariates retained: age, alcohol consumption and tobacco smoking.
Hernberg et al. (1970) Poland NR	Design: cross-sectional Subjects: adult lead workers (n = 166); reference group (n = 16) Outcome measures: blood ALAD Analysis: regression, correlation	Blood lead (μg/dL) range: 5–95	Linear regression for blood ALAD (log-transformed) and blood lead concentration (n = 158): $\alpha = 2.274$, $\beta = -0.018$, $r = -0.90$, $p < 0.001$
Bergdahl et al. (1997) Sweden NR	Design: cross-sectional Subjects: adult smelter worker (n = 89); reference groups (n = 24) Outcome measures: blood lead, erythrocyte ALAD-bound lead, ALAD genotype Analysis: comparison of outcome measures	Blood lead (μg/dL): range 0.8–93 Urine lead (mg/L): range 1–112 Bone lead (μg/g) range −19–101	No association between ALAD genotype and lead measures.
Selander and Cramer (1970) Sweden NR	Design: cross-sectional Subjects: adult battery manufacture workers (n = 177) Outcome measures: urine ALA Analysis: regression, correlation	Blood lead (μg/dL) range: 6–90	Linear regression for urine ALA (log-transformed) and blood lead concentration (n = 150): $\alpha = -1.0985$, $\beta = 0.0157$, $r = 0.74$

Table 6-9.2 (cont'd). Effects of Lead on Biochemical Effects in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Wildt et al. (1987) Sweden NR	Design: longitudinal Subjects: adult battery manufacture workers (n = 234, 37 females) mean age 35 y (range 17–70); reference group (n = 951, 471 females), mean age 39 yr (range 19–67) Outcome measures: EP Analysis: analysis of variability over time, linear regression, correlation	Blood lead (µg/dL) mean (range): lead: 10–80 reference: male: 11.3 (8–27) female: 8.5 (5–21)	Linear regression for EP (log-transformed) and blood lead concentration: males (n = 851): $\alpha = 1.21$, $\beta = 0.0148$, r = 0.72 females (n = 139): $\alpha = 1.48$, $\beta = 0.0113$, r = 0.56
Asia			
Hsieh et al. (2000) China NR	Design: cross-sectional Subjects: Adults in general population (n = 630, 255 females) Outcome measures: blood Hb, Hct, RBC count, ALAD genotype Analysis: comparison of outcome measures between ALAD genotype strata	Blood lead (µg/dL) mean (SD): ALAD 1,1 (n = 630): 6.5 (5.0) ALAD 1,1/2,2 (n = 30) 7.8 (6.0)	Mean blood lead not different between ALAD genotype strata (p = 0.17). RBC count, Hb, Hct not different between ALAD genotype strata (p = 0.7)
Jiun and Hsien (1994) China 1992	Design: longitudinal Subjects: adult male lead workers (n = 62), ages NR; reference group (n = 62, 40 females), ages NR Outcome measures: plasma MDA Analysis: comparison of outcome measures between lead workers and reference group, linear regression	Blood lead (µg/dL) mean (SD, range): lead: 37.2 (12.5, 18.2–76.0) reference: 13.4 (7.5, 4.8–43.9)	Plasma MDA levels significantly ($p < 0.0001$) higher (approximately 2x) in lead workers whose blood lead concentration 35 µg/dL compared to ≤30 µg/dL. In subjects with blood lead >35 µg/dL, blood lead and plasma MDA were significantly correlated: blood lead = 9.584(MDA)+24.412 (r = 0.85)
Froom et al. (1999) Israel 1980–1993	Design: longitudinal survey Subjects: adult male battery manufacturing workers (n = 94), mean age, 38 yr (SD 9, range 26–60) Outcome measures: blood Hb, blood EP Analysis: multivariate linear regression	Blood lead (µg/dL) range of 13-yr individual subject means 20–61 µg/dL	Weak (and probably not significant) covariate-adjusted association between blood Hb and individual sample blood lead ($\beta = -0.0039$, SE 0.0002), subject average blood lead ($\beta = -0.0027$, SE 0.0036), or blood EP ($\beta = -0.001$, SE 0.0007) Covariates retained in model were age and smoking habits.

Table 6-9.2 (cont'd). Effects of Lead on Biochemical Effects in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Krisatal-Boneh et al. (1999) Israel 1994–1995	Design: cross-sectional cohort Subjects: adult male battery manufacture workers (n = 56), mean age 43.1 yr (SD 10.6); reference group (n = 87), mean age 43.2 yr (SD 8.3) Outcome measures: serum total-, HDL-, LDL-cholesterol, HDL:total ratio, triglycerides Analysis: comparison of outcome measures between lead workers and reference group (ANOVA), multivariate linear regression	Blood lead (µg/dL) mean (SD): lead: 42.3 (14.9) reference: 2.7 (3.6)	Covariate-adjusted serum total-cholesterol ($p = 0.016$) and HDL-cholesterol ($p = 0.001$) levels were significantly higher in lead workers compared to reference group. Covariates retained in ANOVA: age, body mass index, season of sampling, nutritional variables (dietary fat, cholesterol, calcium intakes), sport activities, alcohol consumption, cigarette smoking, education, job seniority. Increasing blood lead concentration was significantly associated with covariate-adjusted total cholesterol ($\beta = 0.130$, SE 0.054, $p = 0.017$) and HDL-cholesterol ($\beta = 0.543$, SE 0.173, $p = 0.002$). Covariates retained: age, body mass index. Stepwise inclusion of other potential confounders had no effect.
Solliway et al. (1996) Israel NR	Design: cross-sectional cohort Subjects: adult male battery manufacture workers (n = 34), mean age: 44 yr (SD 13); reference subjects (n = 56), mean age 43 yr (SD 12); cohorts constructed to have similar age, ethnic characteristics, socioeconomic status, education level, and occupation Outcome measures: urinary ALA, erythrocyte GSH-peroxidase Analysis: parametric comparison of outcome measures between lead and reference groups, correlation	Blood lead (µg/dL) mean (SD, range): lead: 40.7 (9.8, 23–63) reference: 6.7 (2.4, 1–13)	Significantly lower mean erythrocyte GSH-peroxidase activity ($p < 0.005$) in and higher urinary ALA ($p < 0.001$) in lead workers compared to reference group.
Ito et al. (1985) Japan NR	Design: cross-sectional cohort Subjects: adult male steel (smelting, casting) workers (n = 712), age range 18–59 yr; reference (office workers) group (n = 155, total), age range 40–59 yr Outcome measures: serum LPO and SOD, total and HDL-cholesterol, phospholipid Analysis: comparison of outcome measures between lead workers and reference group, correlation	Blood lead (µg/dL) range: lead: 5–62 reference: NR	When stratified by age, significantly ($p < 0.05$) higher serum HDL-cholesterol and LPO in lead workers, age range 40–49 yr, compared to corresponding strata of reference group. Serum lipoperoxide levels increased as blood lead increased above 30 µg/dL (p = NR), SOD appeared to decrease with increasing blood lead concentration (p = NR)

Table 6-9.2 (cont'd). Effects of Lead on Biochemical Effects in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Makino et al. (1997) Japan 1990–1994	Design: longitudinal survey Subjects: adult male pigment or vinyl chloride stabilizer manufacture workers (n = 1573) mean age 45 yr Outcome measures: blood Hb, Hct, RBC count Analysis: parametric comparison of outcome measures, stratified by blood lead, linear regression	Blood lead (μg/dL) mean (SD, range): 12.6 (2.0, 1–39) Urine lead (μg/L) mean (SD, range): 10.2 (2.7, 1–239)	Significantly higher ($p < 0.001$) Hct, blood Hb and RBC count in blood lead category 16–39 μg/dL, compared to 1–15 μg/dL category. Significant positive correlation between blood lead concentration and Hct: $\alpha = 42.95$, $\beta = 0.0586$ ($r = 0.1553$, $p < 0.001$), blood Hb: $\alpha = 14.65$, $\beta = 0.0265$ ($r = 0.1835$, $p < 0.001$) and RBC count $\alpha = 457$, $\beta = 0.7120$ ($r = 0.1408$, $p < 0.001$).
Morita et al. (1997) Japan NR	Design: cross-sectional cohort Subjects: male lead workers (n = 76), mean age 42 yr (range 21–62); reference subjects (n = 13, 6 females), mean age, males 41 yr (range 26–52), females 45 yr (range 16–61) Outcome measures: blood NADS, ALAD Analysis: comparison of outcome measures (ANOVA) between blood lead categories, linear regression	Blood lead (μg/dL) mean (SD, range) lead: 34.6 (20.7, 2.2–81.6)	Significantly lower ($p < 0.01$) blood NADS and ALAD in blood lead categories >20 μg/dL compared to <20 μg/dL, with dose trend in magnitude of difference. Significant associations between increasing blood lead and decreasing blood NADS and ALAD in lead workers: NADS: $\alpha = 0.843$, $\beta = -0.00971$, $r = -0.867$, $p < 0.001$, $n = 76$ logALAD: $\alpha = 1.8535$, $\beta = -0.015$, $r = -0.916$, $p < 0.001$, $n = 58$
Oishi et al. (1996) Japan NR	Design: cross-sectional Subjects: adult glass and pigment manufacture workers (n = 418, 165 females), mean age 33 yr (range 18–58); reference workers (n = 227, 89 females), mean age 30 yr (range 17–59) Outcome measures: plasma ALA, urinary ALA Analysis: linear regression, correlation	Blood lead (μg/dL) mean (SD, range): lead: 48.5 (17.0, 10.3–99.4 reference: 9.6 (3.3, 3.8–20.4)	Significant correlation between blood lead concentration and plasma and urinary ALA (both log-transformed): plasma ALA: $\alpha = 0.327$, $\beta = 0.022$, $r = 0.742$ urinary ALA: $\alpha = -0.387$, $\beta = 0.022$, $r = 0.711$ Significant correlation between plasma and urinary ALA: $\alpha = 6.038$, $\beta = 4.962$, $r = 0.897$

Table 6-9.2 (cont'd). Effects of Lead on Biochemical Effects in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Sugawara et al. (1991) Japan NR	Design: cross-sectional cohort Subjects: adult lead workers and reference group (n = 32, total), ages NR Outcome measures: plasma and erythrocyte lipoperoxide and SOD; erythrocyte CAT, GSH, and methemoglobin Analysis: comparisons of outcome measures between lead workers and reference group, linear regression and correlation	Blood lead (µg/dL) mean (SD, range): lead: 57.1 (17.6, 20–96) reference: NR	Significantly ($p < 0.01$) higher erythrocyte LPO and lower SOD, CAT and GSH levels in workers compared to reference group. Erythrocyte lipoperoxide ($r = 0.656$) and GSH ($r = -0.631$) were significantly correlated with blood lead.
Kim et al. (2002) Korea 1996	Design: cross-sectional cohort Subjects: adult male secondary lead smelter workers (n = 83), mean age: 38.7 yr (SD 10.8); reference subjects (n = 24), mean age: 32.0 (SD 10.8) Outcome measures: blood Hb, blood ALAD, blood EP, blood P5N Analysis: parametric comparison (ANOVA) of outcome measures between lead workers and reference group, correlation, multivariate linear regression	Blood lead (µg/dL) mean (SD) lead: 52.4 (17.7) reference: 6.2 (2.8)	Significantly ($p < 0.05$) lower blood P5N, ALAD, and Hb; and higher blood EP in lead workers compared to controls. Significant ($p < 0.001$) correlations (in lead worker group) with blood lead: P5N ($r = -0.704$), log EP ($r = 0.678$), log ALAD ($r = -0.622$). Significant association between increasing EP and decreasing blood Hb: blood lead ≥60 µg/dL: $\beta = -1.546$ (95% CI: -2.387 to -0.704, $r^2 = 0.513$, $p = 0.001$) blood lead <60 µg/dL: $\beta = -1.036$ (95% CI: -1.712 to -0.361, $r^2 = 0.177$, $p = 0.003$) Significant association between increasing P5N and increasing blood Hb (high blood lead group only): blood lead ≥60 µg/dL: $\beta = 0.222$ (95% CI: 0.015 to 0.419, $r^2 = 0.513$, $p = 0.036$) Covariates included in model: P5N, log serum ferritin, log EP
Lee et al. (2000) Korea NR	Design: cross-sectional cohort Subjects: adult male lead workers (n = 95; secondary smelter, PVC-stabilizer manufacture, battery manufacture); mean age 42.8 yr (SD 9.3, range 19–64); reference group (n = 13), mean age 35.1 yr (SD 9.9, range 22–54) Outcome measures: urinary ALA, EP Analysis: correlation	Blood lead (µg/dL) mean (SD, range): lead: 44.6 (12.6, 21.4–78.4) reference: 5.9 (1.2, 4.0–7.2)	Significant correlation between increasing DMSA-provoked urinary lead and urinary ALA ($r = 0.31$, $p < 0.002$) and EP ($r = 0.35$, $p < 0.001$).

Table 6-9.2 (cont'd). Effects of Lead on Biochemical Effects in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Schwartz et al. (1997) Korea 1994-1995	Design: cross-sectional Subjects: adult male battery manufacture workers (n = 57), mean age 32 yrs (SD 6). Outcome measures: blood Hb, Hb A_1, and Hb_{A2}, ALAD genotype Analysis: comparison of outcome measures between ALAD genotype strata	Blood lead (µg/dL) mean (SD): ALAD 1,1 (n = 38): 26.1 (9.8) ALAD 1 2 (n = 19): 24.0 (11.3)	Mean blood lead (p = 0.48) and blood Hb levels (p = 0.34) were not different between ALAD genotype strata.
Gurer-Orhan et al. (2004) Turkey NR	Design: cross-sectional cohort Subjects: adult male battery manufacture workers (n = 20), mean age 35 yr (SD 8); reference workers (n = 16), mean age 32 yr (SD 9) Outcome measures: blood ALAD, EP, erythrocyte MDA, CAT, G6PD, blood GSH:GSSG Analysis: comparison of outcome measures between lead workers and reference group, correlation	Blood lead (µg/dL) mean (SD): lead: 54.6 (17) reference: 11.8 (3.2)	Significant correlation between blood lead concentration and blood ALAD ($r = -0.85$, $p < 0.0001$) and EP ($r = 0.83$, $p < 0.001$). Significant correlation between blood lead concentration and erythrocyte MDA ($r = 0.80$, $p = <0.0001$), erythrocyte G6PD ($r = 0.70$, $p < 0.0001$, erythrocyte CAT ($r = 0.62$, $p < 0.001$), blood GSH ($r = 0.64$, $p < 0.0005$), blood GSSG ($r = 0.67$, $p < 0.0001$). GSH:GSSG ratio lower (p = NR) in lead workers (3.2), compared to controls (8.0).
Suzen et al. (2003) Turkey NR	Design: cross-sectional Subjects: Male lead battery manufacture workers (n = 72), age range 24-45 yrs. Outcome measures: blood ALAD, urine ALA, ALAD genotype Analysis: comparison of outcome measures between ALAD genotype strata	Blood lead (µg/dL) mean (SD, range): All: 34.5 (12.8, 13.4-71.8) ALAD 1,1 (n = 51) 34.4 (13.1, 13.4-71.8) ALAD 2 (n = 21) 34.9 (12.6, 19.2-69.6)	Mean blood lead concentration (p = 0.88) and blood ALAD activity (p = 0.33) were not different between ALAD genotype strata. Mean urinary ALA was significantly higher ($p < 0.05$) in the ALAD 1-1 stratum.

ALA, δ-aminolevulinic acid; ALAD, δ-aminolevulinic acid dehydratase; ANOVA, analysis of variance; CAT, catalase; CP, coproporphryn; DMSA, dimercaptosuccinic acid; EP, erythrocyte protoporphyrin; G6PD, glucose-6-phosphate dehydrogenase; GSH, reduced glutathione; GSSG, glutathione disulfide; Hb, blood hemoglobin; Hct, hematocrit; HDL, high-density lipoprotein; LDH, lactate dehydrogenase; LPO, lipoperoxide; MDA, malondialdehyde; NADS, adenine dinucleotide synthetase; OR, odds ratio; P5N, erythrocyte pyrymidine-5'nucleotidase; R/ALAD, ratio of ALAD activity, before and after reactivation; RBC, red blood cells; ROS reactive oxygen species; SD, standard deviation; SE, standard estimation; SOD, superoxide dismutase; UCP, urinary coproporphyrin

Table AX6-9.3. Effects of Lead on Hematopoietic System in Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States			
Liebelt et al. (1999) Connecticut NR	Design: cross-sectional Subjects: children (n = 86, 31 female), ages 1–6 yr Outcome measures: serum EPO, blood Hb Analysis: ANOVA of outcome measures stratified by blood lead, linear regression	Blood lead (µg/dL) median (range): 18 (2–84) 84% <35	Significant association between increasing blood lead concentration and decreasing serum EPO concentration (β = -0.03, p = 0.02). Covariates included in model were blood Hb (β = -1.36, p < 0.01) (age was not included), R^2 = 0.224. Predicted decrease in serum EPO per 10 µg/dL was 0.03 mIU/mL. No significant association between blood lead and blood Hb.
Schwartz et al. (1990) Idaho 1974	Design: cross-sectional Subjects: children (n = 579), ages 1–5 yr, residing near an active smelter (with uncontrolled emissions) Outcome measures: Hct Analysis: logistic regression	Blood lead (µg/dL) range: 11–164	Significant association between increasing blood lead concentration and probability of anemia (Hct<35%) (β_1: 0.3083, SE 0.0061) and age (β_2: -0.3831, SE 0.1134). A 10% probability of anemia was predicted to be associated with blood lead concentration of approximately 20 µg/dL at age 1 yr, 50 µg/dL at age 3 yr, and 75 µg/dL at age 5 yrs (from Fig. 2 Schwartz et al. (1990). Regression model relating Hct to blood lead (BL µg/dL) and age (AGE, yr): $Hct=A/(1+\exp(\beta_0+\beta_1 BL+\beta_2 AGE))$: A = 39.42 (SE 0.79, p = 0.0001) β_0 = -3.112 (SE 0.446, p = 0.0001) β_1 = 0.0133 (SE 0.0041, p = 0.0005) β_2 = -0.2016 (SE 0.0905, p = 0.0129) Based on above model, a 10% decrease in hematocrit (from 39.5 to 35.5%) is predicted in association with blood lead concentrations of 85, 115, and 145 µg/dL, at ages 1, 3, and 5 yrs, respectively.

Table AX6-9.3 (cont'd.). Effects of Lead on Hematopoietic System in Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe			
Graziano et al. (2004) (Factor Litvak et al. (1999, 1998) Yugoslavia 1985–1998	Design: prospective Subjects: children (n = 311; age range: 4.5–12 yr) from high-lead (smelter/refinery) and low-lead areas Outcome measures: blood Hb, serum EPO. Analysis: multivariate linear regression (GEE for repeated measures)	Blood lead (μg/dL) range: 4.5 yr: 4.6–73.1 6.5 yr: 3.1–71.7 9.0 yr: 2.3–58.1 Blood lead (μg/dL) means for ages 4.5 – 12 yrs: high lead: 30.6–39.3 low lead: 6.1–9.0	Significant association between increasing blood lead concentration and increasing serum EPO concentration at ages 4.5 ($p < 0.0001$) and 6.5 yr ($p < 0.0007$), with decreasing regression slope with age: 4.5 yr: $\beta = 0.21$ (SE 0.043, $p = 0.0001$); 6.5 yr: $\beta = 0.11$ (SE 0.41, $p = 0.0103$); 9.5 yr: $\beta = 0.029$ (SE 0.033, $p = 0.39$); 12 yr: $\beta = 0.016$ (SE 0.031, $p = 0.60$). Covariates retained in regression model were age (α), blood lead (β), and blood Hb (γ). GEE for repeated measures yielded (Factor-Litvak et al. 1998, updated from personal communication from Graziano 07/2005): γ: 0.6097 (95% CI:-0.0915, -0.0479; $p < 0.0001$) 4.5 yr: $\alpha = 1.3421$ (95%CI: 1.0348-1.6194, $p < 0.0001$), $\beta = 0.2142$ (0.1282-0.3003, $p < 0.0001$) 6.5 yr: $\alpha = 1.66201.3737$-1.9503, $p < 0.0001$), $\beta = 0.1167$ (0.0326-0.2008, $p < 0.001$) 9.5 yr: $\alpha = 1.7639$ (1.4586-2.0691, $p < 0.0001$), $\beta = 0.0326$ (-0.0346-0.0998, $p = 0.1645$). 12 yr: $\alpha = 1.8223$ (1.524-2.1121, $p < 0.0001$), $\beta = 0.0112$ (-0.0359-0.0584, $p = 0.1645$). Based on the GEE, the predicted increase in serum EPO per 10 μg/dL increase in blood lead concentration (at Hb=13 g/dL) \ was: 1.25 mIU/mL (36%) at age 4.5 yr and 1.18 (18%) at age 6.5 y. Blood Hb levels were not significantly different in children from high-lead area (mean 25–38 μg/dL) compared to low-lead area (mean: 5–9 μg/dL).

Table AX6-9.3 (cont'd). Effects of Lead on Hematopoietic System in Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Latin America			
Perez-Brava et al. (2004) Chile NR	Design: cross-sectional; Subjects: children (n = 93, 43 males), age range: 5-12 yrs who attended school near a powdered lead storage facility Outcome measures: blood Hb and Hct, ALAD genotype Analysis: comparison of outcome measures between ALAD genotype strata	Blood lead (µg/dL) mean (SE): ALAD 1 (n = 84): 13.5 (8.7) ALAD 2 (n = 9): 19.2 (9.5)	Mean blood lead, blood Hb, and Hct not different between ALAD genotypes

EPO, serum erythropoietin; GEE, generalized estimating equation; Hct, hematocrit; Hb, blood hemoglobin; SE, standard estimation

Table AX6-9.4. Effects of Lead on Hematopoietic System in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States			
Hu et al. (1994) U.S. 1991	Design: survey Subjects: adult male carpentry workers (n = 119), mean age: 48.6 yr (range: 23–67) Outcome measures: blood Hct, blood Hb Analysis: multivariate linear regression	Blood lead (µg/dL) mean (SD, range): 8.3 (4.0, 2–25) Bone lead (µg/g) mean (SD, range) tibia: 9.8 (9.5, -15–39) patella: 13.9 (16.6, -11–78)	Significant association between increasing patella bone lead and decreasing covariate adjusted blood Hb (β = -0.019, SE 0.0069, p = 0.008, R^2 = 0.078) and blood Hct (β = -0.052, SE 0.019, p = 0.009, R^2 = 0.061). After adjustment for bone lead measurement error, a 37 µg/dL increase in patella bone lead level (from the lowest to highest quintile) was associated with a decrease in blood Hb and Hct of 11 g/L (95% CI: 2.7–19.3 g/L) and 0.03 (95% CI, 0.01 - 0.05), respectively. Covariates considered: age, body mass index, tibia lead, patella lead, blood lead, current smoking status, alcohol consumption Covariates retained: patella bone lead, alcohol consumption, body mass index.
Europe			
Osterode et al. (1999) Austria NR	Design: cross-sectional cohort Subjects: adult male lead workers (n = 20), ages 46 yr (SD, 7); age-matched reference group (n = 20) Outcome measures: blood PCV, blood Hb, serum EPO, blood erythroid progenitor (BFU-E) cell count, blood pluripotent progenitor (CFU-GEMM) cell count, blood granulocyte/macrophage progenitor (CFU-GM) cell count. Analysis: parametric and nonparametric comparison of outcomes between lead workers and reference group; correlation	Blood lead (µg/dL) mean (range): lead: 45.5 (16–91) reference: 4.1 (3–14) Urine lead (µg/L) mean (range): lead: 46.6 (7–108) reference: 3.7 (2–16)	Significantly lower (p < 0.001) BFU-E counts in lead workers who had blood lead concentrations ≥60 µg/dL, compared to reference group. Significant negative correlation between blood lead or urine lead and CFU-GM and CFU-E. Serum EPO was not correlated with Hct in lead workers, however, serum EPO increased exponentially with decrease in Hct in reference group.

Table AX6-9.4 (cont'd). Effects of Lead on Hematopoietic System in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Gennart et al. (1992) Belgium NR	Design: cross-sectional cohort Subjects: adult battery manufacture workers (n = 98), mean age, 37.7 yr (range: 22–55); reference group (n = 85), mean age 38.8 yr (24–55) Outcome measures: blood Hb, RBC count, Hct, blood EP Analysis: linear regression	Blood lead (μg/dL) mean (SD, range): lead: 51.0 (8.0, 40–70) reference: 20.9 (11.1, 4.4–30.0	Significant association between increasing blood lead concentration and decreasing blood Hb (β = -0.011, r = 0.22, p = 0.003) or Hct (β = -0.035, r = 0.24, p < 0.01) Significant association between increasing blood lead concentration and increasing blood EP (β = 0.0191, r = 0.87, p = 0.0001) (No apparent analysis of covariables)
Mohammed-Brahim et al. (1985) Belgium NR	Design: cross-sectional cohort Subjects: adult smelter and ceramics manufacture workers (n = 38, 13 females); reference subjects (n = 100) matched with worker group by age, sex, and socioeconomic status Outcome measures: blood P5N, EP, ALAD, R/ALAD (ratio of ALAD before and after reactivation). Analysis: comparison of outcome measures (ANOVA) between lead workers and reference group; correlation	Blood lead (μg/dL) mean (SD, range): lead: 48.5 (9.1, 27.8–66.6) reference: 14.3 (6.7, 5.6–33.6) Urine lead (μg/g creatinine) mean (SD, range): lead: 84.0 (95.9, 21.8–587) reference: 10.5 (8.2, 1.7–36.9)	Significantly lower (p = NR) P5N in lead workers (males or females, or combined) compared to corresponding reference groups. Correlations with blood lead: log P5N r = -0.79 (p < 0.001) log ALAD r = -0.97 (p = NR) R/ALAD r = -0.94 (p < 0.001) log EP r = 0.86 (p = NR) Correlations with urine lead: log P5N r = -0.74 (p = NR) log ALAD r = -0.79 (p = NR) R/ALAD r = -0.84 (p < 0.001) log EP r = 0.80 (p = NR)
Hajem et al. (1990) France NR	Design: cross-sectional Subjects: adult males (n = 129), mean age 36 yr (SD 7.8, range: 24–55), with no environmental exposure to lead Outcome measures: erythrocyte membrane activities of Na+-K+-ATPase, Na+-K+-co-transport, Na+-Li+-antiport, and passive Na+ and K+ permeability Analysis: linear regression, correlation	Blood lead (μg/dL) geometric mean (95% CI range): 16.0 (15.2–16.8, 8.0–33.0) Hair lead (μg/g) geometric mean (95% CI range): 5.3 (4.44–6.23, 0.9–60)	Na+-K+-co-transport activity negatively correlated with blood lead concentration (r = -0.23, p = 0.02); linear regression: α = 583.19, β = -170.70. Na+-K+-ATPase activity negatively correlated with hair lead (r = -0.18, p = 0.04); simple linear regression: α = 3.34, β = -0.02.

Table AX6-9.4 (cont'd). Effects of Lead on Hematopoietic System in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Poulos et al. (1986) Greece NR	Design: cross-sectional cohort Subjects: adult male cable production workers who were exposed to lead (worker 1; n = 50, mean age: 37 yr); male cable workers who had not direct contact with lead (worker 2, n = 75, mean age: 36.5 yr); reference group (n = 35, mean age: 39 yr) Outcome measures: blood Hb, Hct Analysis: simple linear regression in the form: mean Hct = a+β(individual Hct – group mean Hct)	Blood lead (µg/dL) mean (SE): worker 1: 27.0 (0.7) worker 2: 18.3 (0.6) reference: 21.5 (1.5)	Significant association between increasing blood lead and decreasing Hct: worker 1: $\alpha = 46.50$, $\beta = -0.170$, SE 0.079, $p < 0.05$ worker 2: $\alpha = 44.57$, $\beta = -0.180$, SE 0.083, $p < 0.05$ reference: $\alpha = 44.69$, $\beta = -0.255$, SE 0.044, $p < 0.001$ Significant association between increasing blood lead and decreasing blood Hb: worker 1: $\alpha = 15.23$, $\beta = -0.058$, SE 0.028, $p < 0.05$ worker 2: $\alpha = 14.58$, $\beta = -0.071$, SE 0.034, $p < 0.05$ reference: $\alpha = 14.64$, $\beta = -0.087$, SE 0.015, $p < 0.001$
Romeo et al. (1996) Italy NR	Design: cross-sectional cohort Subjects: adult male lead workers (n = 28), age range, 17–73; reference group (n = 113), age range, 21–75 yr Outcome measures: serum EPO, blood Hb Analysis: nonparametric comparison of outcome measures between lead workers and reference group; correlation	Blood lead (µg/dL) mean (SD, range): lead 1: 32.3 (5.6, 30–49) lead 2: 65.1 (16, 50–92) reference: 10.4 (4.3, 3–20)	Significantly ($p = 0.021$) lower serum EPO in lead workers compared to reference group. No significant ($p < 0.05$) lead effect on blood Hb.
Graziano et al. (1990) Yugoslavia 1986	Design: prospective Subjects: pregnant women (n = 1502) from high-lead (smelter/refinery) and low-lead areas Outcome measures: Hb Analysis: comparison of outcome measures between high-and low-lead groups	Blood lead (µg/dL) mean (95% CI): high lead: 17.1 (6.9–42.6) low lead: 5.1 (2.5–10.6)	Mean blood hemoglobin levels (g/dL) in high-lead group (12.4; 95% CI: 10.3–14.5) not different from low-lead group (12.3; 95% CI: 10.0–14.7).

Table AX6-9.4 (cont'd). Effects of Lead on Hematopoietic System in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Graziano et al. (1990) Yugoslavia 1986	Design: prospective Subjects: pregnant women (n = 48) from high-lead (smelter/refinery) and low-lead areas (6 highest and lowest mid-pregnancy blood lead concentrations), within each of 4 Hb strata (g/dL): 9.0–9.9, 10.0–10.9, 11.0–11.9, 12.0–12.9 Outcome measures: Hb, EPO Analysis: ANOVA of outcome measures in subjects stratified by blood lead and blood Hb	Blood lead (µg/dL) mean range for Hb strata high lead: 16.9–38.6 low lead: 2.4–3.6	Significant effect of blood lead (p = 0.049) and blood Hb (p = 0.001) on mid-term and term serum EPO (blood lead p = 0.055, Hb p = 0.009), with significantly lower serum EPO associated with higher blood lead.
Asia			
Hsiao et al. (2001) China 1989–1999	Design: longitudinal Subjects: adult battery manufacture workers (n = 30, 13 females), mean age 38.3 yr Outcome measures: blood Hb, Hct, RBC count Analysis: GEE for repeated measures (models: linear correlation, threshold change, synchronous change, lag change); logistic regression	Blood lead (µg/dL) mean: 1989: 60 1999: 30	Significant association between increasing blood lead and increasing RBC count and Hct: Odds ratios (95% CI): synchronous change model: blood Hb (0.95, 0.52–1.78) RBC count (3.33, 1.78–6.19) Hct (2.19, 1.31–3.66) lag change: blood Hb (1.70, 0.99–2.92) RBC count (2.26, 1.16–4.41) Hct (2.08, 1.16–4.41)
Hsieh et al. (2000) China NR	Design: cross-sectional Subjects: Adults in general population (n = 630, 255 females) Outcome measures: blood Hb, Hct, RBC count, ALAD genotype Analysis: comparison of outcome measures between ALAD genotype strata	Blood lead (µg/dL) mean (SD): ALAD 1,1 (n = 630): 6.5 (5.0) ALAD: 1,1/2,2 (n = 30) 7.8 (6.0)	Mean blood lead not different between ALAD genotype strata (p = 0.17). RBC count, Hb, Hct not different between ALAD genotype strata (p = 0.7)

Table AX6-9.4 (cont'd). Effects of Lead on Hematopoietic System in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Froom et al. (1999) Israel 1980–1993	Design: longitudinal survey Subjects: adult male battery manufacturing workers (n = 94), mean age, 38 yr (SD 9, range: 26–60) Outcome measures: blood Hb, blood EP Analysis: multivariate linear regression	Blood lead (µg/dL) range of 13-yr individual subject means 20–61 µg/dL	Week (and probably not significant) covariate-adjusted association between blood Hb and individual sample blood lead (β = -0.0039, SE 0.0002), subject average blood lead (β = -0.0027, SE 0.0036) or blood EP (β = -0.001, SE 0.0007). Covariates retained in model were age and smoking habits.
Solliway et al. (1996) Israel NR	Design: cross-sectional cohort Subjects: adult male battery manufacture workers (n = 34), mean age: 44 yr (SD 13); reference subjects (n = 56), mean age 43 yr (SD 12); cohorts constructed to have similar age, ethnic characteristics, socioeconomic status, education level, and occupation Outcome measures: blood Hb, RBC count Analysis: parametric comparison of outcome measures between lead and reference groups, correlation	Blood lead (µg/dL) mean (SD, range): lead: 40.7 (9.8, 23–63) reference: 6.7 (2.4, 1–13)	Significantly lower ($p < 0.05$) mean RBC count in lead workers compared to reference group. Significant negative correlation between blood lead concentration and RBC count ($r = -0.29$, $p < 0.05$). Mean comparison for blood Hb ($p = 0.4$); correlation with blood lead concentration ($r = -0.05$, $p = 0.7$).
Horiguchi et al. (1991) Japan NR	Design: cross-sectional cohort Subjects: adult male secondary lead refinery workers (n = 17), mean age: 44.9 yr (range: 24–58); reference male subjects (n = 13), mean age: 33.5 yr (range: 22–44) Outcome measures: RBC deformability (microfiltration at -20 cm H_2O pressure), RBC count, Hct, blood Hb Analysis: comparisons of outcome measures between lead workers and reference group	Blood mead (µg/dL) mean (SD): lead: 53.5 (16.1) reference: NR Urine lead (µg/L) mean (SD): lead: 141.4 (38.1) reference: NR	Significantly lower RBC deformability ($p < 0.01$), RBC count ($p < 0.01$) Hct ($p < 0.01$), and blood Hb ($p > 0.001$) in lead workers compared to reference group.

Table AX6-9.4 (cont'd). Effects of Lead on Hematopoietic System in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Makino et al. (1997) Japan 1990–1994	Design: longitudinal survey Subjects: adult male pigment or vinyl chloride stabilizer manufacture workers (n = 1573) mean age 45 yr Outcome measures: blood Hb, Hct, RBC count Analysis: parametric comparison of outcome measures, stratified by blood lead, linear regression	Blood lead (μg/dL) mean (SD, range): 12.6 (2.0, 1–39) Urine lead (μg/L) mean (SD, range): 10.2 (2.7, 1–239)	Significantly higher ($p < 0.001$) Hct, blood Hb, and RBC count in blood lead category 16–39 μg/dL, compared to 1–15 μg/dL category. Significant positive correlation between blood lead concentration and Hct: $\alpha = 42.95$, $\beta = 0.0586$ ($r = 0.1553$, $p < 0.001$), blood Hb: $\alpha = 14.65$, $\beta = 0.0265$ ($r = 0.1835$, $p < 0.001$), and RBC count $\alpha = 457$, $\beta = 0.7120$ ($r = 0.1408$, $p < 0.001$).
Morita et al. (1997) Japan NR	Design: cross-sectional cohort Subjects: male lead workers (n = 76), mean age 42 yr (range: 21–62); reference subjects (n = 13, 6 females), mean age, males 41 yr (range: 26–52), females 45 yr (range: 16–61) Outcome measures: blood NADS, ALAD Analysis: comparison of outcome measures (ANOVA) between blood lead categories, linear regression	Blood lead (μg/dL) mean (SD, range) lead: 34.6 (20.7, 2.2–81.6)	Significantly lower ($p < 0.01$) blood NADS and ALAD in blood lead categories >20 μg/dL compared to <20 μg/dL, with dose trend in magnitude of difference. Significant associations between increasing blood lead and decreasing blood NADS and ALAD in lead workers: NADS: $\alpha = 0.843$, $\beta = -0.00971$, $r = -0.867$, $p < 0.001$, $n = 76$ logALAD: $\alpha = 1.8535$, $\beta = -0.015$, $r = -0.916$, $p < 0.001$, $n = 58$
Kim et al. (2002) Korea 1996	Design: cross-sectional cohort Subjects: adult male secondary lead smelter workers (n = 83), mean age: 38.7 yr (SD 10.8); reference subjects (n = 24), mean age: 32.0 (SD 10.8) Outcome measures: blood Hb, blood ALAD, blood EP, blood P5N Analysis: parametric comparison (ANOVA) of outcome measures between lead workers and reference group, correlation, multivariate linear regression	Blood lead (μg/dL) mean (SD) lead: 52.4 (17.7) reference: 6.2 (2.8)	Significantly ($p < 0.05$) lower blood P5N, ALAD, and Hb; and higher blood EP in lead workers compared to controls. Significant ($p < 0.001$) correlations (in lead worker group) with blood lead: P5N ($r = -0.704$), log EP ($r = 0.678$), log ALAD ($r = -0.622$). Significant association between increasing EP and decreasing blood Hb: blood lead ≥60 μg/dL: $\beta = -1.546$ (96% CI: -2.387 to -0.704, $r^2 = 0.513$, $p = 0.001$) blood lead <60 μg/dL: $\beta = -1.036$ (96% CI: -1.712 to -0.361, $r^2 = 0.177$, $p = 0.003$) Significant association between increasing P5N and increasing blood Hb (high blood lead group only): blood lead ≥60 μg/dL: $\beta = 0.222$ (96% CI: 0.015 to 0.419, $r^2 = 0.513$, $p = 0.036$) Covariates included in model: P5N, log serum ferritin, log EP

Table AX6-9.4 (cont'd). Effects of Lead on Hematopoietic System in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Schwartz et al. (1997) Korea 1994-1995	Design: cross-sectional Subjects: adult male battery manufacture workers (n = 57), mean age 32 yrs (SD 6). Outcome measures: blood Hb, Hb A_1, and Hb_{A2}, ALAD genotype Analysis: comparison of outcome measures between ALAD genotype strata	Blood lead (µg/dL) mean (SD): ALAD1,1 (n = 38): 26.1 (9.8) ALAD1,2 (n = 19): 24.0 (11.3)	Mean blood lead (p = 0.48) and blood Hb levels (p = 0.34) were not different between ALAD genotype strata.

ALAD, δ-aminolevulinic acid dehydratase; BFU-E, blood erythroid progenitor; CFR-GM, colony forming unit-granulocyte/macrophage progenitor; CFU-E, colony forming unit blood-erythroid progenitor; CFU-GEMM, colony forming unit blood-pluripotent progenitor; EP, erythrocyte protoporphyrin; EPO, serum erythropoietin; GEE, generalized estimation equation; Hb, blood hemoglobin; Hct, blood hematocrit; NADS, nicotinamide adenine dinucleotide; PCV, packed cell volume; P5N, pyrimidine 5'-nucleotidase; R/ALAD, ratio of ALAD activity, before and after reactivation; RBC, red blood cells

Table AX6-9.5. Effects of Lead on the Endocrine System in Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States			
Mahaffey et al. (1982) Wisconsin, New York NR	Design: cross-sectional Subjects: children/adolescents (n = 177), ages 1–16 yr Outcome measures: serum 1,25-OH-D Analysis: comparison of outcome measures between age, location and blood lead strata, linear regression	Blood lead (μg/dL) range: 12–120	Serum 1,25-OH-D levels were significantly ($p = 0.05$) higher in the age group 11–16 yr compared to age groups 1–5 or 6–10 yr. Increasing blood lead (log-transformed) significantly associated with decreasing serum 1,25-OH-D levels in children 1–5 yr of age ($\alpha = 74.5$, $\beta = -34.5$, $r = -0.884$, $n = 50$) Dietary calcium: NR
Rosen et al. (1980) New York NR	Design: cross-sectional Subjects: children (n = 45), ages 1–5 yr Outcome measures: serum calcium, PTH, 25-OH-D, 1,25-OH-D Analysis: comparison of outcome measures between blood lead strata, and before and after chelation, correlation	Blood lead (μg/dL) mean (SE, range): ≤29 (n = 15): 18 (1, 10–26) 30–59 (n = 18): 47 (2, 33–55) ≥60 (n = 12): 74 (98, 62–120)	Significantly higher serum PTH levels and lower 25-OH-D in high-lead group compared to low-lead group; significantly lower 1,25-OH-D levels in moderate- and high-lead group compared to low-lead group. Serum levels of 1,25-OH-D were negatively correlated with blood lead (high lead: $r = -0.71$, moderate: $r = -0.63$, $p < 0.01$). After chelation therapy, blood lead decreased and serum 1,25-OH-D levels increased to levels not significantly different ($p > 0.1$) from low-lead group, 25-OH-D levels were unchanged. Dietary calcium intake (mg/day) mean (SE): low lead: 800 (30) moderate lead: 780 (25) high lead: 580 (15)
Sorrell et al. (1977) New York 1971–1975	Design: cross-sectional Subjects: children (124), ages 1–6 yr Outcome measures: serum calcium, phosphate, 25-OH-D Analysis: comparison of outcome measures between blood lead strata, correlation	Blood lead (μg/dL) mean (SE): ≤29 (n = 40): 23 (1) 30–59 (n = 35): 48 (1) ≥60 (n = 49): 84 (5.0)	Serum calcium and 25-OH-D were significantly lower in high lead group ($p < 0.001$). Significant negative correlation between blood lead and serum calcium (high lead, $r = -0.78$, $p < 0.001$) or calcium intake high lead, ($r = -0.82$, $p < 0.001$) in all three lead strata. Serum 25-OH-D was significantly positively correlated with vitamin D intake, but not with blood lead. Dietary calcium intake (mg/day) mean (SE): low lead: 770 (20) moderate lead: 760 (28) high lead: 610 (20)

Table AX6-9.5 (cont'd). Effects of Lead on the Endocrine System in Children

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States (cont'd)			
Siegel et al. (1989) Connecticut 1987	Design: cross-sectional Subjects: children (n = 68, 32 female), ages 11 mo to 7 yr Outcome measures: serum FT4, TT4 Analysis: linear regression	Blood lead (μg/dL) mean (range): 25 (2–77)	No significant association between blood lead concentration and thyroid hormone outcomes. Linear regression parameters: FT4: $\alpha = 1.55$ (SE 0.05), $\beta = 0.0024$ (SE 0.0016), $r^2 = 0.03$, $p = 0.13$ TT4: $\alpha = 8.960$ (SE 0.39), $\beta = 0.0210$ (SE 0.0127), $r^2 = 0.04$, $p = 0.10$
Koo et al. (1991) Ohio NR	Design: longitudinal (subset of prospective) Subjects: children (n = 105, 56 females), age 21, 27, 33 mo Outcome measures: serum calcium magnesium, phosphorus, PTH, CAL, 25-OH-D, 1,25-OH-D, and bone mineral content Analysis: structural equation modeling	Blood lead (μg/dL) geometric mean (GSD, range): lifetime mean, based on quarterly measurements: 9.74 (1.44, 4.8–23.6) concurrent: 15.01 (1.52, 6–44) maximum observed: 18.53 (1.53, 6–63)	Significant association between increasing blood lead (ln-transformed) and covariate-adjusted decreasing serum phosphorus ($\alpha = 1.83$, $\beta = -0.091$). No other covariate-adjusted outcomes were significantly associated with blood lead. Covariates retained: age, sex, race, and sampling season. Dietary calcium intake (mg/day) ≤600: n = 4 (4%) 600–1200: n = 58 (55%) >1200: n = 43 (41%)

CAL, calcitonin; FT4, free thyroxine; GSD, geometric standard deviation; 25-OH-D, 25-hydroxyvitamin D; 1,25-OH-D, 1,25-dihydroxyvitamin D; PTH, parathyroid hormone; RBP, retinal binding protein; SE, standard estimation; TRH, thyroid releasing hormone; TSH, thyroid stimulating hormone; TT3, total triiodothyronine; TT4, total thyroxine; TTR, transthyretin

Table AX6-9.6. Effects of Lead on the Endocrine System in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
United States			
Cullen et al. (1984) Connecticut 1979 NR	Design: clinical case study Subjects: adult males with neurological symptoms of lead poisoning Outcome measures: serum, FSH, LH, PRL, TES Analysis: clinical outcomes in terms of abnormal values	Blood lead (μg/dL) (range): 66–139	Five subjects with defects in spermatogenesis (including azospermia), with no change in basal serum FSH, LH, PRL, and TES.
Robins et al. (1983 Connecticut NR	Design: cross-sectional Subjects: adult male brass foundry workers (n = 47), age range 20–64 yr Outcome measures: FT4 Analysis: simple linear regression with stratification by age and race.	Blood lead (μg/dL) range: 16–127	Significant association between increasing blood lead concentration and decreasing FT4 (α = 1.22, β = -0.0042; 95% CI: -0.0002, -0.0082; r^2 = 0.085, p = 0.048). Significant interaction between race (black, white) and blood lead. When stratified by race: black: α = 1.13, β = -0.0051, 95% CI: 0.0007, -0.0095, r^2 = 0.21, p = 0.03) white: r^2 = 0.05, p = 0.27 Strength of association not changed by including age in the regression model.
Braunstein et al. (1978) California NR	Design: clinical Subjects: adult male secondary lead smelter (n = 12), mean age 38 yr, reference group, (n = 9), mean age 29 yr Outcome measures: serum EST, FSH, LH, TES, HCG-stimulated EST and TES, GnRH-stimulated serum FSH and LH Analysis: comparisons of outcome measures between patients symptomatic for lead poisoning, lead-exposed patients not symptomatic, reference group	Blood lead (μg/dL) mean (SD): symptomatic (n = 9): time of test: 38.7 (3.0) highest: 88.2 (4.0) asymptomatic (n = 4): time of test: 29.0 (5.0) highest: 80.0 (0.0) reference: 16.1 (1.7)	Statistically significant (p < 0.05) lower basal serum TES, higher TES response to HCG, and significantly reduced LH response to GnRH in workers symptomatic for lead poisoning (including EDTA-provoked urinary lead >500 μg/24 hr).
Refowitz (1984) NR	Design: cross-sectional survey Subjects: secondary copper smelter workers (n = 58) Outcome measures: FT4, TT4 Analysis: linear regression	Blood lead (μg/dL) range: 5–60	No significant association between blood lead and hormone levels: FT4: α = 2.32, β = -0.0067 (95% CI: -0.18 - +0.0043) TT4: α = NR, β = -0.28 (95% CI: -0.059 - +0.0002) No significant association when ratified by race (black, white)

Table AX6-9.6 (cont'd). Effects of Lead on the Endocrine System in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Canada			
Alexander et al. (1998, 1996) British Columbia 1993	Design: cross-sectional Subjects: adult male primary smelter workers (n = 152), mean age 40 yr Outcome measures: serum FSH, LH, TES Analysis: multivariate linear regression	Blood lead (µg/dL) range (n = 81): 5 (DL)-58 (75th %tile: 29) Semen lead (µg/dL) range: 0.3 (DL) -17.6	No significant association between covariate-adjusted blood lead and hormone levels ($p \geq 0.5$) or prevalence of abnormal levels. Significant association between covariate-adjusted increasing semen lead concentration and decreasing serum TES ($\beta = -1.57$, $p = 0.004$). Covariates considered: age, smoking, alcohol, other metals in blood (As, Cd, Cu, Zn), abstinence days prior to sample collection, and sperm count.
Schumacher et al. (1998) British Columbia 1993	Design: cross-sectional Subjects: adult male smelter workers (n = 151) mean age 40 yr (SD 7.2) Outcome measures: serum FT4, TT4, TSH Analysis: linear regression, ANOVA	Blood lead (µg/dL) mean: 24.1 (n = 151) <15 (n = 36) 15–24 (n = 52) 25–39 (n = 41) ≥40 (n = 22)	No significant effect of blood lead (categorical) on covariate-adjusted or unadjusted FT4 ($p = 0.68$), TT4 ($p = 0.13$), TSH ($p = 0.54$). No significant association of blood lead with prevalence of abnormal values of hormones. No significant association between 10-yr average blood lead and hormone levels or prevalence of abnormal values. Covariates considered: age and alcohol consumption.
Europe			
Gennart et al. (1992) Belgium NR	Design: cross sectional cohort Subjects: adult battery manufacture workers (n = 98), mean age 37.7 yr (SD 8.3, range: 22–55); reference worker group (n = 85), mean age 38.8 yr (SD 8.7, range: 22–55) Outcome measures: serum TT3, FT4, TT4, TSH, FSH, LH Analysis: comparison of outcome measures between lead workers and reference group	Blood lead (µg/dL) mean (SD, range): lead: 51.0 (8.0, 40.0–75.0) reference: 20.9 (11.1, 4.4–39.0) Urine lead (µg/g cr) mean (range): lead: 57.8 (1.95, 4.3–399) reference: 9.75 (2.73, 1.45–77.7)	Mean hormone levels in lead workers and reference group not different (p = NR); no association between hormone levels and blood lead or exposure duration quartile.

Table AX6-9.6 (cont'd). Effects of Lead on the Endocrine System in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Assennato et al. (1987) Italy NR	Design: cross-sectional Subjects: adult male battery manufacture workers (n = 39), mean age 41 yr (SD 10); reference cement plant workers (n = 18), mean age 40 yr (SD 10) Outcome measures: serum FSH, LH, PRL, TES; urinary 17-ketosteroids Analysis: parametric comparison of outcome measures between lead and reference groups	Blood lead (µg/dL) mean (SD): lead: 61 (20) reference: 18 (5) Urinary lead (µg/L) mean (SD): lead: 79 (37) reference: 18 (8)	No significant association ($p > 0.05$) between blood lead and hormone levels.
Govoni et al. (1987) Italy NR	Design: cross-sectional Subjects: adult male pewter manufacture workers (n = 78), mean age 35 yr (SD 19, range: 19–52) Outcome measures: serum PRL Analysis: parametric comparison of outcome measures between blood lead and ZPP strata	Blood lead (µg/dL) mean (SD)/blood ZPP (µg/dL) mean (SD): A (n = 22): 28.2 (7.1)/24.4 (8.7) B (n = 33): 60/3(19.3)/131(107) C (n = 13): 33.1(6.7)/77.0(42.2) D (n = 8): 49.1(4.2)/34.0(4.8)	Significantly ($p < 0.02$) higher serum PRL in high ZPP strata (B and C, compared to low ZPP strata A).
Rodamilans et al. (1988) Spain NR	Design: cross-sectional cohort Subjects: adult male lead smelter workers (n = 23), age range 21–44 yr; reference group (n = 20), age range 20–60 yr. Outcome measures: serum: FSH, LH, TES, FTES, SHBG Analysis: comparison of outcome measures between exposure duration strata	Blood lead (µg/dL) mean (SD) lead <1 yr (n = 5): 66(22) lead 1–5 yr (n = 8): 73 (24) lead >5 yr (n = 10): 76(11) reference (n = 20): 17.2 (13)	Serum TES ($p = 0.01$) and FTES ($p = 0.001$) significantly lower and SHBG significantly higher ($p < 0.025$) in >5-yr exposure group compared to reference group; serum LH was significantly ($p < 0.01$) higher in all exposure groups compared to reference group.

Table AX6-9.6 (cont'd). Effects of Lead on the Endocrine System in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Erfurth et al. (2001) Sweden NR	Design: cross-sectional cohort Subjects: adult male active secondary smelter workers (n = 62), mean age 43 yr (range: 21–78) reference worker group (: 26), mean age 43 yr (range: 23–66) Outcome measures: serum FT3, FT4, TSH, TES, SHBG; TRH-stimulated serum TSH; GnRH-stimulated serum FSH, LH, and PRL Analysis: nonparametric comparison of outcome measures between lead workers and reference group; multivariate linear regression	Blood lead (µg/dL) median (range): lead: 31.1 (8.3–93.2) reference: 4.1 (0.8–6.2) Plasma lead (µg/dL) median (range: lead: 31.1 (8.3–93.2) reference: 4.1 (0.8–6.2) Urine lead (µg/g cr) median (range): lead: 19.6 (3.1–80.6) reference: 4.1 (2.4–7.3) Bone (finger) lead (µg/g) median (range): lead: 25 (-13–99) reference: 2 (-21–14)	Basal hormone levels in workers not different from reference group ($p \geq 0.05$); age-adjusted basal hormone levels not associated with plasma lead, blood lead, urine lead, or bone lead. In an age-matched subset of the cohorts (n = 9 lead workers, n = 11 reference), median GnRH-stimulated serum FSH was significantly ($p = 0.014$) lower (77 IU/L x hr) in lead workers than in reference group (162 IU/L x hr). No association between stimulated TSH, LH, FSH or PRL and lead measures.
Gustafson et al. (1989) Sweden NR	Design: cross-sectional cohort Subjects: adult male secondary smelter workers (n = 21) mean age 36.0 yr (SD 10.4); individually matched for age, sex, and work shift (n = 21), Outcome measures: serum FTES, TTES; FSH, LH, PRL, COR, TSH, TT3, TT4 Analysis: nonparametric comparison of outcome measures between lead workers and reference group, correlation	Blood lead (µg/dL) mean (SE): lead: 39.4 (2.1) reference: 5.0 (0.2)	Significantly higher TT4 ($p < 0.02$) and lower serum FSH ($p = 0.009$) in lead workers compared to reference group. When restricted to the age range <40 yr, lead workers had significantly higher TT4 ($p = 0.01$) and lower FSH ($p = 0.03$), LH ($p = 0.04$), and COR ($p = 0.04$), compared to the reference group.
Campbell et al. (1985) UK NR	Design: cross-sectional cohort Subjects: adult male welders (n = 25); reference subjects (n = 8) (ages NR) Outcome measures: plasma ACE, AI, PRA, plasma ALD Analysis: linear regression, nonlinear least squares	Blood lead (µg/dL) mean (SD, range): 35.6 (15.3, 8–62)	Significant positive correlation between blood lead concentration and plasma ALD level ($r = 0.53$, $p < 0.002$), PRA ($r = -0.76$, $p < 0.001$), AI ($r = 0.68$, $p < 0.002$), and ACE ($r = 0.74$, $p < 0.001$).

Table AX6-9.6 (cont'd). Effects of Lead on the Endocrine System in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Europe (cont'd)			
Chalkey et al. (1998) UK 1979–1984	Design: cross-sectional Subjects: adult male primary metal (Cd, Pb, Zn) workers (n = 19), ages NR Outcome measures: blood calcium, serum 25-OH-D, 1,25-OH-D, 24,25-OH-D Analysis: comparison of outcome measures (ANOVA) in group stratified by blood lead and urinary cadmium	Blood lead (µg/dL) mean (SD, range): 47 (21–76)	After stratification by blood lead and urinary cadmium, serum 1,25-OH-D levels in strata were significantly different (p = 0.006), with higher mean values in high blood lead (>40µg/dL)/high blood cadmium (>0.9 µg/L)/high urine cadmium >3.1 µg/L) stratum compared to low blood lead (<40µg/dL)/high blood cadmium (>0.9 µg/L)/high urine cadmium >3.1 µg/L) stratum. Serum 24,25-OH-D levels decreased with increasing urinary cadmium (p = NR)
Mason et al. (1990) UK NR	Design: cross-sectional Subjects: adult male lead workers (n = 63), age range 21–63 yr; reference male subjects (n = 75), age range 22–64 yr Outcome measures: serum calcium phosphate, PTH, 1,25-OH-D Analysis: comparison of al outcome measures between lead workers and reference group, multivariate regression	Blood lead (µg/dL) range: lead (15–94) reference: NR Tibia lead (µg/g) lead: 0–93 reference: NR	Significantly higher ($p < 0.025$) prevalence of elevated 1,25-OH-D (>2 SD of reference mean) in lead workers (8/63, 13%) compared to reference group (1/75, 1.3%). Serum levels of 1,25-OH-D significantly ($p < 0.05$) higher in lead workers compared to reference group. After stratification of lead workers into exposure categories (high: blood lead ≥40 µg/dL and bone lead ≥40 µg/g, low: blood lead ≤40 µg/dL and bone lead ≤40 µg/g), serum 1,25-OH-D levels were significantly ($p < 0.01$) higher in the high lead group. Increasing blood lead was significantly (p = NR) associated with increasing 1,25-OH-D levels ($r^2 = 0.206$; with age and bone lead included, $r^2 = 0.218$). After excluding 12 subjects whose blood lead concentrations >60 µg/dL, $r^2 = 0.162$ (p = 0.26).
McGregor and Mason, (1990) UK NR	Design: cross-sectional cohort Subjects: adult male lead workers (n = 90), mean age (31.5 yr (SD 11.9); reference workers (n = 86), mean age 40.6 yr (SD 11.8) Outcome measures: serum FSH, LH, TES, SHBG Analysis: comparison of outcome means between lead workers and reference groups, multivariate regression, correlation	Blood lead (µg/dL) range: lead: 17–77 reference: <12	Age-adjusted serum FSH was significantly (p = 0.004) higher in lead workers compared to reference group. Increasing serum FSH significantly (p = NR) associated with blood lead and age. Increasing serum LH significantly associated with increasing exposure duration (not blood lead or age). No significant association between serum TES or SHBG and blood lead or exposure duration. No significant difference in prevalence of abnormal hormone levels between groups.

Table AX6-9.6 (cont'd). Effects of Lead on the Endocrine System in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Latin America			
Lopez et al. (2000) Argentina NR	Design: cross sectional Subjects: adult male battery manufacture workers (n = 75), age range 21–56 yr; reference group (n = 62), age NR Outcome measures: serum TT3, FT4, TT4, TSH Analysis: comparison of outcome measures between lead workers and reference group, correlation	Blood lead (µg/dL) mean (range): lead: 50.9 (23.3, 8–98) reference: 19.1 (7.1, 4–39)	Significantly higher serum FT4 ($p < 0.01$) and TT4 ($p < 0.05$) in lead workers compared to reference group. Significant positive correlation between blood lead and serum TT3 ($p < 0.05$), FT4 ($p < 0.01$), TT4 ($p < 0.05$), and TSH ($p < 0.05$), for blood lead range 8–50 µg/dL; and for TSH ($p < 0.05$) for blood lead range 8–26 µg/dL.
Roses et al. (1989) Brazil NR	Design: adult male lead workers (n = 70), age range 20–53 yr; reference group (n = 58), age range 25–37 yr. Outcome measures: serum PRL Analysis: comparison of outcome measure between lead workers and reference group, linear regression	Blood lead (µg/dL) (range): lead: 9–86 reference: 8–28	Serum RL levels in lead workers and reference group not significantly different (p = NR). Correlation between serum PRL and blood lead ($r = 0.57$, p = NR).
Asia			
Dursun and Tutus (1999) Turkey NR	Design: cross-sectional Subjects: adult metal powder manufacture workers (n = 27) mean age 41.1 yr (SD 5.45, range: 25–50); reference group (n = 30), mean age 42 yr (SD 3.42, range: 28–49) Outcome measures: serum FT4, TT4, FT3, TT3, TSH Analysis: parametric comparison of outcome measures between lead and reference groups, simple and multivariate linear regression	Blood lead (µg/dL) mean (range): lead: 17.1 (9.0, 6–36) reference: 2.4 (0.1, 1–4)	Significantly ($p < 0.0001$) higher mean TT4, FT4, and FT3 in lead workers compared to reference group. Significant association between TT4, age ($\beta = 0.23$, $p < 0.006$), and exposure duration (β; -0.20, $p > 0.01$), but not blood lead ($\beta = 0.00$, NR) in linear regression model that included age, blood lead, and exposure duration ($\alpha = 2.76$, $r^2 = 0.3$, $p = 0.03$).

Table AX6-9.6 (cont'd). Effects of Lead on the Endocrine System in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Kristal-Boneh et al. (1998) Israel NR	Design: cross-sectional cohort Subjects: adult male battery manufacture/recycling workers (n = 56), mean age 43.4 yr (SD 11.2); reference workers (n = 90), mean age 41.5 yr (SD 9.3) Outcome measures: serum calcium, magnesium, phosphorus, PTH, 25-OH-D, 1,25-OH-D Analysis: parametric comparison of outcome measures between lead workers and reference group, multivariate linear regression	Blood lead (µg/dL) mean (SD, range): lead: 42.6 (14.5, 20–77) reference: 4.5 (2.6, 1.4–19)	Serum 1,25-OH-D ($p = 0.0001$) and PTH ($p = 0.042$) were significantly higher in lead workers compared to reference group Increasing blood lead concentration (ln-transformed) was significantly associated with covariate-adjusted increasing serum PTH and 1,25-OH-D levels: PTH: $\beta = 4.8$ (95% CI: 0.8–8.8, $r^2 = 0.12$) 1,25-OH-D: $\beta = 4.8$ (95% CI: 2.7–6.9, $r^2 = 0.10$) Occupational lead exposure (yes) significantly associated with increasing PTH and 1,25-OH-D levels. Covariates retained: age, alcohol consumption, smoking; calcium, magnesium, and calorie intake: PTH: $\beta = 7.81$ (95% CI: 3.7–11.5) 1,25-OH-D: $\beta = 12.3$ (95% CI: 3.84–20.8)
Horiguchi et al. (1987) Japan NR	Design: cross-sectional Subjects: adult secondary lead refinery (n = 60, 8 females), mean age 49 yr (range: 15–69) Outcome measures: serum TT3, TT4, TSH Analysis: comparison of outcome measures (method NR), between job categories, correlation	Blood lead (µg/dL) mean (SD): male: 31.9 (20.4) female: 13.5 (9.5) Urine lead (µg/L) mean (SD): male: 59.3 (76.3) female: 26.0 (19.7)	No significant differences (p = NR) between hormone levels in job lead categories: mean blood lead (µg/dL, SD): 17.9 (10.7), 25.6 (15.4), 49.9 (18.7). No significant correlations (p = NR) between hormone levels and blood or urine lead levels.
Ng et al. (1991) China NR	Design: cross-sectional cohort Subjects: adult male battery manufacture workers (n = 122), mean age 32.6 (SD 8,2, range: 17–54); reference group (n = 49), mean age 43.4 yr (SD 13.4, range: 18–74) Outcome measures: serum FSH, LH, PRL, TES Analysis: multivariate linear regression ANCOVA	Blood lead (µg/dL) mean (SD, range): lead: 35.2 (13.2, 9.6–77.4) reference: 8.3 (2.8, 2.6–14.8)	When cohorts were stratified by age serum FSH and LH were significantly ($p < 0.02$) higher in lead workers <40 yrs of age compared to corresponding age strata of the reference group; serum TES was significantly ($p < 0.01$) lower in lead workers ≥40 yr of age. Covariate-adjusted serum TES were significantly lower ($p < 0.01$) in lead workers in the ≥10-yr exposure duration category, compared to the reference group. Covariate-adjusted serum FSH and LH were significantly higher ($p < 0.01$) in lead workers in the <10-yr exposure duration category, compared to the reference group. Covariates: age and tobacco smoking.

Table AX6-9.6 (cont'd). Effects of Lead on the Endocrine System in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Zheng et al. (2001) China NR	Design: retrospective cross-sectional Subjects: adult hospital patients (n = 82, 32 females) mean age 49.6 yr (SD 18.7) Outcome measures: serum and CSF TTR, TT4 Analysis: simple and multivariate linear regression	Blood lead (µg/dL) mean (SD): all: 14.9 (8.3) female: 14.2 (8.76) male: 15.4 (8.07)	No significant association between blood lead and serum TTR ($r = -0.114$, $p = 0.307$), TT4 ($r = -0.160$, $p = 0.152$). Significant association between age-adjusted CSF lead and CSF TTR ($r = -030$, $p = 0.023$). No significant association between CSF lead and CSF TT4 ($r = -0.22$, $p = 0.090$).
Singh et al. (2000) India NR	Design: cross-sectional cohort Subjects: adult male petrol pump attendants (n = 58), mean age 31.7 yr (SD 10.6); reference group (n = 35), mean age 28.9 yr (SD 4.20) Outcome measures: serum TT3, TT4, TSH Analysis: parametric comparison of outcome measures between lead workers and reference group, stratified by blood lead or exposure duration	Blood lead (µg/dL) mean (SD): lead: 51.6 (9.3) reference: 9.5 (8.7)	Serum TSH significantly higher ($p < 0.01$) in lead workers compared to reference group, significantly higher in high blood lead category (≤ 70 µg/dL, mean 54.5 µg/dL) compared to low worker group ≤41 µg/dL, mean 31.3 µg/dL). Serum TSH significantly higher in lead workers who were exposed for ≤60 mo, compared to workers exposed for >60 mo.
Africa			
Tuppurainen et al. (1988) Kenya 1984	Design: cross-sectional Subjects: adult male battery manufacture workers (n = 176), mean age 34.1 yr (SD 8.1, range: 21–54) Outcome measures: serum TT3, FT4, TT4, TSH Analysis: multivariate linear regression and correlation	Blood lead (µg/dL) mean (SD, range): 55.9 (23.8, 14.5–133.6)	Increasing exposure duration significantly associated with decreasing FT4 ($r^2 = 0.071$, $p = 0.001$) and TT4 ($r^2 = 0.059$, $p = 0.021$); regression not improved by including age or blood lead. Strength of association greater when restricted to workers who had an exposure duration >7.6 yrs: FT4: $r^2 = 0.33$, $p < 0.002$; TT4: $r^2 = 0.21$, $p < 0.001$. No significant association between blood lead and hormone levels.

1,25-OH-D, 1,25-dihydroxyvitamin D; 25-OH-D, 25-hydroxyvitamin D; ACE, angiotensin converting enzyme; AI, angiotensin I; ALD, aldosterone; ANOVA, analysis of variance; CAL, calcitonin; COR, cortisol; cr, creatinine; CSF, cerebral spinal fluid; EDTA, ethylenediaminetetraacetic acid; EST, estradiol; FSH, follicle stimulating hormone; FT4, free thyroxine; FTES, free testosterone; GnRH, gonadotropin releasing hormone; HCG, human chorionic gonadotropin; LH, luteinizing hormone; NR, not reported; PRL, prolactin; PTH, parathyroid hormone; RBP, retinal binding protein; SD, standard deviation; SE, standard estimation; SHBG, sex hormone binding globulin; SHBG, sex hormone binging globulin; TES, testosterone; TRH, thyroid releasing hormone; TSH, thyroid stimulating hormone; TT3, total triiodothyronine; TT4, total thyroxine; TTES, total testosterone; TTES, total testosterone; TTR, transthyretin; ZPP, zinc protoporphyrin

Table AX6-9.7. Effects of Lead on the Hepatic System in Children and Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Children			
United States			
Saenger et al. (1984) New York NR	Design: clinical cases Subjects: children (n = 26) ages 2–9 yr; age-matched reference group (n = NR) Outcome measures: urinary cortisol and 6β-OH-cortisol (CYP3A metabolite of cortisol) Analysis: comparison of outcome measure between children who qualified for EDTA treatment (EDTA provocation >500 µg/24 hr)	Blood lead (µg/dL) mean (SE, range): chelated: 46 (2, 33–60) not chelated: 42 (3, 32–60) Urinary lead (µg/24 hr) mean (SE, range), EDTA-provocation: chelated: 991 (132, 602–2247) not chelated: 298 (32, 169–476)	Significantly lower (~45% lower) urinary excretion of 6β-OH-cortisol ($p = 0.001$) and urinary 6β-OH-cortisol: cortisol ratio ($p < 0.001$) in children who qualified for chelation than in children who did not qualify and significantly lower than age-matched reference group. Urinary 6β-OH-cortisol: cortisol ratio was significantly correlated with blood lead ($r = -0.514$, $p < 0.001$), urinary lead, and EDTA provocation urinary lead ($r = -0.593$, $p < 0.001$).
Adults			
Asia			
Al-Neamy et al. (2001) United Arab Emirates 1999	Design: cross-sectional cohort Subjects: adult male (n = 100) workers (e.g., gas pump attendants, garage workers, printing workers, construction workers), mean age 34.6 yr (SD 8.0); reference group (n = 100) matched with lead workers for age, sex, nationality. Outcome measures: serum protein, albumin, ALT, AP, AST, BUN, γGT, LDH Analysis: comparison of outcome measures between lead workers and reference group	Blood lead (µg/dL) mean (SD): lead: 77.5 (42.8) reference: 19.8 (12.3)	Significantly higher serum AP ($p = 0.012$) and LDH ($p = 0.029$) in lead workers compared to reference group (values within normal range).

Table AX6-9.7 (cont'd). Effects of Lead on the Hepatic System in Children and Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
***Adults,* Asia** (cont'd)			
Hsiao et al. (2001) China 1989–1999	Design: longitudinal Subjects: adult battery manufacture workers (n = 30, 13 females), mean age 38.3 yr Outcome measures: serum ALT Analysis: GEE for repeated measures (models: linear correlation, threshold change, synchronous change, lag change); logistic regression	Blood lead (μg/dL) mean: 1989: 60 (~25–100) 1999: 30 (~10–60)	No association between blood lead and ALT. Odds ratios (95% CI): synchronous change model: 1.25 (0.69–2.25) lag change: 1.76 (0.76–4.07)
Satarug et al. (2004) Thailand NR	Design: cross-sectional Subjects: adults from general population (n = 118, 65 female), age range, 21–57 yr Outcome measures: coumarin-induced urinary 7-OH-coumarin (marker for CYP2A6 activity) Analysis: multivariate linear regression	Urinary lead (μg/g cr) mean (SD, range): males: 1.3 (1.8, 0.1–12) females: 2.4 (1.1, 0.6–6.8) Serum lead (μg/L) mean (SD, range): males: 4.2 (5.4, 1–28) females: 3.0 (2.2, 1–12)	Significant association between increasing urinary lead and decreasing covariate-adjusted urinary 7-OH-coumarin (β = -0.29, p = 0.003) in males, but not in females. Covariates retained: age and zinc excretion. Significant association in opposite direction between urinary cadmium and urinary 7-OH-coumarin (β = 0.38, p = 0.006).

γGT, γ-glutamyl transferase; 6β-OH-cortisol, 6-β-hydroxycortisol; ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CI, confidence interval; cr, creatinine; EDTA, ethylenediaminetetraacetic acid; GEE, generalized estimating equations; LDH, lactate dehydrogenase; SD, standard deviation; UAE, United Arab Emirates

Table AX6-9.8. Effects of Lead on the Gastrointestinal System

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Canada			
Holness and Nethercott (1988) Ontario 1982–1984	Design: longitudinal Subjects: adult male demolition workers (n = 119), age NR Outcome measures: prevalence of symptoms Analysis: comparison of prevalence of symptoms (questionnaire) stratified by job phase or blood lead	Blood lead (µg/dL) mean (range): phase 1: 59 (15–99) phase 2: 30 phase 3: 19 phase 4: 17	Prevalence of reporting of symptoms of abdominal cramps or constipation increased with increasing blood lead concentration ($p < 0.05$): <50 µg/dL: 8%, 6% 50–70 µg/dL: 37%, 42% >70 µg/dL: 77%, 62%
Caribbean			
Matte et al. (1989) Jamaica 1987	Design: survey Subjects: battery manufacture/repair workers (n = 63), mean age ~30 yr (range: 11–47) Outcome measures: prevalence of symptoms Analysis: comparison of GI symptoms (questionnaire) between blood lead strata	Blood lead (µg/dL) geometric mean site range: 40–64 Blood lead distribution: >60: 60% <60: 40%	When stratified by blood lead, <60 µg/dL (low) or ≥60 µg/dL (high), prevalence ratio (high/low) was not significant for abdominal pain (1.5, 95% CI: 0.5–4.6), or for any other lead symptom (e.g. muscle weakness).
Asia			
Bercovitz and Laufer (1991) Israel NR	Design: cross-sectional Subjects: health individuals (n = 12), peptic ulcer patients (n = 11), and individuals with heart disease (n = 11) with environmental exposure Analysis: one-way ANOVA used to compare tooth lead concentrations in the three groups	Tooth lead (µg/g dry dentine) mean (SE): Healthy: 25.62 (10.15) Peptic ulcer = 75.02 (8.15) Heart disease: 20.30 (2.70)	Tooth lead levels in patients with gastrointestinal ulcers (n = 11), were significantly higher than that in healthy subjects ($p = 0.001$)). Ten of the 11 peptic ulcer patients had a higher lead level than the health subjects. In these 10 patients, increased severity of the ulcer and longevity of suffering was associated with increased tooth lead levels. There was no significant difference between the tooth lead levels in the healthy subjects and in the heart disease patients.

Table AX6-9.8 (cont'd). Effects of Lead on the Gastrointestinal System

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia (cont'd)			
Lee et al. (2000) Korea NR	Design: cross-sectional cohort Subjects: adult male lead workers (n = 95; secondary smelter, PVC-stabilizer manufacture, battery manufacture); mean age 42.8 yr (SD 9.3, range: 19–64); reference group (n = 13), mean age 35.1 yr (SD 9.9, range: 22–54) Outcome measures: prevalence of GI symptoms (self-administered questionnaire) Analysis: multivariate logistic regression	Blood lead (µg/dL) mean (SD, range): lead: 44.6 (12.6, 21.4–78.4) reference: 5.9 (1.2, 4.0–7.2)	Covariate-adjusted OR for GI symptoms (loss of appetite, constipation or diarrhea, abdominal pain) in workers (referents not included in model) were not significant: blood lead: 45.7 µg/dL vs. <45.7 µg/dL: OR 1.8 (95% CI: 0.7–4.5) DMSA-provoked urinary lead: >260.5 vs. <260.5 µg: OR = 1.1 (95% CI: 0.4–2.5) OR for neuromuscular symptoms were significantly associated with DMSA-provoked lead (OR = 7.8 (95% CI: 2.8–24.5), but not with blood lead. Covariates retained: age, tobacco smoking, and alcohol consumption.
Africa			
Awad el Karim et al. (1986) Sudan NR	Design: cross-sectional cohort Subjects: adult male battery manufacture workers (n = 92), mean age 31.1 yr (SD 8.2); reference group (n = 40), mean age 33.7 yr (SD 9.7) Outcome measures: clinical evaluation Analysis: comparison of prevalence of symptoms of lead poisoning between lead workers and reference group	Blood lead (µg/dL) mean (SD, range): lead: 55–81 (mean range for various jobs), range: 39–107 Blood lead distribution >80: 23% 40–80: 72% <40: 5% reference: 21 (8.5, 7.4–33.1)	Prevalences of abdominal colic (pain) and constipation were 41.3% and 41.4 % in lead workers and 7.5% and 10%, respectively, in the reference group.

DMSA, dimercaptosuccinic acid; GI, gastrointestinal; NR, not reported; OR, odds ratio; PAR, population attributable risk; PVC, polyvinyl chloride; SD, standard deviation; SE, standard error

Table AX6-9.9. Effects of Lead on the Respiratory Tract in Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Asia			
Bagci et al. (2004) Turkey NR	Design: cross-sectional cohort Subjects: adult male battery manufacture and automobile exhaust repair workers (n = 62), mean age 32.6 yr; reference hospital workers (n = 24), mean age 28.8 yr Outcome measures: VC, FVC, FEV_1, PEF, FEF, MVV Analysis: comparison of mean outcomes (ANOVA) between lead workers and reference group, multivariate (Pearson partial) correlation	Blood lead (μg/dL) mean (SD, 95% CI): battery (n = 22): 36.8 (8.1, 33.2-40.3) exhaust (n = 40): 26.9 (9.2, 24.0-29.9) reference (n = 24): 14.8 (3.0, 13.5-16.1)	Battery manufacture workers had significantly lower FEV ($p < 0.05$), FEV: VC ratio ($p < 0.05$), FEV: FVC ratio ($p < 0.01$), FEF ($p < 0.01$), and MVV ($p < 0.01$) compared to the hospital workers. Significant negative (partial) correlation between blood lead and FEV/FVC ($r = -0.31$, $p = 0.006$) and FEF ($r = -0.30$, $r = 0.009$), adjusted for age, cigarette smoking, and exposure duration.

ANOVA, analysis of variance; CI, confidence interval; FEF, forced expiratory flow; FEV, forced expiratory volume; FVC, forced vital capacity; MVV, maximum voluntary ventilation; PEF, expiratory peak flow; SD, standard deviation; VC, vital capacity

Table AX6-9.10. Effects of Lead on Bone and Teeth in Children and Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Children			
United States			
Moss et al. (1999) U.S. 1988–1994	Design: cross-sectional national survey (NHANES III) Subjects: general population (n = 24,901), ages 2–5 yr (n = 3,547), 6–11 yr (n = 2,894), ≥12 yr (18,460) Outcome measures: number of caries (dfs, DFS, DMFS) Analysis: multivariate linear regression and logistic regression	Blood lead (μg/dL) geometric mean (SE): 2–5 yr: 2.90 (0.12) 6–11 yr: 2.07 (0.08) ≥12 yr: 2.49 (0.06)	Increasing blood lead concentration (log-transformed) significantly associated with covariate adjusted increases in dfs: 2–5 yr: β = 1.78 (SE 0.59, p = 0.004) 6–11 yr: β = 1.42 (SE 0.51, p = 0.007) and increases in DFS: 6–11 yr: β = 0.48 (SE 0.22, p = 0.03) ≥12 yr: β = 2.50 (SE 0.69, p < 0.001) and increases in DMFS: ≥ 12 yr: β = 5.48 (SE 1.44, p = 0.01) Odds ratio (OR) for caries (≥1 DMFS, ages 5–17 yr) and population attributable risk (PAR) in association with 2nd or 3rd blood lead tertiles, compared to 1st tertile were: 1st tertile (≤1.66 μg/dL) 2nd tertile (1.66–3.52 μg/dL): OR 1.36 (95% CI: 1.01–2.83); PAR 9.6% 3rd tertile (>3.52 μg/dL): OR 1.66 (95% CI: 1.12–2.48); PAR 13.5% For an increase of blood lead of 5 μg/dL, OR 1.8 (95% CI: 1.3–2.5) Covariates retained were age, gender, race/ethnicity, poverty income ratio, exposure to cigarette smoke, geographic region, educational level of head of household, carbohydrate and calcium intakes, and dental visits.

Table AX6-9.10 (cont'd). Effects of Lead on the Gastrointestinal System in Children and Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Children, **United States** (cont'd)			
Schwartz et al. (1986) U.S. 1976–1980	Design: cross-sectional national survey (NHANES II) Subjects: ages <7 yr (n = 2,695) Outcome measures: variables of stature, including height, weight, and chest circumference Analysis: multivariate weighted linear regression	Blood lead (μg/dL) range: 5-35	Blood lead levels were a statistically significant predictor of children's height ($p < 0.0001$), weight ($p < 0.001$), and chest circumference ($p < 0.026$), after controlling for age in months, race, sex, and nutrition. Height: $\beta = -0.119$ (SE 0.0005) Weight: $\beta = -1.0217$ (SE 0.08) for log-transformed blood lead Chest circumference: $\beta = -0.6476$ (SE 0.077) for log-transformed blood lead There are several explanations for the inverse correlation between blood lead and growth in children. First, blood lead level may be a composite factor for genetic, ethnic, nutritional, environmental, and sociocultural factors. Second, nutritional deficits that retard growth also enhance lead absorption. Finally, there may be a direct effect of low level lead on growth in children.
Gemmel et al. (2002) Boston/Cambridge, MA NR	Design: cross-sectional Subjects: children (n = 543), ages 6–10 yr Outcome measures: number of caries (dfs, DFS) Analysis: multivariate linear regression	Blood lead (μg/dL) mean (SD, max): urban (n = 290): 2.9 (2.0, 13) rural (n = 253): 1.7 (1.0, 7)	Increasing blood lead (ln-transformed) was significantly associated with covariate-adjusted number of caries (dfs + DFS) (ln-transformed) in the urban ($\beta = 0.22$, SE 0.08, $p = 0.005$) group, but not in the rural group ($\beta = -0.15$, SE 0.09, $p = 0.09$). When dfs numbers were stratified by permanent or deciduous teeth, the blood lead association in the urban group was significant for deciduous teeth ($\beta = 0.28$, SE 0.09, $p = 0.002$), but not for permanent teeth ($\beta = 0.02$, SE 0.07, $p = 0.8$). Covariates retained: age, sex, ethnicity, family income, education of female guardian, maternal smoking, frequency of tooth brushing, firmness of toothbrush bristles, and frequency of chewing gum.

Table AX6-9.10 (cont'd). Effects of Lead on the Gastrointestinal System in Children and Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
***Children,* United States** (cont'd)			
Campbell et al. (2000) New York 1995–1997	Design: retrospective cohort Subjects: children (n = 154), ages 6.9–12 yr Outcome measures: prevalence of caries (dfs, DMFS) Analysis: multivariate logistic regression	Blood lead (μg/dL) mean (range): 10.7 (18.0–36.8) (measured at ages 18 and 37 mo)	Covariate-adjusted odds ratios for caries in association with blood lead <10 or ≥10 μg/dL, were: permanent teeth (DMFS): OR 0.95 (95% CI: 0.43–2.09) deciduous teeth (dfs): OR 1.77 (95% CI: 0.97–3.24) Covariates retained: age, grade in school, number of tooth surfaces at risk. Other covariates explored, that had no effect on strength of association with blood lead were: sex, ethnicity, and oral hygiene score.
Adults			
United States			
Dye et al. (2002) U.S. 1988–1994	Design: cross-sectional national survey (NHANES III) Subjects: adults in general population (n = 10,033; 5,255 females), ages 20–69 yr Outcome measures: symptoms of periodontal bone loss (attachment loss, periodontal pocket depth) Analysis: multivariate linear regression	Blood lead (μg/dL) geometric mean (SE, range): 2.5 (0.08) (2.36%> 10)	Increasing blood lead (log-transformed) was significantly associated with increasing prevalence of covariate-adjusted dental furcation (β = 0.13, SE 0.05, p = 0.005). Covariates retained: age, sex, race/ethnicity, education, smoking, and age of home. Smoking status interaction was significant when included in the model as an interaction term (β = 0.10, SE 0.05, p = 0.034). When stratified by smoking status, association between dental furcation and blood lead was significant for current smokers (β = 0.21, SE 0.07, p = 0.004) and former smokers (β = 0.17, SE 0.07, p = 0.015), but not for nonsmokers (β = -0.02, SE 0.07, p = 0.747).
Europe			
Tvinnereim et al. (2000) Norway 1990–1994	Design: cross-sectional Subjects: 1,271 teeth samples collected by dentists in all 19 counties in Norway Analysis: Student's t-test comparing metal concentrations in teeth with caries, roots, and in different tooth groups	Tooth lead (μg/g tooth) geometric mean (SD, range): 1.16 (1.72, 0.12-18.76)	Also examined mercury, cadmium, and zinc. All tooth groups had higher lead concentrations in carious than in non-carious teeth. The geometric mean lead concentration in carious teeth was 1.36 μg/g compared to 1.10 μg/g (p = 0.001).

Table AX6-9.11. Effects of Lead on Ocular Health in Children and Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Children			
Latin America			
Rothenberg et al., 2002 Mexico 1987–1997	Design = longitudinal (subset of prospective) Subjects: children (n = 45, 24 female), ages 7–10 yr Outcome measures: ERG Analysis: comparison of outcome measures between blood lead tertiles (ANOVA for repeated measures)	Blood lead (μg/dL) median (range) at 85–124 mo: 1st tertile: 4.0 (2.0–4.5) 2nd tertile: 6.0 (5.0–6.5) 3rd tertile: 7.5 (7.0–16.0) Blood lead (μg/dL) median (range), maternal at 12 wk of gestation = 1st tertile: 4.0 (2.0–5.5) 2nd tertile: 8.5 (6.0–10.0) 3rd tertile: 14.0 (10.5–32.5)	Significant association between increasing maternal blood lead at 12 wk of gestation and increasing ERG a-wave ($p = 0.025$) and b-wave amplitude ($p = 0.007$), with significant increases in a-wave in the 2nd blood lead tertile (6.0–10.0 μg/dL), and a-wave and b-wave in the 3rd blood lead tertile (10.5–32.5 μg/dL), compared to the 1st blood lead tertile.
Adults			
United States			
Schaumberg et al., 2004 Massachusetts 1991–2002	Design = longitudinal (subset of Normative Aging Study) Subjects: adult male (n = 642), mean age 69 yr (range: 60–93) Outcome measures: cataract diagnosis Analysis: multivariate logistic regression, odds ratio (vs. 1st quintile)	Blood lead (μg/dL) median (range): 5 (0–35) Bone lead (μg/g) median (range): patella : 29 (0–165) tibia: 20 (0–126)	Significant covariate adjusted odds ratio (OR) for cataracts in 5th tibia bone lead quintile (31.0–125 μg/g): OR 3.19 (95% CI: 1.48–6.90, $p = 0.01$). OR for cataracts were not significantly associated with patella bone lead (5th quintile: 43.0–165 μg/g): OR 1.88 (95% CI: 0.88–4.02) or blood lead (5th quintile: 8.17–35.0 μg/dL): OR 0.89 (95% CI: 0.46–1.72, $p = 0.73$). Covariates retained: age, smoking, history of diabetes, daily intake of vitamin C, vitamin E, and carotenoids.

Table AX6-9.11 (cont'd). Effects of Lead on Ocular Health in Children and Adults

Reference, Study Location, and Period	Study Description	Lead Measurement	Findings, Interpretation
Adults			
Europe			
Cavalleri et al., 1982 Italy NR	Design = cross-sectional cohort Subjects: adult male vinyl chloride pipe manufacture workers, exposed to lead stearate (n = 35), mean age 45 yr (SD 14, range: 21–59); reference group (n = 35) matched for age, smoking, and alcohol consumption. Outcome measures: visual field Analysis: comparison of outcome measures between lead workers and reference group	Blood lead (μg/dL) mean (SD, range): lead: 46 (14, 21–82) reference: 23 (4, 21–37) Urine lead (μg/L) mean (SD, range): lead: 71 (18, 44–118) reference: 30 (5, 21–42)	Visual sensitivity was significantly ($p = 0.003$) lower in lead workers compared to the reference group; however, visual sensitivity index was not significantly associated with blood or urine lead. Mesopic field scotoma prevalence was 10 of 35 (28%) in lead workers and 0% in the reference group.

ERG, electroretinogram

CHAPTER 6 ANNEX

ANNEX SECTION AX6-10

The analyses fitting both the linear and log-linear models assumed that the error in the response variable was constant across the range of values of the independent variable. Violations of this assumption (heteroscedasticity) could potentially bias the estimated slope of the model. Two models were considered:

Linear model: IQ = 90.0 – 0.4 × (blood lead level – 10.0), and
Log-linear model: IQ = 90.0 – 4.0 × [ln(blood lead level) – ln(10.0)].

The standard deviation of IQ was assumed to be equal to 15 × (blood lead level / 10)^{h} where h is the heteroscedasticity factor. When h = 0 there is no heteroscedasticity, and when h = 1 the standard deviation is proportional to the value of the blood lead. The value of h = 1 would be comparable to the situation where there is a lognormal error.

The linear regression models described above were simulated for a sample size of 200 subjects and a lognormal distribution of blood lead levels with a geometric mean of 10.0 and a geometric standard deviation of 1.5. For each set of models and values of h, 100,000 simulations were performed.

Table AX6-10.1. Average Estimated Slopes for Linear and Log-linear Models in the Presence of Heteroscedasticity

Heteroscedasticity (h)	Linear Model (True slope = -0.4)	Log-Linear Model (True slope = -4.0)
0.0	−0.400	−4.00
0.5	−0.400	−4.00
1.0	−0.399	−4.01

The simulations indicated that any presence of heteroscedasticity would have no noticeable bias on the estimation of the slopes of the models.

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