ECAO-CD-81-2 IIA.K.1

United States
Environmental Protection
Agency

Environmental Criteria and
Assessment Office
Research Triangle Park NC 27711

EPA-600/8-83-028A
October 1983
External Review Draft

Research and Development

EPA

Air Quality Criteria for Lead

Review Draft

(Do Not
Cite or Quote)

Volume IV of IV

FILE COPY

NOTICE

This document is a preliminary draft. It has not been formally released by EPA and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implications.

EPA-600/8-83-028A
October 1983
External Review Draft

Draft
Do Not Quote or Cite

Air Quality Criteria for Lead

Volume IV

NOTICE

This document is a preliminary draft. It has not been formally released by EPA and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implications.

Environmental Criteria and Assessment Office
Office of Health and Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, N.C. 27711

NOTICE

Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

ABSTRACT

The document evaluates and assesses scientific information on the health and welfare effects associated with exposure to various concentrations of lead in ambient air. The literature through 1983 has been reviewed thoroughly for information relevant to air quality criteria, although the document is not intended as a complete and detailed review of all literature pertaining to lead. An attempt has been made to identify the major discrepancies in our current knowledge and understanding of the effects of these pollutants.

Although this document is principally concerned with the health and welfare effects of lead, other scientific data are presented and evaluated in order to provide a better understanding of this pollutant in the environment. To this end, the document includes chapters that discuss the chemistry and physics of the pollutant; analytical techniques; sources, and types of emissions; environmental concentrations and exposure levels; atmospheric chemistry and dispersion modeling; effects on vegetation; and respiratory, physiological, toxicological, clinical, and epidemiological aspects of human exposure.

PRELIMINARY DRAFT

CONTENTS

		Page
VOLUME I		
Chapter 1.	Executive Summary and Conclusions	1-1
VOLUME II		
Chapter 2.	Introduction	2-1
Chapter 3.	Chemical and Physical Properties	3-1
Chapter 4.	Sampling and Analytical Methods for Environmental Lead	4-1
Chapter 5.	Sources and Emissions	5-1
Chapter 6.	Transport and Transformation	6-1
Chapter 7.	Environmental Concentrations and Potential Pathways to Human Exposure	7-1
Chapter 8.	Effects of Lead on Ecosystems	8-1
VOLUME III		
Chapter 9.	Quantitative Evaluation of Lead and Biochemical Indices of Lead Exposure in Physiological Media	9-1
Chapter 10.	Metabolism of Lead	10-1
Chapter 11.	Assessment of Lead Exposures and Absorption in Human Populations	11-1
Volume IV		
Chapter 12.	Biological Effects of Lead Exposure	12-1
Chapter 13.	Evaluation of Human Health Risk Associated with Exposure to Lead and Its Compounds	13-1

TABLE OF CONTENTS

Page

LIST OF FIGURES ix
LIST OF TABLES ix

12. BIOLOGICAL EFFECTS OF LEAD EXPOSURE 12-1
12.1 INTRODUCTION 12-1
12.2 SUBCELLULAR EFFECTS OF LEAD IN HUMANS AND EXPERIMENTAL ANIMALS 12-3
12.2.1 Effects of Lead on the Mitochondrion 12-4
12.2.1.1 Effects of Lead on Mitochondrial Structure 12-4
12.2.1.2 Effects of Lead on Mitochondrial Function 12-4
12.2.1.3 *In Vivo* Studies 12-4
12.2.1.4 *In Vitro* Studies 12-6
12.2.2 Effects of Lead on the Nucleus 12-7
12.2.3 Effects of Lead on Membranes 12-8
12.2.4 Other Organellar Effects of Lead 12-9
12.2.5 Summary of Subcellular Effects of Lead 12-9
12.3 EFFECTS OF LEAD ON HEME BIOSYNTHESIS AND ERYTHROPOIESIS/ERYTHROCYTE PHYSIOLOGY IN HUMANS AND ANIMALS 12-12
12.3.1 Effects of Lead on Heme Biosynthesis 12-12
12.3.1.1 Effects of Lead on δ-Aminolevulinic Acid Synthetase 12-13
12.3.1.2 Effects of Lead on δ-Aminolevulinic Acid Dehydrase and ALA Accumulation/Excretion 12-14
12.3.1.3 Effects of Lead on Heme Formation from Protoporphyrin 12-19
12.3.1.4 Other Heme-Related Effects of Lead 12-24
12.3.2 Effects of Lead on Erythropoiesis and Erythrocyte Physiology 12-25
12.3.2.1 Effects of Lead on Hemoglobin Production 12-25
12.3.2.2 Effects of Lead on Erythrocyte Morphology and Survival 12-26
12.3.2.3 Effects of Lead on Pyrimidine-5'-Nucleotidase Activity and Erythropoietic Pyrimidine Metabolism 12-28
12.3.3 Effects of Alkyl Lead on Heme Synthesis and Erythopoiesis 12-30
12.3.4 The Interrelationship of Lead Effects on Heme Synthesis and the Nervous System 12-30
12.3.5 Summary and Overview 12-33
12.3.5.1 Lead Effects on Heme Biosynthesis 12-33
12.3.5.2 Lead Effects on Erythropoiesis and Erythrocyte Physiology 12-37
12.3.5.3 Effects of Alkyl Lead Compounds on Heme Biosynthesis and Erythropoiesis 12-38
12.3.5.4 Relationships of Lead Effects on Heme Synthesis and Neurotoxicity 12-38
12.4 NEUROTOXIC EFFECTS OF LEAD 12-40
12.4.1 Introduction 12-40
12.4.2 Human Studies 12-41
12.4.2.1 Neurotoxic Effects of Lead Exposure in Adults 12-44
12.4.2.2 Neurotoxic Effects of Lead Exposure in Children 12-50
12.4.3 Animal Studies 12-76
12.4.3.1 Behavioral Toxicity: Critical Periods for Exposure and Expression of Effects 12-77

TABLE OF CONTENTS (continued).

Page

12.4.3.2 Morphological Effects 12-100
12.4.3.3 Electrophysiological Effects 12-102
12.4.3.4 Biochemical Alterations 12-105
12.4.3.5 Accumulation and Retention of Lead in the Brain 12-110
12.4.4 Integrative Summary of Human and Animal Studies of Neurotoxicity .. 12-110
12.4.4.1 Internal Exposure Levels at Which Adverse Neurobehavioral Effects Occur 12-114
12.4.4.2 The Question of Irreversibility 12-115
12.4.4.3 Early Development and the Susceptibility to Neural Damage 12-116
12.4.4.4 Utility of Animal Studies in Drawing Parallels to the Human Condition 12-116
12.5 EFFECTS OF LEAD ON THE KIDNEY 12-121
12.5.1 Historical Aspects 12-121
12.5.2 Lead Nephropathy in Childhood 12-121
12.5.3 Lead Nephropathy in Adults 12-122
12.5.3.1 Lead Nephropathy Following Childhood Lead Poisoning 12-122
12.5.3.2 "Moonshine" Lead Nephropathy 12-124
12.5.3.3 Occupational Lead Nephropathy 12-124
12.5.3.4 Lead and Gouty Nephropathy 12-129
12.5.3.5 Lead and Hypertensive Nephrosclerosis 12-132
12.5.3.6 General Population Studies 12-133
12.5.4 Mortality Data 12-134
12.5.5 Experimental Animal Studies of the Pathophysiology of Lead Nephropathy 12-135
12.5.5.1 Lead Uptake By the Kidney 12-135
12.5.5.2 Intracellular Binding of Lead in the Kidney 12-137
12.5.5.3 Pathological Features of Lead Nephropathy 12-137
12.5.5.4 Functional Studies 12-139
12.5.6 Experimental Studies of the Biochemical Aspects of Lead Nephrotoxicity 12-140
12.5.6.1 Membrane Marker Enzymes and Transport Functions 12-140
12.5.6.2 Mitochondrial Respiration/Energy-Linked Transformation 12-140
12.5.6.3 Renal Heme Biosynthesis 12-141
12.5.6.4 Lead Alteration of Renal Nucleic Acid/Protein Synthesis 12-142
12.5.6.5 Lead Effects on the Renin-Angiotension System 12-144
12.5.6.6 Effects of Lead on Uric Acid Metabolism 12-145
12.5.6.7 Effects of Lead on Vitamin D Metabolism in the Kidney ... 12-145
12.5.7 General Summary and Comparison of Lead Effects in Kidneys of Humans and Animal Models 12-146
12.6 EFFECTS OF LEAD ON REPRODUCTION AND DEVELOPMENT 12-147
12.6.1 Human Studies 12-147
12.6.1.1 Historical Evidence 12-147
12.6.1.2 Effects of Lead Exposure on Reproduction 12-148
12.6.1.3 Placental Transfer of Lead 12-152
12.6.1.4 Effects of Lead on the Developing Human 12-152
12.6.1.5 Summary of the Human Data 12-156
12.6.2 Animal Studies 12-156
12.6.2.1 Effects of Lead on Reproduction 12-156
12.6.2.2 Effects of Lead on the Offspring 12-160

TABLE OF CONTENTS (continued).

		Page
	12.6.2.3 Effects of Lead on Avian Species	12-171
	12.6.3 Summary	12-171
12.7	GENETOXIC AND CARCINOGENIC EFFECTS OF LEAD	12-173
	12.7.1 Introduction	12-173
	12.7.2 Carcinogenesis Studies with Lead and its Compounds	12-176
	12.7.2.1 Human Epidemiological Studies	12-176
	12.7.2.2 Induction of Tumors in Experimental Animals	12-180
	12.7.2.3 Cell Transformation	12-185
	12.7.3 Genotoxicity of Lead	12-188
	12.7.3.1 Chromosomal Aberrations	12-188
	12.7.3.2 Effect of Lead on Bacterial and Mammalian Mutagenesis Systems	12-193
	12.7.3.3 Effect of Lead on Parameters of DNA Structure and Function	12-194
	12.7.4 Summary and Conclusions	12-195
12.8	EFFECTS OF LEAD ON THE IMMUNE SYSTEM	12-196
	12.8.1 Development and Organization of the Immune System	12-196
	12.8.2 Host Resistance	12-197
	12.8.2.1 Infectivity Models	12-198
	12.8.2.2 Tumor Models and Neoplasia	12-200
	12.8.3 Humoral Immunity	12-200
	12.8.3.1 Antibody Titers	12-200
	12.8.3.2 Enumeration of Antibody Producing Cells (Plaque-Forming Cells)	12-202
	12.8.4 Cell-Mediated Immunity	12-204
	12.8.4.1 Delayed-Type Hypersensitivity	12-204
	12.8.4.3 Interferon	12-206
	12.8.5 Lymphocyte Activation by Mitogens	12-206
	12.8.5.1 *In Vivo* Exposure	12-206
	12.8.5.2 *In Vitro* Exposure	12-208
	12.8.6 Macrophage Function	12-209
	12.8.7 Mechanisms of Lead Immunomodulation	12-211
	12.8.8 Summary	12-211
12.9	EFFECTS OF LEAD ON OTHER ORGAN SYSTEMS	12-212
	12.9.1 The Cardiovascular System	12-212
	12.9.2 The Hepatic System	12-214
	12.9.3 The Endocrine System	12-216
	12.9.4 The Gastrointestinal System	12-218
12.10	CHAPTER SUMMARY	12-218
	12.10.1 Introduction	12-218
	12.10.2 Subcellular Effects of Lead	12-218
	12.10.3 Effects of Lead on Heme Biosynthesis, Erythropoiesis, and Erythrocyte Physiology in Humans and Animals	12-221
	12.10.4 Neurotoxic Effects of Lead	12-227
	12.10.4.1 Internal Exposure Levels at Which Adverse Neurobehavioral Effects Occur	12-228
	12.10.4.2 The Question of Irreversibility	12-229
	12.10.4.3 Early Development and the Susceptibility to Neural Damage	12-230
	12.10.4.4 Utility of Animal Studies in Drawing Parallels to the Human Condition	12-230

TABLE OF CONTENTS (continued).

	Page
12.10.5 Effects of Lead on the Kidney	12-232
12.10.6 Effects of Lead on Reproduction and Development	12-233
12.10.7 Genotoxic and Carcinogenic Effects of Lead	12-234
12.10.8 Effects of Lead on the Immune System	12-234
12.10.9 Effects of Lead on Other Organ Systems	12-235
12.11 REFERENCES	12-236
APPENDIX 12-A	12A-1
APPENDIX 12-B	12B-1
APPENDIX 12-C	TBA
APPENDIX 12-D	12D-1
13.1 INTRODUCTION	13-1
13.2 EXPOSURE ASPECTS	13-2
13.2.1 Sources of Lead Emission in the United States	13-2
13.2.2 Environmental Cycling of Lead	13-4
13.2.3 Levels of Lead in Various Media of Relevance to Human Exposure	13-5
13.2.3.1 Ambient Air Lead Levels	13-6
13.2.3.2 Levels of Lead in Dust	13-6
13.2.3.3 Levels of Lead in Food	13-7
13.2.3.4 Lead Levels in Drinking Water	13-7
13.2.3.5 Lead in Other Media	13-11
13.2.3.6 Cumulative Human Lead Intake From Various Sources	13-11
13.3 LEAD METABOLISM: KEY ISSUES FOR HUMAN HEALTH RISK EVALUATION	13-11
13.3.1 Differential Internal Lead Exposure Within Population Groups	13-12
13.3.2 Indices of Internal Lead Exposure and Their Relationship to External Lead Levels and Tissue Burdens/Effects	13-13
13.4 DEMOGRAPHIC CORRELATES OF HUMAN LEAD EXPOSURE AND RELATIONSHIPS BETWEEN EXTERNAL AND INTERNAL LEAD EXPOSURE INDICES	13-16
13.4.1 Demographic Correlates of Lead Exposure	13-16
13.4.2 Relationships Between External and Internal Lead Exposure Indices	13-18
13.4.3 Proportional Contributions of Lead in Various Media to Blood Lead in Human Populations	13-23
13.5 BIOLOGICAL EFFECTS OF LEAD RELEVANT TO THE GENERAL HUMAN POPULATION	13-27
13.5.1 Introduction	13-27
13.5.2 Dose-Effect Relationship for Lead-Induced Health Effects	13-29
13.5.2.1 Human Adults	13-29
13.5.2.2 Children	13-31
13.6 DOSE-RESPONSE RELATIONSHIPS FOR LEAD IN HUMAN POPULATIONS	13-36
13.7 POPULATIONS AT RISK	13-40
13.7.1 Children as a Population at Risk	13-40
13.7.1.1 Inherent Susceptibility of the Young	13-40
13.7.1.2 Exposure Consideration	13-41
13.7.2 Pregnant Women and the Conceptus as a Population at Risk	13-41
13.7.3 Description of the United States Population in Relation to Potential Lead Exposure Risk	13-42
13.8 SUMMARY AND CONCLUSIONS	13-44
13-9 REFERENCES	13-46

LIST OF FIGURES

Figure		Page
12-1	Lead effects on heme biosynthesis	12-13
12-2	Maximal motor nerve conduction velocity (NCV) of the median nerve plotted against the actual Pb-B level (μg/100 ml) for 78 workers occupationally exposed to lead and for 34 control subjects	12-49
12-3	(a) Predicted SW voltage and 95% confidence bounds in 13- and 75-month-old children as a function of blood lead level. (b) Scatter plots of SW data from children aged 13-47 months with predicted regression lines for ages 18, 30, and 42 months. (c) Scatter plots for children aged 48-75 months with predicted regression lines for ages 54 and 66 months. These graphs depict the linear interaction of blood lead level and age	12-73
12-4	Peroneal nerve conduction velocity versus blood lead level, Idaho, 1974	12-75
12-5	Probit plot of incidence of renal tumors in male rats	12-186
13-1	Pathways of lead from the environment to man	13-3
13-2	Geometric mean blood lead levels by race and age for younger children in the NHANES II study, and the Kellogg/Silver Valley and New York childhood screening studies	13-17
13-3	Dose-response for elevation of EP as a function of blood lead level using probit analysis	13-38
13-4	Dose-response curve for FEP as a function of blood lead level: in subpopulations	13-38
13-5	EPA calculated dose-response curve for ALA-U	13-39

LIST OF TABLES

Table		Page
12-1	Summary of results of human studies on neurobehavioral effects	12-55
12-2	Effects of lead on activity in rats and mice	12-81
12-3	Recent animal toxicology studies of lead effects on learning in rodent species	12-83
12-4	Recent animal toxicology studies of lead effects on learning in primates	12-89
12-5	Summary of key studies of morphological effects of *in vivo* lead exposure	12-101
12-6	Summary of key studies of electrophysiological effects of *in vivo* lead exposure	12-103
12-7	Summary of key studies on biochemical effects of *in vivo* lead exposure	12-106
12-8	Index of blood lead and brain lead levels following exposure	12-111
12-9	Summary of key studies of *in vitro* lead exposure	12-119
12-10	Morphological features of lead nephropathy in various species	12-138
12-11	Effects of lead exposure on renal heme biosynthesis	12-143
12-12	Statistics on the effect of lead on pregnancy	12-148
12-13	Effects of prenatal exposure to lead on the offspring of laboratory and domestic animals	12-161
12-14	Effects of prenatal lead exposure on offspring of laboratory animals	12-163
12-15	Reproductive performance of F_1 lead-intoxicated rats	12-166

LIST OF TABLES (continued).

Table		Page
12-16	Expected and observed deaths for malignant neoplasms Jan. 1, 1947 - Dec. 31, 1979 for lead smelter and battery plant workers	12-177
12-17	Expected and observed deaths resulting from specified malignant neoplasms for lead smelter and battery plant workers and levels of significance by type of statistical analysis according to one-tailed tests	12-178
12-18	Examples of studies on the incidence of tumors in experimental animals exposed to lead compounds	12-181
12-19	Mortality and kidney tumors in rats fed lead acetate for two years	12-185
12-20	Cytogenetic investigations of cells from individuals exposed to lead: 10 positive studies	12-189
12-21	Cytogenetic investigations of cells from individuals exposed to lead: 6 negative studies	12-190
12-22	Effect of lead on host resistance to infectious agents	12-197
12-23	Effect of lead on antibody titers	12-200
12-24	Effect of lead on the development of antibody-producing cells (PFC)	12-202
12-25	Effect of lead on cell-mediated immunity	12-204
12-26	Effect of lead exposure on mitogen activation of lymphocytes	12-206
12-27	Effect of lead on macrophage and reticuloendothelial system function	12-209
12-B	Tests commonly used in a psycho-educational battery for children	12-B2
13-1	Summary of baseline human exposures to lead	13-8
13-2	Relative baseline human lead exposures expressed per kilogram body weight	13-9
13-3	Summary of potential additive exposures to lead	13-11
13-4	Summary of blood inhalation slopes, (β)	13-19
13-5	Estimated contribution of leaded gasoline to blood lead by inhalation and non-inhalation pathways	13-22
13-6	Direct contributions of air lead to blood lead in adults at fixed inputs of water and food lead	13-24
13-7	Direct contributions of air lead to blood lead in children at fixed inputs of food and water lead	13-25
13-8	Contributions of dust/soil lead to blood lead in children at fixed inputs of air, food, and water lead	13-26
13-9	Summary of lowest observed effect levels for key lead-induced health effects in adults	13-30
13-10	Summary of lowest observed effect levels for key lead-induced health effects in children	13-32
13-11	EPA-estimated percentage of subjects with ALA-U exceeding limits for various blood lead levels	13-39
13-12	Provisional estimate of the number of individuals in urban and rural population segments at greatest potential risk to lead exposure	13-43

LIST OF ABBREVIATIONS

AAS	Atomic absorption spectrometry
Ach	Acetylcholine
ACTH	Adrenocoticotrophic hormone
ADCC	Antibody-dependent cell-mediated cytotoxicity
ADP/O ratio	Adenosine diphosphate/oxygen ratio
AIDS	Acquired immune deficiency syndrome
AIHA	American Industrial Hygiene Association
AII	Angiotensin II
ALA	Aminolevulinic acid
ALA-D	Aminolevulinic acid dehydrase
ALA-S	Aminolevulinic acid synthetase
ALA-U	Aminolevulinic acid in urine
APDC	Ammonium pyrrolidine-dithiocarbamate
APHA	American Public Health Association
ASTM	Amercian Society for Testing and Materials
ASV	Anodic stripping voltammetry
ATP	Adenosine triphosphate
B-cells	Bone marrow-derived lymphocytes
Ba	Barium
BAL	British anti-Lewisite (AKA dimercaprol)
BAP	benzo(a)pyrene
BSA	Bovine serum albumin
BUN	Blood urea nitrogen
BW	Body weight
C.V.	Coefficient of variation
CaBP	Calcium binding protein
CaEDTA	Calcium ethylenediaminetetraacetate
CBD	Central business district
Cd	Cadmium
CDC	Centers for Disease Control
CEC	Cation exchange capacity
CEH	Center for Environmental Health
CFR	reference method
CMP	Cytidine monophosphate
CNS	Central nervous system
CO	Carbon monoxide
COHb	Carboxyhemoglobin
CP-U	Urinary coproporphyrin
C_{Pah}	plasma clearance of p-aminohippuric acid
Cu	Copper
D.F.	Degrees of freedom
DA	Dopamine
DCMU	[3-(3,4-dichlorophenyl)-1,1-dimethylurea
DDP	Differential pulse polarography
DNA	Deoxyribonucleic acid
DTH	Delayed-type hypersensitivity
EEC	European Economic Community
EEG	Electroencephalogram
EMC	Encephalomyocarditis
EP	Erythrocyte protoporphyrin
EPA	U.S. Environmental Protection Agency

LIST OF ABBREVIATIONS (continued).

FA	Fulvic acid
FDA	Food and Drug Administration
Fe	Iron
FEP	Free erythrocyte protoporphyrin
FY	Fiscal year
G.M.	Grand mean
G-6-PD	Glucose-6-phosphate dehydrogenase
GABA	Gamma-aminobutyric acid
GALT	Gut-associated lymphoid tissue
GC	Gas chromatography
GFR	Glomerular filtration rate
HA	Humic acid
Hg	Mercury
hi-vol	High-volume air sampler
HPLC	High-performance liquid chromatography
i.m.	Intramuscular (method of injection)
i.p.	Intraperitoneally (method of injection)
i.v.	Intravenously (method of injection)
IAA	Indol-3-ylacetic acid
IARC	International Agency for Research on Cancer
ICD	International classification of diseases
ICP	Inductively coupled plasma
IDMS	Isotope dilution mass spectrometry
IF	Interferon
ILE	Isotopic Lead Experiment (Italy)
IRPC	International Radiological Protection Commission
K	Potassium
LAI	Leaf area index
LDH-X	Lactate dehydrogenase isoenzyme x
LC_{50}	Lethyl concentration (50 percent)
LD_{50}	Lethal dose (50 percent)
LH	Luteinizing hormone
LIPO	Laboratory Improvement Program Office
ln	National logarithm
LPS	Lipopolysaccharide
LRT	Long range transport
mRNA	Messenger ribonucleic acid
ME	Mercaptoethanol
MEPP	Miniature end-plate potential
MES	Maximal electroshock seizure
MeV	Mega-electron volts
MLC	Mixed lymphocyte culture
MMD	Mass median diameter
MMED	Mass median equivalent diameter
Mn	Manganese
MND	Motor neuron disease
MSV	Moloney sarcoma virus
MTD	Maximum tolerated dose
n	Number of subjects
N/A	Not Available

LIST OF ABBREVIATIONS

NA	Not Applicable
NAAQS	National ambient air quality standards
NADB	National Aerometric Data Bank
NAMS	National Air Monitoring Station
NAS	National Academy of Sciences
NASN	National Air Surveillance Network
NBS	National Bureau of Standards
NE	Norepinephrine
NFAN	National Filter Analysis Network
NFR-82	Nutrition Foundation Report of 1982
NHANES II	National Health Assessment and Nutritional Evaluation Survey II
Ni	Nickel
OSHA	Occupational Safety and Health Administration
P	Potassium
p	Significance symbol
PAH	Para-aminohippuric acid
Pb	Lead
PBA	Air lead
$Pb(Ac)_2$	Lead acetate
PbB	concentration of lead in blood
PbBrCl	Lead (II) bromochloride
PBG	Porphobilinogen
PFC	Plaque-forming cells
pH	Measure of acidity
PHA	Phytohemagglutinin
PHZ	Polyacrylamide-hydrous-zirconia
PIXE	Proton-induced X-ray emissions
PMN	Polymorphonuclear leukocytes
PND	Post-natal day
PNS	Peripheral nervous system
ppm	Parts per million
PRA	Plasma renin activity
PRS	Plasma renin substrate
PWM	Pokeweed mitogen
Py-5-N	Pyrimide-5'-nucleotidase
RBC	Red blood cell; erythrocyte
RBF	Renal blood flow
RCR	Respiratory control ratios/rates
redox	Oxidation-reduction potential
RES	Reticuloendothelial system
RLV	Rauscher leukemia virus
RNA	Ribonucleic acid
S-HT	Serotonin
SA-7	Simian adenovirus
scm	Standard cubic meter
S.D.	Standard deviation
SDS	Sodium dodecyl sulfate
S.E.M.	Standard error of the mean
SES	Socioeconomic status
SGOT	Serum glutamic oxaloacetic transaminase

LIST OF ABBREVIATIONS (continued).

sIg	Surface immunoglobulin
SLAMS	State and local air monitoring stations
SMR	Standardized mortality ratio
Sr	Strontium
SRBC	Sheep red blood cells
SRMs	Standard reference materials
STEL	Short-term exposure limit
SW voltage	Slow-wave voltage
T-cells	Thymus-derived lymphocytes
t-tests	Tests of significance
TBL	Tri-n-butyl lead
TEA	Tetraethyl-ammonium
TEL	Tetraethyllead
TIBC	Total iron binding capacity
TML	Tetramethyllead
TMLC	Tetramethyllead chloride
TSH	Thyroid-stimulating hormone
TSP	Total suspended particulate
U.K.	United Kingdom
UMP	Uridine monophosphate
USPHS	U.S. Public Health Service
VA	Veterans Administration
V_d	Deposition velocity
VER	Visual evoked response
WHO	World Health Organization
XRF	X-Ray fluorescence
X^2	Chi squared
Zn	Zinc
ZPP	Erythrocyte zinc protoporphyrin

MEASUREMENT ABBREVIATIONS

dl	deciliter
ft	feet
g	gram
g/gal	gram/gallon
g/ha·mo	gram/hectare·month
km/hr	kilometer/hour
l/min	liter/minute
mg/km	milligram/kilometer
$\mu g/m^3$	microgram/cubic meter
mm	millimeter
µmol	micrometer
ng/cm^2	nanograms/square centimeter
nm	namometer
nM	nanomole
sec	second

Chapter 12: Biological Effects of Lead Exposure

Contributing Authors

Dr. Max Costa
Department of Pharmacology
University of Texas Medical School
Houston, TX 77025

Dr. J. Michael Davis
Environmental Criteria and Assessment Office
MD-52
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Dr. Jack Dean
Immunobiology Program and Immunotoxicology/
Cell Biology Program
CIIT
P.O. Box 12137
Research Triangle Park, NC 27709

Dr. Bruce Fowler
Laboratory of Pharmacology
NIEHS
P.O. Box 12233
Research Triangle Park, NC 27709

Dr. Lester Grant
Director, Environmental Criteria and
Assessment Office
MD-52
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Dr. Ronald D. Hood
Department of Biology
The University of Alabama
P.O. Box 1927
University, AL 35486

Dr. Loren Koller
School of Veterinary Medicine
University of Idaho
Moscow, ID 83843

Dr. David Lawrence
Microbiology and Immunology
Department
Albany Medical College of Union
University
Albany, NY 12208

Dr. Paul Mushak
Department of Pathology
UNC School of Medicine
Chapel Hill, NC 27514

Dr. Dr. David Otto
Clinical Studies Division
MD-58
U.S. Environmental Protection
Agency
Research Triangle Park, NC 27711

Dr. Magnus Piscator
Department of Environmental Hygiene
The Karolinska Institute 104 01
Stockholm
Sweden

Dr. Stephen R. Schroeder
Division for Disorders of
Development and Learning
Biological Sciences Research Center
University of North Carolina
Chapel Hill, NC 27514

Dr. Richard P. Wedeen
V.A. Medical Center
Tremont Avenue
East Orange, NJ 07019

Dr. David Weil
Environmental Criteria and
Assessment Office
MD-52
U.S. Environmental Protection
Agency
Research Triangle Park, NC 27711

The following persons reviewed this chapter at EPA's request. The evaluations and conclusions contained herein, however, are not necessarily those of the reviewers.

Dr. Carol Angle
Department of Pediatrics
University of Nebraska
College of Medicine
Omaha, NE 68105

Dr. Lee Annest
Division of Health Examin. Statistics
National Center for Health Statistics
3700 East-West Highway
Hyattsville, MD 20782

Dr. Donald Barltrop
Department of Child Health
Westminister Children's Hospital
London SW1P 2NS
England

Dr. Irv Billick
Gas Research Institute
8600 West Bryn Mawr Avenue
Chicago, IL 60631

Dr. Joe Boone
Clinical Chemistry and
Toxicology Section
Center for Disease Control
Atlanta, GA 30333

Dr. Robert Bornschein
University of Cincinnati
Kettering Laboratory
Cincinnati, OH 45267

Dr. A. C. Chamberlain
Environmental and Medical Sciences Division
Atomic Energy Research Establishment
Harwell OX11
England

Dr. Neil Chernoff
Division of Developmental Biology
MD-67
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Dr. Julian Chisolm
Baltimore City Hospital
4940 Eastern Avenue
Baltimore, MD 21224

Dr. Jerry Cole
International Lead-Zinc Research
Organization
292 Madison Avenue
New York, NY 10017

Dr. Anita Curran
Commissioner of Health
Westchester County
White Plains, NY 10607

Dr. Cliff Davidson
Department of Civil Engineering
Carnegie-Mellon University
Schenley Park
Pittsburgh, PA 15213

Dr. H. T. Delves
Chemical Pathology and Human
Metabolism
Southampton General Hospital
Southampton SO9 4XY
England

Dr. Fred deSerres
Associate Director for Genetics
NIEHS
P.O. Box 12233
Research Triangle Park, NC 27709

Dr. Joseph A. DiPaolo
Laboratory of Biology, Division
of Cancer Cause and Prevention
National Cancer Institute
Bethesda, MD 20205

Dr. Robert Dixon
Laboratory of Reproductive and
Developmental Toxicology
NIEHS
P.O. Box 12233
Research Triangle Park, NC 27711

Dr. Clair Ernhart
Department of Psychiatry
Cleveland Metropolitan General Hospital
3395 Scranton Road
Cleveland, OH 44109

Dr. Sergio Fachetti
Section Head - Isotope Analysis
Chemistry Division
Joint Research Center
121020 Ispra
Varese, Italy

Dr. Virgil Ferm
Department of Anatomy and Cytology
Dartmouth Medical School
Hanover, NH 03755

Dr. Alf Fischbein
Environmental Sciences Laboratory
Mt. Sinai School of Medicine
New York, NY 10029

Dr. Jack Fowle
Reproductive Effects Assessment Group
U.S. Environmental Protection Agency
RD-689
Washington, DC 20460

Dr. Bruce Fowler
Laboratory of Pharmocology
NIEHS
P.O. Box 12233
Research Triangle Park, NC 27709

Dr. Warren Galke
Department of Biostatistics and Epidemiology
School of Allied Health
East Carolina University
Greenville, NC 27834

Mr. Eric Goldstein
Natural Resources Defense Council, Inc.
122 E. 42nd Street
New York, NY 10168

Dr. Harvey Gonick
1033 Gayley Avenue
Suite 116
Los Angeles, CA 90024

Dr. Robert Goyer
Deputy Director
NIEHS
P.O. Box 12233
Research Triangle Park, NC 27711

Dr. Philippe Grandjear
Department of Environmental Medicine
Institute of Community Health
Odense University
Denmark

Dr. Stanley Gross
Hazard Evaluation Division
Toxicology Branch
U.S. Environmental Protection Agency
Washington, DC 20460

Dr. Paul Hammond
University of Cincinnati
Kettering Laboratory
Cincinnati, OH 45267

Dr. Kari Hemminki
Institute of Occupational Health
Tyoterveyslaitos-Haartmaninkatu
1 SF-00290 Helsinki 29
Finland

Dr. V. Houk
Center for Disease Control
1600 Clifton Road, NE
Atlanta, GA 30333

Dr. Carole A. Kimmel
Perinatal and Postnatal Evaluation Branch
National Center for Toxicological Research
Jefferson, AR 72079

Dr. Kristal Kostial
Institute for Medical Research and Occupational Health
YU-4100 Zagreb
Yugoslavia

Dr. Lawrence Kupper
Department of Biostatistics
UNC School of Public Health
Chapel Hill, NC 27514

Dr. Phillip Landrigan
Division of Surveillance,
Hazard Evaluation and Field Studies
Taft Laboratories - NIOSH
Cincinnati, OH 45226

Dr. Alais-Yves Leonard
Centre Betude De L'Energie Nucleaire
B-1040 Brussels
Belgium

Dr. Jane Lin-Fu
Office of Maternal and Child Health
Department of Health and Human Services
Rockville, MD 20857

Dr. Don Lynam
Air Conservation
Ethyl Corporation
451 Florida Boulevard
Baton Rouge, LA 70801

Dr. Kathryn Mahaffey
Division of Nutrition
Food and Drug Administration
1090 Tusculum Avenue
Cincinnati, OH 45226

Dr. Ed McCabe
Department of Pediatrics
University of Wisconsin
Madison, WI 53706

Dr. Chuck Nauman
Exposure Assessment Group
U.S. Environmental Protection Agnecy
Washington, DC 20460

Dr. Herbert L. Needleman
Children's Hospital of Pittsburgh
Pittsburgh, PA 15213

Dr. Forrest H. Nielsen
Grand Forks Human Nutrition Research Center
USDA
Grand Forks, ND 58202

Dr. Stephen Overman
Toxicology Institute
New York State Department of
Health
Empire State Plaza
Albany, NY 12001

Dr. H. Mitchell Perry
V.A. Medical Center
St. Louis, MO 63131

Dr. Jack Pierrard
E.I. duPont de Nemours and
Company, Inc.
Petroleum Laboratory
Wilmington, DE 19898

Dr. Sergio Piomelli
Columbia University Medical School
Division of Pediatric Hematology
and Oncology
New York, NY 10032

Dr. Robert Putnam
International Lead-Zinc
Research Organization
292 Madison Avenue
New York, NY 10017

Dr. Rabinowitz
Children's Hospital Medical Center
300 Longwood Avenue
Boston, MA 02115

Dr. Dr. Larry Reiter
Neurotoxicology Division
MD-74B
U.S. Environmental Protection
Agency
Research Triangle Park, NC 27711

Dr. Cecil R. Reynolds
Department of Educational Psychology
Texas A & M University
College Station, TX 77843

Dr. Patricia Rodier
Department of Anatomy
University of Rochester Medical
Center
Rochester, NY 14642

Dr. Harry Roels
Unite de Toxicologie Industrielle et Medicale
Universite de Louvain
Brussels, Belgium

Dr. John Rosen
Head, Division of Pediatric Metabolism
Montefiore Hospital and Medical Center
111 East 210 Street
Bronx, NY 10467

Dr. Michael Rutter
Department of Psychology
Institute of Psychiatry
DeCrespigny Park
London SE5 8AL
England

Dr. Anna-Maria Seppalainen
Institutes of Occupational Health
Tyoterveyslaitos
Haartmanikatu 1
00290 Helsinki 29
Finland

Dr. Ellen Silbergeld
Environmental Defense Fund
1525 18th Street, NW
Washington, DC 20036

Dr. Ron Snee
E.I. duPont de Nemours and
Company, Inc.
Engineering Department L3167
Wilmington, DE 19898

Dr. J. William Sunderman, Jr.
Department of Pharmacology
University of Connecticut
School of Medicine
Farmington, CT 06032

Dr. Gary Ter Haar
Toxicology and Industrial
Hygiene
Ethyl Corporation
451 Florida Boulevard
Baton Rouge, LA 70801

Dr. Hugh A. Tilson
Laboratory of Behavioral and
Neurological Toxicology
NIEHS
Research Triangle Park, NC 27709

Mr. Ian von Lindern
Department of Chemical Engineering
University of Idaho
Moscow, ID 83843

Chapter 13: Risk Assessment

Principal Authors

Dr. Lester Grant
Director, Environmental Criteria and Assessment Office
MD-52
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Dr. Paul Mushak
Department of Pathology
UNC School of Medicine
Chapel Hill, NC 27514

Contributing Authors

Dr. Robert Elias
Environmental Criteria and Assessment Office
MD-52
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Dr. Alan Marcus
Department of Mathematics
Washington State University
Pullman, Washington 99164-2930

Dr. Vic Hasselblad
Biometry Division
MD-55
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Dr. David Weil
Environmental Criteria and Assessment Office
U.S Environmental Protection Agency
Research Triangle Park, NC 27711

Dr. Dennis Kotchmar
Environmental Criteria and Assessment Office
MD-52
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

12. BIOLOGICAL EFFECTS OF LEAD EXPOSURE

12.1 INTRODUCTION

As noted in Chapter 2, air quality criteria documents evaluate scientific knowledge of relationships between pollutant concentrations and their effects on the environment and public health. Early chapters of this document (Chapters 3-7) discuss: physical and chemical properties of lead; measurement methods for lead in environmental media; sources of emissions; transport, transformation, and fate; and ambient concentrations and other aspects of the exposure of the U.S. population to lead in the environment. Chapter 8 evaluates the projected impact of lead on ecosystems. Chapters 9-11, immediately proceeding this one, discuss: measurement techniques for lead in biologic media; aspects related to the uptake, distribution, toxicokinetics, and excretion of lead; and the relationship of various external and internal lead exposure indices to each other. This chapter assesses information regarding biological effects of lead exposure, with emphasis on (1) the qualitative characterization of various lead-induced effects and (2) the delineation of dose-effect relationships for key effects most likely of health concern at ambient exposure levels presently encountered by the general population of the United States.

In discussing biological effects of lead, one should note at the outset that, to date, lead has not been demonstrated to have any beneficial biological effect in humans. Some investigators have hypothesized that lead may serve as an essential element in certain mammalian species (e.g., the rat) and have reported experimental data interpreted as supporting such an hypothesis. However, a critical evaluation of these studies presented in Appendix 12-A of this chapter raises serious questions regarding interpretation of the reported findings; and the subject studies are currently undergoing intensive evaluation by an expert committee convened by EPA. Therefore, pending the final report from that expert committee, the present chapter does not address the issue of potential essentiality of lead.

It is clear from the evidence evaluated in this chapter that there exists a continuum of biological effects associated with lead across a broad range of exposure. At rather low levels of lead exposure, biochemical changes, e.g., disruption of certain enzymatic activities involved in heme biosynthesis and erythropoietic pyrimidine metabolism, are detectable. With increasing lead exposure, there are sequentially more pronounced effects on heme synthesis and a broadening of lead effects to additional biochemical and physiological mechanisms in various tissues, such that increasingly more severe disruption of the normal functioning of many different organ systems becomes apparent. In addition to impairment of heme biosynthesis, signs of disruption of normal functioning of the erythropoietic and nervous systems are among the earliest effects observed in response to increasing lead exposure. At increasingly higher exposure levels, more severe disruption of the erythropoietic and nervous systems occurs; and

other organ systems are also affected so as to result in the manifestation of renal effects, disruption of reproductive functions, impairment of immunological functions, and many other biological effects. At sufficiently high levels of exposure, the damage to the nervous system and other effects can be severe enough to result in death or, in some cases of non-fatal lead poisoning, long-lasting sequelae such as permanent mental retardation.

The etiologies of many of the different types of functional disruption of various mammalian organ systems derive (at least in their earliest stages) from lead effects on certain subcellular organelles, which result in biochemical derangements (e.g., disruption of heme synthesis processes) common to and affecting many tissues and organ systems. Some major effects of lead on subcellular organelles common to numerous organ systems in mammalian species are discussed below in Section 12.2, with particular emphasis on lead effects on mitochondrial functions. Subsequent sections of the chapter discuss biological effects of lead in terms of various organ systems affected by that element and its compounds (except for Section 12.7, which assesses genotoxic and carcinogenic effects of lead). Additional cellular and subcellular aspects of the biological effects of lead are discussed within respective sections on particular organ systems.

Sections 12.3 to 12.9 have been sequenced generally according to the degree of known vulnerability of each organ system to lead. Major emphasis is placed first on discusssion of the three systems classically considered to be most sensitive to lead (i.e., the hematopoietic, the nervous, and the renal systems). The next sections then discuss the effects of lead on reproduction and development (in view of the impact of lead on the fetus and pregnant women), as well as gametotoxic effects of lead. Genotoxic effects of lead and evidence for possible carcinogenic effects of lead are then reviewed, followed by discussion of lead effects on the immune system and, lastly, other organ systems.

This chapter is subdivided mainly according to organ systems to facilitate presentation of information. It must be noted, however, that, in reality, all systems function in delicate concert to preserve the physiological integrity of the whole organism and all systems are interdependent. Thus, not only do effects in a critical organ often exert impacts on other organ systems, but low-level effects that might be construed as unimportant in a single specific system may be of concern in terms of their cumulative or aggregate impact.

Special emphasis is placed on the discussion of lead exposure effects in children. They are particularly at risk due to sources of exposure, mode of entry, rate of absorption and retention, and partitioning of lead in soft and hard tissues. The greater sensitivity of children to lead toxicity, their inability to recognize symptoms, and their dependence on parents and health care professionals all make them an especially vulnerable population requiring special consideration in developing criteria and standards for lead.

12.2 SUBCELLULAR EFFECTS OF LEAD IN HUMANS AND EXPERIMENTAL ANIMALS

The biochemical or molecular basis for lead toxicity is the ability of the toxicant, as a metallic cation, to bind to ligating groups in biomolecular substances crucial to normal physiological functions, thereby interfering with these functions via such mechanisms as competition with native essential metals for binding sites, inhibition of enzyme activity, and inhibition or other alterations of essential ion transport. The relationship of this basis for lead toxicity to organ- and organelle-specific effects is modulated by: (1) the inherent stability of such binding sites for lead; (2) the compartmentalization kinetics governing lead distribution among body compartments, among tissues, and within cells; and (3) differences in biochemical and physiological organization across tissues and cells due to their specific function. Given complexities introduced by factors 2 and 3, it is not surprising that no single, unifying mechanism of lead toxicity has been demonstrated to apply across all tissues and organ systems.

In the 1977 Air Quality Criteria Document for Lead, cellular and subcellular effects of lead were discussed, including effects on various classes of enzymes. Much of the literature detailing the effects of lead on enzymes *per se* has entailed study of relatively pure enzymes *in vitro* in the presence of added lead. This was the case for data discussed in the earlier document and such information continues to appear in the literature. Much of this material is of questionable relevance for effects of lead *in vivo*. On the other hand, lead effects on certain enzymes or enzyme systems are recognized as integral mechanisms of action underlying the impact of lead on tissues *in vivo* and are logically discussed in later sections below on effects at the organ system level.

This subsection is mainly concerned with organellar effects of lead, especially those that provide some rationale for lead toxicity at higher levels of biological organization. While a common mechanism at the subcellular level that would account for all aspects of lead toxicity has not been identified, one fairly common cellular response to lead is the impairment of mitochondrial structure and function; thus, mitochondrion effects are accorded major attention here. Lead effects on other organelles have not been as extensively studied as mitochondrion effects; and, in some cases, it is not clear how the available information, e.g., that on lead-containing nuclear inclusion bodies, relates to organellar dysfunction.

12.2.1 Effects of Lead on the Mitochondrion

The mitochondrion is clearly the target organelle for toxic effects of lead on many tissues, the characteristics of vulnerability varying somewhat with cell type. Given early recognition of this sensitivity, it is not surprising that an extensive body of *in vivo* and *in vitro* data has accumulated, which can be characterized as evidence of: (1) structural injury to the mitochondrion; (2) impairment of basic cellular energetics and other mitochondrial functions; and (3) uptake of lead by mitochondria *in vivo* and *in vitro*.

12.2.1.1 Lead Effects on Mitochondrial Structure. Changes in mitochondrial morphology with lead exposure have been well documented in humans and experimental animals and, in the case of the kidney, are a rather early response to such exposure. Earlier studies have been reviewed by Goyer and Rhyne (1973), followed by later updates by Fowler (1978) and Bull (1980).

Chronic oral exposure of adult rats to lead (1 percent lead acetate in diet) results in structural changes in renal tubule mitochondria, including swelling with distortion or loss of cristae (Goyer, 1968). Such changes have also been documented in renal biopsy tissue of lead workers (Wedeen et al., 1975; Biagini et al., 1977) and in tissues other than kidney, i.e., heart (Malpass et al., 1971; Moore et al., 1975b), liver (Hoffman et al., 1972), and the central (Press, 1977) and peripheral (Brashear et al., 1978) nervous systems.

While it appears that relatively high level lead exposures are necessary to detect structural changes in mitochondria in some animal models (Goyer, 1968; Hoffman et al., 1972), changes in rat heart mitochondria have been seen at blood lead levels as low as 42 μg/dl. Also, in the study of Fowler et al. (1980), swollen mitochondria or renal tubule cells were seen in rats chronically exposed to lead from gestation to 9 months of age at a dietary lead dosing level as low as 50 ppm and a median blood lead level of 26 μg/dl (range 15-41 μg/dl). Taken collectively, these differences point out relative tissue sensitivity, dosing protocol, and the possible effect of developmental status (Fowler et al., 1980) as important factors in determining lead exposure levels at which mitochondria are affected in various tissues.

12.2.1.2 Lead Effects on Mitochondrial Function. Both *in vivo* and *in vitro* studies dealing with such effects of lead as the impact on energy metabolism, intermediary metabolism, and intracellular ion transport have been carried out in various experimental animal models. Of particular interest for this section are such effects of lead in the developing versus the adult animal, given the greater sensitivity of the young organism to lead.

12.2.1.3 In Vivo Studies. Uncoupled energy metabolism, inhibited cellular respiration using succinate and NAD-linked substrates, and altered kinetics of intracellular calcium have all been documented for animals exposed to lead *in vivo*, as reviewed by Bull (1980).

Adult rat kidney mitochondria, following chronic oral feeding of lead in the diet (1 percent lead acetate, 10 or more weeks) showed a marked sensitivity of the pyruvate-NAD reductase system (Goyer, 1971), as indicated by impairment of pyruvate-dependent respiration indexed by ADP/O ratio and respiratory control rates (RCRs). Succinate-mediated respiration in these animals, however, was not different from controls. In contrast, Fowler et al. (1980) found in rats exposed *in utero* (dams fed 50 or 250 ppm lead) and for 9 months postnatally (50 or 250 ppm lead in their diet) renal tubule mitochondria that exhibited decreased state 3 respiration and RCRs for both succinate and pyruvate/malate substrates. This may have been due to longer exposure to lead or a differential effect of lead exposure during early development.

Intraperitoneal administration of lead to adult rats at doses as low as 12 mg/kg over 14 days was associated with inhibition of potassium-stimulated respiration in cerebral cortex slices with impairment of NAD(P)H oxidation using glucose but not pyruvate as substrate (Bull et al., 1975). This effect occurred at a corresponding blood lead of 72 μg/dl and a brain lead content of 0.4 μg/g, values below those associated with overt neurotoxicity. Bull (1977), in a later study, demonstrated that the respiratory response of cerebral cortical tissue from lead-dosed rats receiving a total of 60 mg Pb/kg (10 mg/kg x 6 dosings) over 14 days was associated with a marked decrease in turnover of intracellular calcium in a cellular compartment that appears to be the mitochondrion. This is consistent with the observation of Bouldin et al. (1975) that lead treatment leads to increased retention of calcium in guinea pig brain.

Numerous studies have evaluated relative effects of lead on mitochonodria of developing vs. adult animals, particularly in the nervous system. Holtzman and Shen Hsu (1976) exposed rat pups at 14 days of age to lead via milk of mothers fed lead in the diet (4 percent lead carbonate) with exposure lasting for 14 days. A 40 percent increase in state 4 respiratory rate of cerebellar mitochonodria was seen within one day of treatment and was lost at the end of the exposure period. However, at this later time (28 days of age), a substantial inhibition of state 3 respiration was observed. This early effect of lead on uncoupling oxidative phosphorylation is consistent with the results of Bull et al. (1979) and McCauley et al. (1979). In these investigations, female rats received lead in drinking water (200 ppm) from 14 days before breeding through weaning of the pups. At 15 days of age, cerebral cortical slices showed alteration of potassium-stimulated respiratory response and glucose uptake.

Holtzman et al. (1980a) compared mitochondrial respiration in cerebellum and cerebrum in rat pups exposed to lead beginning at 14 days of age (via milk of mothers fed 4 percent lead carbonate) and in adult rats maintained on the same diet. Cerebellar mitochondria showed a very early loss (by 2 days of exposure) of respiratory control in the pups with inhibition of phosphorylation-coupled respiration for NAD-linked substrates but not for succinate. Such changes were less pronounced in mitochondria of the cerebrum and were not seen for either brain region in the adult rat. This regional and age dependency of mitochondrial impairment parallels features of lead encephalopathy.

In a second study addressing this issue, Holtzman et al. (1981) measured the cytochrome contents of cerebral and cerebellar mitochondria from rat pups exposed either from birth or at 14 days of age via the same dosing protocol noted above. These were compared to adult animals exposed in like fashion. Pups exposed to lead from birth showed statistically significant reductions of cytochrome b, cytochromes $c + c_1$, and cytochromes $a + a_3$ in cerebellum by 4 weeks of exposure. Changes in cerebral cytochromes, in contrast, were marginal. When lead exposure began at 14 days of age, little effect was observed, and adult rats showed little

change. This study indicates that the most vulnerable period for lead effects on developing brain oxidative metabolism is the same period where a major burst in such activity begins.

Related to effects of lead on energy metabolism in the developing animal mitochondrion is the effect on brain development. In the study of Bull et al. (1979) noted earlier, cerebral cytochrome c + c_1 levels between 10 and 15 days of age decreased in a dose-dependent fashion at all maternal dosing levels (5-100 mg Pb/liter drinking water) and corresponding blood lead values for the rat pups (11.7-35.7 μg/dl). Delays in synaptic development in these pups also occurred, as indexed by synaptic counts taken in the parietal cortex. As the authors concluded, uncoupling of energy metabolism appears to be causally related to delays in cerebral cortical development.

Consistent with the effects of lead on mitochondrial structure and function are in vivo data demonstrating the selective accumulation of lead in mitochondria. Studies in rats using radioisotopic tracers ^{210}Pb (Castallino and Aloj, 1969) and ^{203}Pb (Barltrop et al., 1971) demonstrate that mitochondria will accumulate lead in significant relative amounts, the nature of the accumulation seeming to vary with the dosing protocol. Sabbioni and Marafante (1976) as well as Murakami and Hurosawa (1973) also found that lead is selectively lodged in mitochondria. Of interest in regard to the effects of lead on brain mitochondria are the data of Moore et al. (1975a) showing uptake of lead by guinea pig cerebral mitochondria, and the results of Krigman et al. (1974c) demonstrating that mitochondria in brain from 6-month-old rats exposed chronically to lead since birth showed the highest uptake of lead (34 percent), followed by the nuclear fraction (31 percent). While the possibility of translocation of lead during subcellular fractionation can be raised, the distribution pattern seen in the reports of Barltrop et al. (1971) and Castallino and Aloj (1969) over multiple time points make this unlikely. Also, findings of in vivo uptake of lead in brain mitochondria are supported by in vitro data discussed below.

12.2.1.4 In Vitro Studies. In vitro studies of both the response of mitochondrial function to lead and its accumulation by the organelle have been reported, using both isolated mitochondria and tissues. Bull (1980) reviewed such data published up to 1979.

Significant reductions in mitochondrial respiration, using both NAD-linked and succinate substrates have been reported for isolated heart and brain mitochondria. The lowest levels of lead associated with such effects appear to be 5 μM or, in some cases, less. Available evidence suggests that the sensitive site for lead in isolated mitochondria is before cytochrome b in the oxidative chain and involves either tricarboxylic acid enzymes or non-heme protein/ubiquinone steps. If substrate specificity is compared, e.g., succinate vs. glutamate/malate oxidation, there appear to be organ-specific differences. As Bull (1980) noted, tissue-specific effects of lead on cellular energetics may be one bases for differences in toxicity across organs. Also, several enzymes involved in intermediary metabolism in isolated mito-

chondria have been observed to undergo significant inhibition in activity in the presence of lead, and these have been tabulated by Bull (1980).

One focus of studies dealing with lead effects on isolated mitochondria has been ion transport--particularly that of calcium. Scott et al. (1971) have shown that lead movement into rat heart mitochondria involves active transport, with characteristics similar to those of calcium, thereby establishing a competitive relationship. Similarly, lead uptake into brain mitochondria is also energy dependent (Holtzman et al., 1977; Goldstein et al., 1977). The recent elegant studies of Pounds and coworkers (Pounds et al., 1982a,b), using labeled calcium or lead and desaturation kinetic studies of these labels in isolated rat hepatocytes, have elucidated the intracellular relationship of lead to calcium in terms of cellular compartmentalization. In the presence of graded amounts of lead (10, 50, or 100 μM), the kinetic analysis of desaturation curves of calcium-45 label showed a lead dose-dependent increase in the size of all three calcium compartments within the hepatocyte, particularly that deep compartment associated with the mitochondrion (Pounds et al., 1982a). Such changes were seen to be relatively independent of serum calcium or endogenous regulators of systemic calcium metabolism. Similarly, the use of lead-210 label and analogous kinetic analysis (Pounds et al., 1982b) showed the same three compartments of intracellular distribution as noted for calcium, including the deep component (which has the mitochondrion). Hence, there is striking overlap in the cellular metabolism of calcium and lead. These studies not only further confirm easy entry of lead into cells and cellular compartments, but also provide a basis for perturbation by lead of intracellular ion transport, particularly in neural cell mitochondria, where there is a high capability for calcium transport. Such capability is approximately 20-fold higher than in heart mitochondria (Nicholls, 1978).

Given the above observations, it is not surprising that a number of investigators have noted the *in vitro* uptake of lead into isolated mitochondria. Walton (1973) noted that lead is accumulated within isolated rat liver mitochondria over the range of 0.2-100 μM lead; and Walton and Buckley (1977) extended this observation to mitochondria in rat kidney cells in culture. Electron microprobe analyses of isolated rat synaptosomes (Silbergeld et al., 1977) and capillaries (Silbergeld et al., 1980b) incubated with lead ion have established that significant accumulation of lead, along with calcium, occurs in the mitochondrion. These observations are consistent with the kinetic studies of Pounds et al. (1982a,b), and the *in vitro* data for isolated capillaries are in accord with the observations of Toews et al. (1978), who found significant lead accumulation in brain capillaries of the suckling rat.

12.2.2 Effects of Lead on the Nucleus

With lead exposure, a cellular reaction typical of many species (including humans) is the formation of intranuclear lead-containing inclusion bodies, early data for which have been sum-

marized by Goyer and Moore (1974). In brief, these lead-bearing inclusion bodies: (1) have been verified as to lead content by X-ray microanalysis (Carroll et al., 1970); (2) consist of a rather dense core encapsulated by a fibrillary envelope; (3) are a complex of lead and the acid fractions of nuclear protein; (4) can be disaggregated in vitro by EDTA; (5) can appear very rapidly after lead exposure (Choie et al., 1975); (6) consist of a protein moiety in the complex which is synthesized de novo; and (7) have been postulated to serve a protective role in the cell, given the relative amounts of lead accumulated and presumably rendered toxicologically inert.

Based on renal biopsy studies, Cramer et al. (1974) concluded that such inclusion body formation in renal tubule cells in lead workers is an early response to lead entering the kidney, in view of decreased presence as a function of increased period of employment. Schumann et al. (1980), however, observed a continued exfoliation of inclusion-bearing tubule cells into urine of workers having a variable employment history.

Any protective role played by the lead inclusion body appears to be an imperfect one, to the extent that both subcellular organelle injury and lead uptake occur simultaneously with such inclusion formation, often in association with severe toxicity at the organ system level. For example, Osheroff et al. (1982), observed lead inclusion bodies in the anterior horn cells of the cervical spinal cord and neurons of the substantia nigra (as well as in renal tubule cells) in the adult rhesus monkey, along with damage to the vascular walls and glial processes and ependymal cell degeneration. At the light- and electron-microscope level, there were no signs of neuronal damage or altered morphology except for the inclusions. As noted by the authors, these inclusions in the large neurons of the substantia nigra show that the neuron will take up and accumulate lead. In the study of Fowler et al. (1980), renal tubule inclusions were observed simultaneously with evidence of structural and functional damage to the mitochondrion, all at relatively low levels of lead. Hence, it appears that a limited protective role for these inclusions may extend across a range of lead exposure.

Chromosomal effects and other indices of genotoxicity in humans and animals are discussed in Section 12.7 of this chapter.

12.2.3 Effects of Lead on Membranes

In theory, the cell membrane is the first organelle to encounter lead, and it is not surprising that cellular effects can be ascribed to interactions at cellular and intracellular membranes, mainly appearing to be associated with ion transport processes across membranes. In Section 12.3 a more detailed discussion is accorded the effects of lead on the membrane as they relate to the erythrocyte in terms of increased cell fragility and increased osmotic resistance. These effects can be rationalized, in large part, by the documented inhibition by lead of erythrocyte membrane (Na^+, K^+)-ATPase.

Lead also appears to interfere with the normal processes of calcium transport across membranes of various tissue types. Silbergeld and Adler (1978) have described lead-induced retardation of the release of the neurotransmitter, acetylcholine, in peripheral cholinergic synaptosomes, due to a blockade of calcium binding to the synaptosomal membrane reducing calcium-dependent choline uptake and subsequent release of acetylcholine from the nerve terminal. Calcium efflux from neurons is mediated by the membrane (Na^+, K^+)-ATPase via an exchange process with sodium. Inhibition of the enzyme by lead, as also occurs with the erythroctye (see above), increases the concentration of calcium within nerve endings (Goddard and Robinson, 1976). As seen from the data of Pounds et al. (1982a), lead can also elicit retention of calcium in neural cells by easy entry into the cell and by directly affecting the deep calcium compartment within the cell, of which the mitochondrion is a major component.

12.2.4 Other Organellar Effects of Lead

Studies of morphological alterations of renal tubule cells in the rat (Chang et al., 1981) and rabbit (Spit et al., 1981) with varying lead treatments have demonstrated lead-induced lysosomal changes. In the rabbit, with relatively modest levels of lead exposure (0.25 or 0.5 mg Pb/kg, 3 times weekly over 14 weeks) and corresponding blood lead values of 50 and 60 μg/dl, there was a dose-dependent increase in the amount of lysosomes in proximal convoluted tubule cells, as well as increased numbers of lysosomal inclusions. In the rat, exposure to 10 mg Pb/kg i.v. (daily over 4 weeks) resulted in the accumulation of lysosomes, some gigantic, in the pars recta segment of renal tubules. These animal data are consistent with observations made in lead workers (Cramer et al., 1974; Wedeen et al., 1975) and appear to represent a disturbance in normal lysosomal function, with the accumulation of lysosomes being due to enhanced degradation of proteins arising from effects of lead elsewhere within the cell.

12.2.5 Summary of Subcellular Effects of Lead

The biological basis of lead toxicity is closely linked to the ability of lead to bind to ligating groups in biomolecular substances crucial to normal physiological functions. This binding interferes with physiological processes by such mechanisms as: competition with native essential metals for binding sites; inhibition of enzyme activity; and inhibition or other changes in essential ion transport.

The main target organelle for lead toxicity in a variety of cell and tissue types clearly is the mitochondrion, followed probably by cellular and intracellular membranes. Mitochondrial effects take the form of structural changes and marked disturbances in mitochondrial function within the cell, especially energy metabolism and ion transport. These effects are

associated, in turn, with demonstrable accumulation of lead in mitochondria, both in vivo and in vitro. Structural changes include mitochondrial swelling in many cell types, as well as distortion and loss of cristae, which occur at relatively moderate levels of lead exposure. Similar changes have been documented in lead workers across a wide range of exposure levels.

Uncoupled energy metabolism, inhibited cellular respiration using both succinate and nicotinamide adenine dinucleotide (NAD)-linked substrates, and altered kinetics of intracellular calcium have been demonstrated in vivo using mitochondria of brain and non-neural tissue. In some cases, relatively moderate lead exposure levels have been associated with such changes, and several studies have documented the relatively greater sensitivity of this organelle in young versus adult animals in terms of mitochondrial respiration. The cerebellum appears to be particularly sensitive, providing a connection between mitochondrial impairment and lead encephalopathy. Impairment by lead of mitochondrial function in the developing brain has also been associated with delayed brain development, as indexed by content of various cytochromes. In the rat pup, ongoing lead exposure from birth is required for this effect to be expressed, indicating that such exposure must occur before, and is inhibitory to, the burst of oxidative metabolism activity that normally occurs in the young rat during 10 to 21 days postnatally.

In vivo lead exposure of adult rats has also been observed to markedly inhibit cerebral cortex intracellular calcium turnover (in a cellular compartment that appears to be the mitochondrion) at a brain lead level of 0.4 ppm. These results are consistent with a separate study showing increased retention of calcium in the brain of lead-dosed guinea pigs. A number of reports have described the in vivo accumulation of lead in mitochondria of kidney, liver, spleen, and brain tissue, with one study showing that such uptake was slightly more than occurred in the nucleus. These data are not only consistent with the various deleterious effects of lead on mitochondria but are also supported by other, in vitro findings.

Significant decreases in mitochondrial respiration in vitro using both NAD-linked and succinate substrates have been observed for brain and non-neural tissue mitochondria in the presence of lead at micromolar levels. There appears to be substrate specificity in the inhibition of respiration across different tissues, which may be a factor in differential organ toxicity. Also, a number of enzymes involved in intermediary metabolism in isolated mitochondria have been observed to undergo significant inhibition of activity with lead.

A major focus of research on lead effects on isolated mitochondria has concerned ion (especially calcuim) transport. Lead movement into brain and other tissue mitochondria, as does calcium movement, involves active transport. Recent sophisticated kinetic analyses of desaturation curves for radiolabeled lead or calcium indicate that there is striking overlap in the cellular metabolism of calcium and lead. These studies not only establish a basis for easy entry of lead into cells and cell compartments, but also provide a basis for impairment

by lead of intracellular ion transport, particularly in neural cell mitochondria, where the capacity for calcium transport is 20-fold higher than even in heart mitochondria.

Lead is also selectively taken up in isolated mitochondria in vitro, including the mitochondria of synaptosomes and brain capillaries. Given the diverse and extensive evidence of lead's impairment of mitochondrial structure and function as viewed from a subcellular level, it is not surprising that these derangements are logically held to be the basis of dysfunction of heme biosynthesis, erythropoiesis, and the central nervous system. Several key enzymes in the heme biosynthetic pathway are intramitochondrial, particularly ferrochelatase. Hence, it is to be expected that entry of lead into mitochondria will impair overall heme biosynthesis, and in fact this appears to be the case in the developing cerebellum. Furthermore, the levels of lead exposure associated with entry of lead into mitochondria and expression of mitochondrial injury can be relatively moderate.

Lead exposure provokes a typical cellular reaction in human and other species that has been morphologically characterized as a lead-containing nuclear inclusion body. Although it has been postulated that such inclusions constitute a cellular protection mechanism, such a mechanism is an imperfect one. Other organelles, e.g., the mitochondrion, also take up lead and sustain injury in the presence of nuclear inclusion bodies. Chromosomal effects and other indices of genotoxicity in humans and animals are considered later, in Section 12.7.

In theory, the cell membrane is the first organelle to encounter lead and it is not surprising that cellular effects of lead can be ascribed to interactions at cellular and intracellular membranes in the form of distrubed ion transport. The inhibition of membrane (Na^+,K^+)-ATPase of erythrocytes as a factor in lead-impaired erythropoiesis is noted elsewhere. Lead also appears to interfere with the normal processes of calcium transport across membranes of different tissues. In peripheral cholinergic synaptosomes, lead is associated with retarded release of acetylcholine owing to a blockade of calcium binding to the membrane, while calcium accumulation within nerve endings can be ascribed to inhibition of membrane (Na^+,K^+)-ATPase.

Lysosomes accumulate in renal proximal convoluted tubule cells of rats and rabbits given lead over a wide range of dosing. This also appears to occur in the kidneys of lead workers and seems to represent a disturbance in normal lysosomal function, with the accumulation of lysosomes being due to enhanced degradation of proteins because of the effects of lead elsewhere within the cell.

In so far as effects of lead on the activity of various enzymes are concerned, many of the available studies concern in vitro behavior of relatively pure enzymes with marginal relevance to various effects in vivo. On the other hand, certain enzymes are basic to the effects of lead at the organ or organ system level, and discussion is best reserved for such effects in ensuing sections of the document dealing with these systems.

12.3 EFFECTS OF LEAD ON HEME BIOSYNTHESIS AND ERYTHROPOIESIS/ERYTHROCYTE PHYSIOLOGY IN HUMANS AND ANIMALS

Lead has well-recognized effects not only on heme biosynthesis, a crucial process common to many organ systems, but also on erythropoiesis and erythrocyte physiology. This section is therefore divided for purposes of discussion into: (1) effects of lead on heme biosynthesis and (2) effects of lead on erythropoiesis and erythrocyte physiology. Discussion of the latter is further subdivided into effects of lead on hemoglobin production, cell morphology and survival, and erythropoietic nucleotide metabolism. The interrelationship of effects of lead on heme biosynthesis and neurotoxic effects of lead are discussed in a final subsection. Attention is accorded to discussion of effects of both inorganic lead and alkyl lead compounds used as gasoline additives.

12.3.1 Effects of Lead on Heme Biosynthesis

The effects of lead on heme biosynthesis are very well known because of both their prominence and the large number of studies of these effects in humans and experimental animals. In addition to being a constituent of hemoglobin, heme is a prosthetic group of a number of tissue hemoproteins having diverse functions, such as myoglobin, the P-450 component of the mixed function oxidase system, and the cytochromes of cellular energetics. Hence, any effects of lead on heme biosynthesis will, perforce, pose the potential for multi-organ toxicity.

At present, much of the available information concerning the effects of lead on heme biosynthesis have been obtained by measurements in blood, due in large part to the relative ease of assessing such effects via measurements in blood and in part to the fact that blood is the vehicle for movement of metabolites from other organ systems. On the other hand, a number of reports have been concerned with lead effects on heme biosynthesis in tissues such as kidney, liver, and brain. In the discussion below, various steps in the heme biosynthetic pathway affected by lead are discussed separately, with information describing erythropoietic effects usually appearing first, followed by studies involving other tissues.

The process of heme biosynthesis results in formation of the porphyrin, protoporphyrin IX, starting with glycine and succinyl-coenzyme A. It culminates with the insertion of iron at the center of the porphyrin ring. As may be noted in Figure 12-1, lead interferes with heme biosynthesis by disturbing the activity of three major enzymes: (1) it indirectly stimulates, by feedback derepression, the mitochondrial enzyme delta-aminolevulinic acid synthetase (ALA-S), which mediates the condensation of glycine and succinyl-coenzyme A to form delta-aminolevulinic acid (δ-ALA); (2) it directly inhibits the cytosolic enzyme delta-aminolevulinic acid dehydrase (ALA-D), which catalyzes the cyclocondensation of two units of ALA to porphobilinogen; (3) it disturbs the mitochondrial enzyme ferrochelatase, found in liver, bone

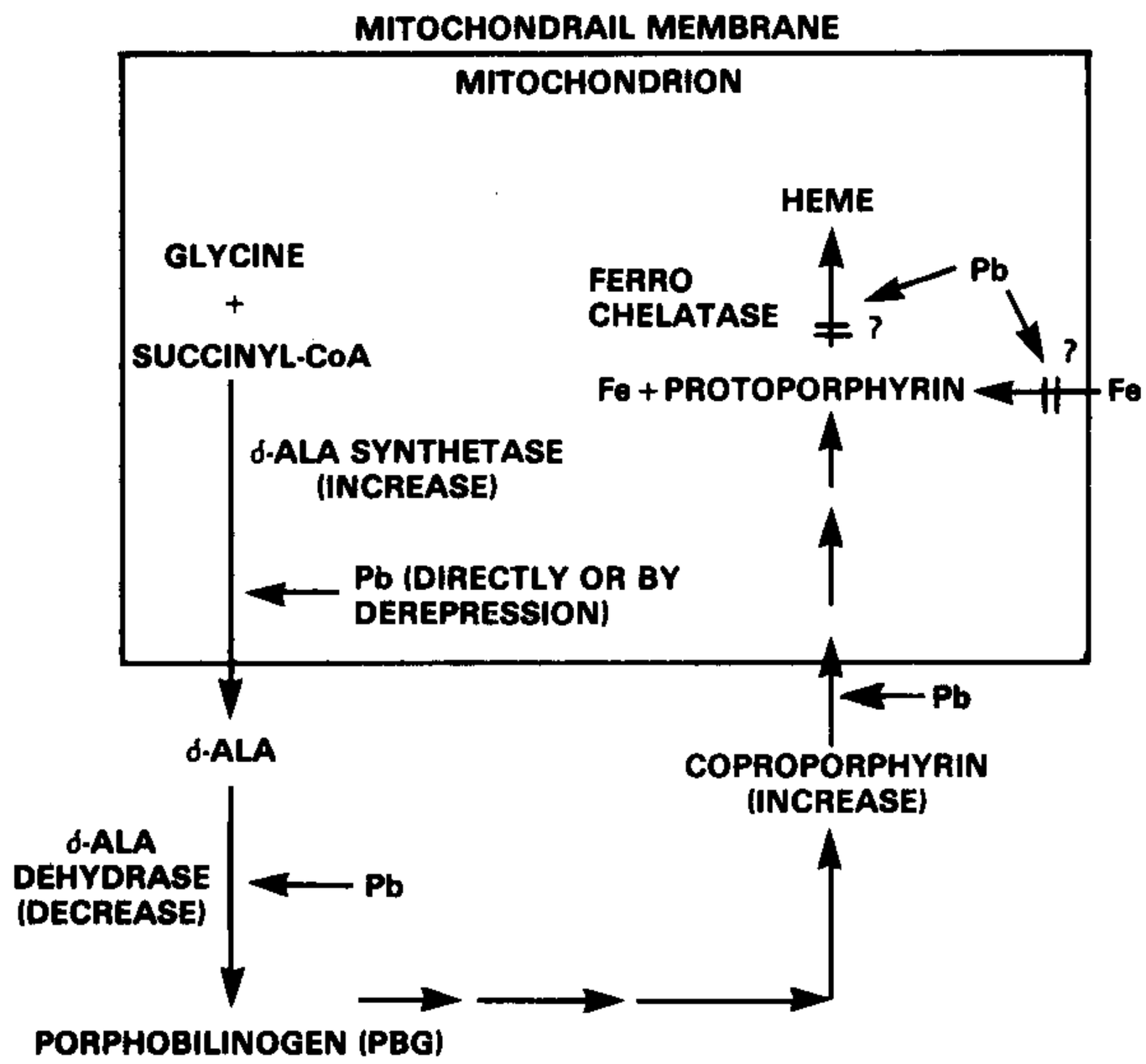

Figure 12-1. Lead effects on heme biosynthesis.

marrow, and other tissues, by either direct inhibition or alteration of intermitochondrial transport of iron ferrochelatase, which catalyzes the insertion of iron (II) into the protoporphyrin ring to form heme, the enzyme situated in mammals in the inner mitochondrial membrane (McKay et al., 1969).

12.3.1.1 Effects of Lead on δ-Aminolevulinic Acid Synthetase. The activity of the enzyme ALA-S is the rate-limiting step in the heme biosynthetic pathway. With decreased heme formation at other steps downstream or with increased heme oxygenase activity, compensatory increase of ALA-S activity occurs through feedback derepression and enhances the rate of heme formation. Hence, excess ALA formation is due to both stimulation of ALA-S and direct inhibition of ALA-D (see below).

Increased ALA-S activity has been reported in lead workers (Takaku et al., 1973; Campbell et al., 1977; Meredith et al., 1978), with leukocyte ALA-S stimulated at a blood lead value of 40 μg/dl (Meredith et al., 1978), a level at which ALA-D activity is significantly inhibited. To the extent that mitochondria in leukocytes show a dose-effect relationship comparable to the bone marrow and hepatic systems, it appears that most of the excess ALA formation below the observed threshold value is due to ALA-D inhibition. From the authors' data, blood ALA had increased about 2-fold in these workers over the blood lead range 18 μg/dl to 40 μg/dl.

In vitro and *in vivo* experimental data have provided mixed results in terms of the direction of the effect of lead on ALA-S activity. Silbergeld et al. (1982) observed that ALA-S activity was increased in kidney with acute lead exposure in rats, while chronic treatment was associated with increased activity of the enzyme in spleen. In liver, however, ALA-S activity was reduced under both acute and chronic dosing. Fowler et al. (1980) reported that renal ALA-S activity was significantly reduced in rats continuously exposed to lead *in utero*, through development, and up to 9 months of age. Meredith and Moore (1979) noted a steady increase in hepatic ALA-S activity when rats were given lead parenterally over an extended period of time. Maxwell and Meyer (1976) and Goldberg et al. (1978) also noted increased ALA-S activity in rats given lead parenterally. It appears that the type and time-frame of dosing influences the observed effect of lead on the enzyme activity. Using a rat liver cell line (RLC-GAI) in culture, Kusell et al. (1978) demonstrated that lead could produce a time-dependent increase in ALA-S activity. Stimulation of activity was observed at lead levels as low as 5×10^{-6} M, with maximum stimulation at 10^{-5} M. The authors report that the activity increase was associated with biosynthesis of more enzyme, rather than stimulation of the pre-existing enzyme. Lead-stimulated ALA-S formation was also not limited to liver cells; rat gliomas and mouse neuroblastomas showed similar results.

12.3.1.2 Effects of Lead on δ-Aminolevulinic Acid Dehydrase and ALA Accumulation/Excretion. Delta-aminolevulinic acid dehydrase (5-aminolevulinate hydrolase; porphobilinogen synthetase; E.C. 4.2.1.24; ALA-D) is a sulfhydryl, zinc-requiring allosteric enzyme in the heme biosynthetic pathway which catalyzes the conversion of two units of ALA to porphobilinogen. The enzyme's activity is very sensitive to inhibition by lead, the inhibition being reversed by reactivation of the sulfhydryl group with agents such as dithiothreitol (Granick et al., 1973), zinc (Finelli et al., 1975), or zinc plus glutathione (Mitchell et al., 1977).

The activity of ALA-D appears to be inhibited at virtually all blood lead levels studied so far, and any threshold for this effect remains to be identified (see discussion below). Dresner et al. (1982) found that ALA-D activity in rat bone marrow suspensions was significantly inhibited to 35 percent of control levels in the presence of 5×10^{-7} M (0.5 μM) lead. This potency, on a comparative molar basis, was unmatched by any other metals tested. Recently, Fujita et al. (1981) showed evidence of an increase in the amount of ALA-D in erythrocytes in lead-exposed rats, ascribed to an increased rate of ALA-D synthesis in bone marrow cells. Hence, the commonly observed net inhibition of activity occurs even in the face of an increase in ALA-D synthesis.

Hernberg and Nikkanen (1970) found that enzyme activity was correlated inversely with (logarithmic) blood lead values in a group of urban, non-exposed subjects. Enzyme activity inhibition was 50 percent at a blood lead level of 16 μg/dl. Other reports have confirmed

these observations across age groups and exposure categories (Alessio et al., 1976b; Roels et al., 1975b; Nieberg et al., 1974; Wada et al., 1973). A ratio of activated to inhibited enzyme activity (versus a single activity measurement, which does not accommodate intersubject genetic variability) measured against blood lead in children with values between 20 and 90 μg/dl was employed by Granick et al. (1973) to obtain an estimated threshold of 15 μg/dl for an effect of lead. On the other hand, Hernberg and Nikkanen (1970) observed no threshold in their subjects, all of whom were at or below 16 μg/dl. The lowest blood lead actually measured by Granick et al. (1973) was higher than the values measured by Hernberg and Nikkanen (1970).

Kuhnert et al. (1977) reported that ALA-D activity measures in erythrocytes from both pregnant women and cord blood of infants at delivery are correlated with the corresponding blood lead values, using the activated/inhibited activity ratio method of Granick et al. (1973). The correlation coefficient of activity with lead level was higher in fetal erythrocytes ($r = -0.58$, $p < 0.01$) than in the mothers ($r = -0.43$, $p < 0.01$). The mean inhibition level was 28 percent in mothers vs. 12 percent in the newborn. A study by Lauwerys et al. (1978) in 100 pairs of pregnant women and infant cord blood samples confirms this observation, i.e., for fetal blood $r = 0.67$ ($p < 0.001$) and for maternal blood $r = -0.56$ ($p < 0.001$).

While several factors other than lead may affect the activity of erythrocyte ALA-D, much of the available information suggests that most of these factors do not materially compromise the interpretation of the relationship between enzyme activity and lead or the use of this relationship for screening purposes. Border et al. (1976) questioned the reliability of ALA-D activity measurement in subjects concurrently exposed to both lead and zinc, since zinc also affects the activity of the enzyme. The data of Meredith and Moore (1980) refute this objection. In subjects without exposure, having serum zinc values of 80-120 μM, there was only a minor activating effect with increasing zinc when contrasted to the correlation of activity and blood lead in these same subjects. In workers exposed to both lead and zinc, serum zinc values were greater than in subjects with just lead exposure, but the mean level of enzyme activity was still much lower than in controls ($p < 0.001$).

The preceding discussion indicates that neither differences within the normal range of physiological zinc in humans nor combined excessive zinc and lead exposure in workers materially affects ALA-D activity. The obverse of this, lead exposure in the presence of zinc deficiency, is probably the more significant issue, but one that has not been well studied. Since ALA-D is a zinc-requiring enzyme, one would expect that optimal activity would be governed by *in vivo* zinc availability. Furthermore, zinc deficiency could potentially have a dual deleterious effect on ALA-D activity, first by reduced activity with reduced zinc availability and second, by enhanced lead absorption in the presence of zinc deficiency (see Section 10.5), the increased lead burden further inhibiting ALA-D activity.

The recent study of Roth and Kirchgessner (1981) indicates that ALA-D activity is significantly decreased in the presence of zinc deficiency. In zinc-deficient rats showing reduced serum and urinary zinc levels, the level of erythrocyte ALA-D activity was only 50 percent that of pair-fed controls, while urinary ALA was significantly elevated. Although these investigators did not measure blood lead in deficient and control animal groups, it would appear that the level of inhibition is more than could be accounted for just on the basis of increased lead absorption from diet. Given the available information documenting zinc deficiency in children (Section 10.5) as well as the animal study of Roth and Kirchgessner (1981), the relationship of lead, zinc deficiency, and ALA-D activity in young children merits further, careful study.

Moore and Meredith (1979) noted the effects of carbon monoxide on the activity of ALA-D, comparing moderate or heavy smokers with non-smokers. At the highest level of carboxyhemoglobin measured in their smoker groups, the depression of ALA-D activity was 2.1 percent. In these subjects, a significant inverse correlation of ALA-D activity and blood lead existed, but there was no significant correlation of such activity and blood carboxyhemoglobin levels.

While blood ethanol has been reported to affect ALA-D activity (Moore et al., 1971; Abdulla et al., 1976), its effect is significant only with intake corresponding to acute alcohol intoxication. Hence, relevance of this observation to screening is limited, particularly in children. The effect is reversible, declining with clearing of alcohol from the blood stream.

The inhibition of ALA-D activity in erythrocytes by lead apparently reflects a similar effect in other tissues. Secchi et al. (1974) observed that there was a clear correlation in 26 lead workers between hepatic and erythrocyte ALA-D activity as well as the expected inverse correlation between such activity and blood lead in the range 12-56 μg/dl. In suckling rats, Millar et al. (1970) noted decreased enzyme activity in brain and liver as well as red cells when lead was administered orally. In the study of Roels et al. (1977), tissue ALA-D changes were not observed when dams were administered 1, 10, or 100 ppm lead in drinking water. However, the recent report of Silbergeld et al. (1982) described moderate inhibition of ALA-D activity in brain and significant inhibition in kidney, liver, and spleen when adult rats were acutely exposed to lead given intraperitoneally; chronic exposure was associated with reduced activity in kidney, liver, and spleen. Gerber et al. (1978) found that neonatal mice exposed to lead from birth through 17 days of age at a level of 1.0 mg/ml in water showed significant decreases in brain ALA-D activity ($p < 0.01$) at all time points studied. These results support the data of Millar et al. (1970) for the suckling rat. In this study by Millar et al., rats exposed from birth through adulthood only showed significant decreases of brain ALA-D activity at 15 and 30 days, which also supports other data for the developing rodent. It would appear, therefore, that brain ALA-D activity is more sensitive to lead in the developing animal than in the adult.

The study of Dieter and Finley (1979) sheds light on the relative sensitivity of ALA-D activity in several regions of the brain and permits comparison of blood vs. brain ALA-D activity as a function of lead level. Mallard ducks given a single pellet of lead showed, by 4 weeks, 1 ppm lead in blood, 2.5 ppm lead in liver, and 0.5 ppm lead in brain. Cerebellar ALA-D activity was reduced by 50 percent at a lead level below 0.5 ppm; erythrocyte enzyme activity was lowered by 75 percent. Hepatic ALA-D activity was comparable to cerebellar activity or somewhat less, although the lead level in the liver was 5-fold higher. Cerebellar ALA-D activity was significantly below that for cerebrum. In an avian species, then, at blood lead levels where erythrocyte ALA-D activity is significantly depressed, activity of the enzyme in cerebellum was even more affected relative to lead concentration. The Roels et al. (1977) data may reflect a lower effective dose delivered to the rat pups in maternal milk as well as the dose taken in by the dams themselves, since they similarly showed no tissue enzyme activity changes.

The inhibition of ALA-D is reflected by increased levels of its substrate, ALA, in urine (Haeger, 1957) as well as in whole blood or plasma (Meredith et al., 1978; MacGee et al., 1977; Chisolm, 1968; Haeger-Aronsen, 1960). The detailed study of Meredith et al. (1978), which involved both control subjects and lead workers, indicated that in elevated lead exposure the increase in urinary ALA is preceded by a significant rise in circulating levels of ALA. The overall relationship of plasma ALA to blood lead was exponential and showed a perceptible continuation of an ALA-blood lead correlation into the control group to include the lowest value, 18 μg/dl. The relationship of plasma ALA to urinary levels of the precursor was found to be exponential, indicating that as plasma ALA increases, a greater proportion undergoes excretion into urine. Inspection of the plot of urinary vs. plasma ALA in these subjects shows that the correlation persists down to the plasma ALA concentration corresponding to the lowest blood lead level, 18 μg/dl. Cramer et al. (1974) have demonstrated that ALA clearance into urine parallels glomerular filtration rate across a range of lead exposures, suggesting that increased urinary output with increasing circulating ALA is associated with decreased tubular reabsorption (Moore et al., 1980). This study employed the method of Haeger-Aronsen (1960), which does not account for the presence of amino-acetone. If amino-acetone were interfering at low blood lead levels, however, one might expect an obliteration of the association, since this metabolite is not affected by lead exposure and its concentration should be randomly distributed in plasma and urine of the subjects.

Urinary ALA has been employed extensively as an indicator of excessive lead exposure, particularly in occupational settings (e.g., Davis et al., 1968; Selander and Cramér, 1970; Alessio et al., 1976a). The reliability of this test in initial screening of children for lead exposure has been questioned by Specter et al. (1971) and Blanksma et al. (1969), who

pointed out the failure of urinary ALA analysis to detect lead exposure when compared with blood lead values. This is due to the fact that an individual subject will show a wide variation in urinary ALA with random sampling. Chisolm et al. (1976) showed that reliable levels could only be obtained with 24-hour collections. In children with blood lead levels above 40 μg/dl the relationship of ALA in urine to blood lead becomes similar to that observed in lead workers (see below).

A correlation exists between blood lead and the logarithm of urinary ALA in workers (Meredith et al., 1978; Alessio et al., 1976a; Roels et al., 1975a; Wada et al., 1973; Selander and Cramér, 1970) and in children (National Academy of Sciences, 1972). Selander and Cramér (1970) reported that two different correlation curves were obtained, one for individuals below 40 μg/dl blood lead, and a different one for values above this, although the degree of correlation was less than with the entire group. A similar observation has been reported by Lauwerys et al. (1974) from a study of 167 workers (10-75 μg/dl). Meredith et al. (1978) found that the correlation curve for blood ALA vs. urinary ALA was linear below a blood lead of 40 μg/dl, as was the relationship of blood ALA to blood lead. Hence, there was also a linear relationship between blood lead and urinary ALA below 40 μg/dl, i.e., a continuation of the correlation below the commonly accepted threshold blood lead value of 40 μg/dl (see below). Tsuchiya et al. (1978) have questioned the relevance of using single correlation curves to describe the blood lead-urinary ALA relationship across a broad range of exposure, because they found that this relationship in workers showing moderate, intermediate, and high lead exposure could be described by three correlation curves of differing slope. This finding is consistent with the observations of Selander and Cramér (1970) as well as the results of Meredith et al. (1978) and Lauwerys et al. (1974). Chisolm et al. (1976) described an exponential correlation between blood lead and urinary ALA in children 5 years old or younger, with blood lead ranging from 25 to 75 μg/dl. In adolescents with blood lead below 40 μg/dl, no clear correlation was observed.

It is apparent from the above reports (Tsuchiya et al., 1978; Meredith et al., 1978; Selander and Cramér, 1970) that circulating ALA and urinary ALA are elevated and correlated at blood lead values below 40 μg/dl in humans. This is consistent, as in the Meredith et al. study, with the significant and steady increase in ALA-D inhibition concomitant with rising blood levels of ALA, even at blood lead values considerably below 40 μg/dl. Increases of ALA in tissues of experimental animals exposed to lead have also been documented. In the study of Silbergeld et al. (1982), acute administration of lead to adult rats was associated with an elevation in spleen and kidney ALA vs. that of controls, while in chronic exposure there was a moderate increase in ALA in the brain and a large increase (9-15 fold) in kidney and spleen. Liver levels with either form of exposure were not materially affected, although there was inhibition of liver ALA-D, particularly in the acute dose group.

12.3.1.3 Effects of Lead on Heme Formation from Protoporphyrin. The accumulation of protoporphyrin in the erythrocytes of individuals with lead intoxication has been recognized since the 1930s (Van den Bergh and Grotepass, 1933), but it has only recently been possible to study this effect through the development of sensitive and specific analytical techniques that permit quantitative measurement. In particular, the development of laboratory microtechniques and the hematofluorometer have allowed the determination of dose-effect relationships as well as the use of such measurements to screen for lead exposure.

In humans under normal circumstances, about 95 percent of the protoporphyrin in circulating erythrocytes is zinc protoporphyrin (ZPP) with the remaining 5 percent present as "free" protoporphyrin (Chisolm and Brown, 1979). Accumulation of protoporphyrin IX in the erythrocytes is the result of impaired iron (II) placement in the porphyrin moiety to form heme, an intramitochondrial process. In lead exposure, the porphyrin acquires a zinc ion, in lieu of the native iron, with the resulting ZPP tightly bound in the available heme pockets for the life of the erythrocyte, about 120 days (Lamola et al., 1975a,b).

In lead poisoning, the accumulation of protoporphyrin differs from that seen in the congenital disorder, erythropoietic protoporphyria. In the latter case, there is a defect in ferrochelatase function, leading to loose attachment of the porphyrin, accumulated without uptake of zinc, on the surface of the hemoglobin. Loose attachment permits diffusion into plasma and ultimately into the skin, where photosensitivity is induced. This behavior is absent in lead-associated porphyrin accumulation. The two forms of porphyrin, free and zinc-containing, differ sufficiently in fluorescence spectra to permit a laboratory distinction. With iron deficiency, there is also accumulation of protoporphyrin in the heme pocket as the zinc complex, resembling in large measure the characteristics of lead intoxication.

The elevation of erythrocyte ZPP has been extensively documented as being exponentially correlated with blood lead in children (Piomelli et al., 1973; Kammholz et al., 1972; Sassa et al., 1973; Lamola et al., 1975a,b; Roels et al., 1976) and in adult workers (Valentine et al., 1982; Lilis et al., 1978; Grandjean and Lintrup, 1978; Alessio et al., 1976b; Roels et al., 1975a, 1979; Lamola et al., 1975a,b). Reigart and Graber (1976) and Levi et al. (1976) have demonstrated that ZPP elevation can predict which children tend to increase their blood lead levels, a circumstance which probably rests on the nature of chronic lead exposure in certain groups of young children where a pulsatile blood lead curve is superimposed on some level of ongoing intake of lead which continues to elevate the ZPP values.

Accumulation of ZPP only occurs in erythrocytes formed during lead's presence in erythropoietic tissue, resulting in a lag of several weeks before the fraction of new ZPP-rich cells is large enough to influence total cell ZPP level. On the other hand, elevated ZPP in erythrocytes long after significant lead exposure has ceased appears to be a better indicator of

resorption of stored lead in bone than other measurements. Alessio et al. (1976b) reported that former lead workers, removed from exposure at the workplace for more than 12 months in all cases, still showed the typical logarithmic correlation with blood or urinary lead. However, the best correlation was observed between ZPP and chelatable lead, that fraction of total body burden considered toxicologically active (see Chapter 10). This post-exposure relationship for adults clearly indicates that significant levels of hematologically toxic lead continue to circulate long after exposure to lead has ceased.

In a report relevant to the problem of multi-indicator measurement to assess the degree of lead exposure, Hesley and Wimbish (1981) studied changes in blood lead and ZPP in two groups, newly exposed lead workers or those removed from significant exposure. In new workers, blood lead achieved a plateau at 9-10 weeks, while ZPP continued to rise over the entire study interval of 24 weeks. Among workers removed from exposure, both blood lead and ZPP values remained elevated up to the end of this study period (33 weeks), but the decline in ZPP concentration lagged behind blood lead in reaching a plateau. These investigators logically concluded that the difficulty in demonstrating reliable blood lead-ZPP relationships may reflect differences in when the two measures reach plateau. Similarly, more reliance should be placed on ZPP vs. blood lead levels before permitting re-entry into areas of elevated lead exposure.

The threshold for the effect of lead on ZPP accumulation is affected by the relative spread of blood lead values and the corresponding concentrations of ZPP. In many cases these range from "normal" levels in non-exposed subjects up to values reflecting considerable exposure. Furthermore, iron deficiency is also associated with ZPP elevation, particularly in children 2-3 years or younger.

In adults, Roels et al. (1975b) found that a cutoff for the relationship of erythrocyte protoporphyrin (EP) elevation to blood lead was 25-30 μg/dl, confirmed by the log-transformed data of Joselow and Flores (1977), Grandjean and Lintrup (1978), Odone et al. (1979), and Herber (1980).

In older children, 10-15 years of age, the data of Roels et al. (1976) indicate a threshold for effect of 15.5 μg/dl. The population dose-response relationship between EP and blood lead in these children indicated that EP levels were significantly higher (>2 SDs) than the reference mean in 50 percent of the children at a blood lead level of 25 μg/dl. In the age range of children studied here, iron deficiency is uncommon and these investigators did not note any significant hematocrit change in the exposure group. In fact, it was lower in the control group, although these subjects had lower ZPP levels. In this study, then, iron deficiency was unlikely to be a confounding factor in the primary relationship. Piomelli et al. (1977) obtained a comparable threshold value (15.5 μg/dl) for lead's effect on ZPP elevation

in children who were older than 4 years as well as those who were 2-4 years old. Were iron deficiency a factor in the results for this large study population (1816 children), one would expect a greater impact in the younger group, where the deficiency is more common.

Within the blood lead range considered "normal," i.e., below 30-40 μg/dl, assessment of any ZPP-blood lead relationship is strongly influenced by the relative analytical proficiency of the laboratory carrying out both measurements, particularly for blood lead at lower values. The type of statistical treatment of the data is also a factor, as are some biological sources of variability. With respect to subject variability, Grandjean (1979) has documented that ZPP increases throughout adulthood while hemoglobin remains relatively constant. Hence, age matching is a prerequisite. Similarly, the relative degree of ZPP response is sexually dichotomous, being greater in females for a given blood lead level (see discussion below).

Suga et al. (1981) claimed no apparent correlation between blood lead levels below 40 μg/dl and blood ZPP content in an adult population of 395 male and female subjects. The values for males and females were combined, based on no measured differences in ZPP response, which is at odds with the studies of Stuik (1974), Roels et al. (1975b), Zielhuis et al. (1978), Odone et al. (1978), and Toriumi and Kawai (1981). Also, EP was found to increase with increasing age, despite the fact that the finding of no correlation between blood lead and ZPP was based on a study population with all age groups combined.

Piomelli et al. (1982) investigated both the threshold for the effect of lead on ZPP accumulation and a dose-response relationship in 2004 children, 1852 of whom had blood lead values below 30 μg/dl. In this study, blood lead and EP measurements were done in facilities with a high proficiency for both blood lead and ZPP analyses. The study employed two statistical approaches (segmental line techniques and probit analysis), both of which revealed an average threshold blood lead level of 16.5 μg/dl in either the full group or the children with blood values below 30 μg/dl. In this report, the effect of iron deficiency and other non-lead factors was tested and removed using the Abbott formula (Abbott, 1925). With respect to population dose-response relationships, it was found that blood lead values corresponding to significant EP elevation at more than 1 or 2 standard deviations above a reference mean in 50 percent of the subjects were 28.6 or 35.6 μg/dl blood lead, respectively. At a blood lead level of 30 μg/dl, furthermore, it was determined that 27 percent of children would have an EP greater than 53 μg/dl.

Comparison of ZPP elevation among adult males and females and children at a given blood lead level generally indicates that children and adult females are more sensitive to this effect of lead. Lamola et al. (1975a,b) demonstrated that the slope of ZPP vs. blood lead was steeper in children than in adults. Roels et al. (1976) found that women and children were equally more sensitive in response than adult males, a finding also observed in the population

studied by Odone et al. (1979). Other comparisons between adults, either as groups studied at random or in a voluntary lead exposure study, also document the sensitivity of females vs. males to this effect of lead (Stuik, 1974; Roels et al., 1975b, 1976, 1979; Toriumi and Kawai, 1981). The heightened response of females to lead-associated EPP elevation was investigated in rats (Roels et al., 1978a) and shown to relate to hormonal interactions with lead, confirming the human data of Roels et al. (1975b, 1976, 1979) that iron status is not a factor in the phenomenon.

The effect of lead on iron incorporation into protoporphyrin in the heme biosynthetic pathway is not restricted to the erythropoietic system. Evidence of a generalized effect of lead on tissue heme synthesis at low levels of lead exposure comes from the recent studies of Rosen and coworkers (Rosen et al., 1980, 1981; Mahaffey et al., 1982). Children with blood lead levels in the range 12-120 μg/dl showed a strong negative correlation ($r = -0.88$) with serum 1,25-dihydroxy vitamin D (1,25-$(OH)_2D$). The slopes of the regression lines for subjects having blood lead below 30 μg/dl were not materially different from those over this level. Furthermore, when lead-intoxicated children were subjected to chelation therapy, it was observed that the depressed levels of serum 1,25-$(OH)_2D$ returned to normal, while values of serum 25-hydroxy vitamin D (the precursor to 1,25-$(OH)_2D$) remained the same. This indicates that lead has an inhibitory effect on renal 1-hydroxylase, a cytochrome P-450 mediated mitochondrial enzyme system that converts 25-(OH)D to 1,25-$(OH)_2D$. The low end of the blood lead range associated with lowered 1,25-$(OH)_2D$ levels and accompanying 1-hydroxylase activity inhibition corresponds to the lead level associated with the onset of EP accumulation in erythropoietic tissue (see above). Sensitivity of renal mitochondrial 1-hydroxylase activity to lead is consistent with a large body of information showing the susceptibility of renal tubule cell mitochondria to injury by lead and with the chronic lead exposure animal model of Fowler et al. (1980), discussed in more detail below.

Formation of the heme-containing protein cytochrome P-450, which is an integral part of the hepatic mixed function oxygenase system, has been documented as being affected by lead exposure, particularly acute lead intoxication, in animals (Alvares et al., 1972; Scappa et al., 1973; Chow and Cornish, 1978; Goldberg et al., 1978; Meredith and Moore, 1979) and humans (Alvares et al., 1975; Meredith et al., 1977; Fischbein et al., 1977). Many of these studies used altered drug detoxification rates as a functional measure of such effects. In the work of Goldberg et al. (1978), increasing level of lead exposure in rats was correlated with both steadily decreasing P-450 content of hepatic microsomes and decreased activity in the detoxifying enzymes aniline hydroxylase and aminopyrine demethylase, while the data of Meredith and Moore (1979) showed that continued dosing of rats with lead results in steadily decreased microsomal P-450 content, decreased total heme content of microsomes, and increased ALA-S activity.

Of interest in this regard are data relating to neural tissue. Studies of organotypic chick dorsal root ganglion in culture document that the nervous system has heme biosynthetic capability (Whetsell et al., 1978) and that this cell system, in the presence of lead, elaborates increased porphyrinic material (Sassa et al., 1979). Chronic administration of lead to neonatal rats indicates that at low levels of exposure, with modest elevations of blood lead, there is a retarded growth in the respiratory chain hemoprotein cytochrome C and disturbed electron transport function in the developing rat cerebral cortex (Holtzman and Shen Hsu, 1976; Bull et al., 1979). These effects on the developing organism are accentuated by increased whole body lead retention in both developing children and experimental animals, as well as by higher retention of lead in brain of suckling rats compared with adults (see Chapter 10).

Heme oxygenase activity is elevated in lead-intoxicated animals (Maines and Kappas, 1976; Meredith and Moore, 1979) in which relatively high dosing is employed, indicating that normal repression of this enzyme's activity is lost, further adding to heme reduction and loss of regulatory control on the heme biosynthetic pathway.

The mechanism(s) underlying derangement of heme biosynthesis leading to ZPP accumulaton in lead intoxication rests with either ferrochelatase inhibition, impaired mitochondrial transport of iron, or a combination of both. Inferentially, the resemblance of lead-associated ZPP accumulation to a similar effect of iron deficiency is consistent with the unavailability of iron to ferrochelatase rather than direct enzyme inhibition, while the porphyrin pattern seen in the congenital disorder, erythropoietic porphyria, where ferrochelatase itself is affected, is different from that seen in lead intoxication. Similarly, lead-induced effects on mitochondrial morphology and function are well known (Goyer and Rhyne, 1973; Fowler, 1978), and such disturbances may include impaired iron transport (Borová et al., 1973).

Several animal studies indicate that the effects of lead on heme formation may involve both ferrochelatase inhibition and impaired mitochondrial transport of iron. Hart et al. (1980) observed that acute lead exposure in rabbits is associated with a two-stage hematopoietic response, the earlier one resulting in significant formation of free vs. zinc protoporphyrin with considerable hemolysis, and a later phase (where ZPP is formed) which otherwise resembles the common features of lead intoxication. Subacute exposure shows more of the typical porphyrin response reported with lead. These data may suggest that acute lead poisoning is quite different from chronic exposure in terms of the nature of hematological derangement.

Fowler et al. (1980) maintained rats on a regimen of oral lead, starting with exposure of their dams to lead in water and continuing through 9 months after birth at levels up to 250 ppm lead. The authors observed that the activity of kidney mitochondrial ALA-S and ferro-

chelatase, but not that of the cytosolic enzyme ALA-D, was inhibited. Ferrochelatase activity was inhibited at 25, 50, and 250 ppm exposure levels, being 63 percent of the control values at the 250 ppm level. Depression of state-3 respiration control ratios was observed for both succinate and pyruvate. Ultrastructurally, the mitochondria were swollen and lysosomes were rich in iron. In this study, reduced ferrochelatase activity was observed while there was concomitant mitochondrial injury and disturbance of function. The accumulation of iron may be the result of phagocytized dead mitochondria or it may represent intracellular accumulation of iron owing to the inability of mitochondria to use the element. Ibrahim et al. (1979) have shown that excess intracellular iron under conditions of iron overload is stored in cytoplasmic lysosomes. The observation of disturbed mitochondrial respiration suggests, as do the mitochondrial function data of Holtzman and Shen Hsu (1976) and Bull et al. (1979) for the developing nervous system, that intramitochondrial transport of iron would be impaired. Flatmark and Romslo (1975) demonstrated that iron transport in mitochondria is energy linked and requires an intact respiration chain at the level of cytochrome C, whereby iron (III) on the C-side of the mitochondrial inner membrane is reduced before transport to the M-side and utilization in heme formation.

The above results are particularly interesting in terms of relative tissue response. While the kidney was affected, there was no change in blood indices of hematological derangement in terms of inhibited ALA-D activity or accumulation of ZPP. This suggests that there is a difference in dose-effect functions among different tissues, particularly with lead exposure during development of the organism. It appears that while indices of erythropoietic effects of lead may be more accessible, they may not be the most sensitive as indicators of heme biosynthesis derangement in other organs.

12.3.1.4 Other Heme-Related Effects of Lead. An increased excretion of coproporphyrin in the urine of lead workers and children with lead poisoning has long been recognized, and urinary coproporphyrin measurement has been used as an indicator of lead poisoning. The mechanism of such accumulation is not understood in terms of differentiating among direct enzyme inhibition, accumulation of substrate secondary to inhibition of heme formation, or impaired movement of the coproporphyrin intramitochondrially. Excess coproporphyrin excretion differs as an indicator of lead exposure from EP accumulation in that the former is a measure of ongoing lead intoxication without the lag in response seen with EP (Piomelli and Graziano, 1980).

In lead intoxication, there is an accumulation of porphobilinogen with elevated excretion in urine, owing to inhibition by lead of the enzyme uroporphyrinogen URO-I-synthetase (Piper and Tephly, 1974). *In vitro* studies of Piper and Tephly (1974) using rat and human erythrocyte and liver preparations indicate that it is the erythrocyte URO-I-synthetase in both rats and humans that is sensitive to the inhibitory effect of lead; activity of the hepatic enzyme

is relatively insensitive. Significant inhibition of the enzyme's activity occurs at 5 μM lead with virtually total inhibition of activity in human red cell hemolysates at 10^{-4} M. According to Piper and van Lier (1977), the lower sensitivity of hepatic URO-I-synthetase activity to lead is due to a protective effect afforded by a pteridine derivative, pteroylpolyglutamate. It appears that the protection does not occur through lead chelation, since hepatic ALA-D activity was reduced in the presence of lead. The studies of Piper and Tephly (1974) indicate that it is inhibition of URO-I-synthetase in erythroid tissue or erythrocytes that accounts for the accumulation of its substrate, porphobilinogen.

12.3.2 Effects of Lead on Erythropoiesis and Erythrocyte Physiology

12.3.2.1 Effects of Lead on Hemoglobin Production. Anemia is a manifestation (sometimes an early one) of chronic lead intoxication. Typically, the anemia is mildly hypochromic and usually normocytic. It is associated with reticulocytosis, owing to shortened cell survival, and the irregular presence of basophilic stippling. Its genesis lies in both decreased hemoglobin production and increased rate of erythrocyte destruction. Not only is anemia commonly seen in children with lead poisoning, but it appears to be more severe and frequent among those with severe lead intoxication (World Health Organization, 1977; National Academy of Sciences, 1972; Lin-Fu, 1973; Betts et al., 1973).

While the anemia associated with lead intoxication in children shows features of iron-deficiency anemia, there are differences in cases of severe intoxication. These differences include reticulocytosis, basophilic stippling, and a significantly lower total iron binding capacity (TIBC). These latter features suggest that iron-deficiency anemia in young children is exacerbated by lead. The reverse is also true.

In young children, iron deficiency occurs at a significant rate, based on national (Mahaffey and Michaelson, 1980) and regional (Owen and Lippman, 1977) surveys and is known to be correlated with increased lead absorption in humans (Yip et al., 1981; Chisolm, 1981; Watson et al., 1980; Szold, 1974; Watson et al., 1958) and animals (Hamilton, 1978; Barton et al., 1978; Mahaffey-Six and Goyer, 1972). Hence, prevalent iron deficiency can be seen to potentiate the effects of lead in reduction of hemoglobin by both increasing lead absorption and exacerbating the degree of anemia.

Also in young children, there is a negative correlation between hemoglobin level and blood lead levels (Adebonojo, 1974; Rosen et al., 1974; Betts et al., 1973; Pueschel et al., 1972). These studies generally involved children under 6 years of age where iron deficiency may have been a factor. In adults, a negative correlation at blood lead values usually below 80 μg/dl has been observed (Grandjean, 1979; Lilis et al., 1978; Roels et al., 1975a; Wada, 1976), while several studies did not report any relationship below 80 μg/dl (Valentine et al.,

1982; Roels et al., 1979; Ramirez-Cervantes et al., 1978). In adults, iron deficiency would be expected to play less of a role in this relationship; Lilis et al. (1978) reported that the significant correlation between lead in blood and hemoglobin level was observed in workers where serum iron and TIBC were indistinguishable from controls.

The blood lead threshold for effects on hemogloblin has not been conclusively established. In children, this value appears to be about 40 μg/dl (World Health Organization, 1977), which is somewhat lower than in adults (Adebonojo, 1974; Rosen et al., 1974; Betts et al., 1973; Pueschel et al., 1972). Tola et al. (1973) observed no effect of lead on new workers until the blood lead had risen to a value of 50 μg/dl after about 100 days. The regression analysis data of Grandjean (1979), Lilis et al. (1978), and Wada (1976) show persistence of the negative correlation of blood lead and hemoglobin below 50 μg/dl. Human population dose-response data for the lead-hemoglobin relationship are limited. For lead workers, Baker et al. (1979) have calculated the corresponding dose-response (<14.0 g Hb/dl): 5 percent at blood lead of 40-59 μg/dl; 14 percent at blood lead of 60-79 μg/dl; and 36 percent at values above 80 μg/dl. In 202 lead workers, Grandjean (1979) noted the following percentage of workers having a hemoglobin level below 14.4 g/dl as a function of blood lead: <25 μg/dl, 17 percent; 25-60 μg/dl, 26 percent; >60 μg/dl, 45 percent.

The underlying mechanisms of lead-associated anemia appear to be a combination of reduced hemoglobin production and shortened erythrocyte survival because of direct cell damage. Effects of lead on hemoglobin production, furthermore, rest with disturbances of both heme and globin biosynthesis.

Biosynthesis of globin, the protein moiety of hemoglobin, also appears to be inhibited in lead exposure (Dresner et al., 1982; Wada et al., 1972; White and Harvey, 1972; Kassenaar et al., 1957). White and Harvey (1972) reported a decrease of globin synthesis in reticulocytes *in vitro* in the presence of lead at levels as low as 1.0 μM, corresponding to a blood lead level of 20 μg/dl. These data are in accord with the observation of Dresner et al. (1982), who noted a reduced globin synthesis (76 percent of controls) in rat bone marrow suspensions exposed to 1.0 μM lead. White and Harvey (1972) also noted that there was a decreased synthesis of alpha chains vs. beta chains.

Disturbance of globin biosynthesis is a consequence of lead's effects on heme formation since cellular heme regulates protein synthesis in erythroid cells (Levere and Granick, 1967) and regulates the translation of globin messenger RNA (Freedman and Rosman, 1976). The disturbance in the translation of mRNA in erythroid tissue may also reflect the effect of lead on pyrimidine metabolism.

12.3.2.2 Effects of Lead on Erythrocyte Morphology and Survival. It is clear that there is a hemolytic component to lead-induced anemia in humans owing to shortened erythrocyte survival,

and the various aspects of this effect have been reviewed by Waldron (1966), Goldberg (1968), Moore et al. (1980), Valentine and Paglia (1980), and Angle and McIntire (1982).

The relevant studies of shortened cell life with lead intoxication include observations of the behavior of red cells to mechanical and osmotic stress under in vivo and in vitro conditions. Waldron (1966) has discussed the frequent reports of increased mechanical fragility of erythrocytes from lead-poisoned workers, beginning with the work of Aub et al. (1926). Increased osmotic resistance of erythrocytes from subjects with lead intoxication is a parallel finding, both in vivo (Aub and Reznikoff, 1924; Harris and Greenberg, 1954; Horiguchi et al., 1974) and in vitro (Qazi et al., 1972; Waldron, 1964; Clarkson and Kench, 1956). Using an apparatus called a coil planet centrifuge, Karai et al. (1981) studied erythrocytes of lead workers and found significant increases in osmotic resistance; at the same time mean corpuscular volume and reticulocyte counts were not different from controls. Karai et al. suggest that one mechanism of increased resistance involves impairment of hepatic lecithin-cholesterol acyltransferase, leading to a build-up of cholesterol in the cell membrane. This resembles the increased osmotic resistance seen in obstructive jaundice where increased membrane cholesterol has been observed (Cooper et al., 1975). Karai et al. (1981) also reported an increased cholesterol-phospholipid ratio in lead workers' erythrocytes.

Erythrokinetic data in lead workers and children with lead-associated anemia have been reported. Shortening of erythrocyte survival has been demonstrated by Hernberg et al. (1967a) using tritium-labeled difluorophosphonate. Berk et al. (1970) used detailed isotope studies of a subject with severe lead intoxication to determine shorter erythrocyte life span, while Leiken and Eng (1963) observed shortened cell survival in three of seven children. These studies, as well as the reports of Landaw et al. (1973), White and Harvey (1972), Albaharry (1972), and Dagg et al. (1965), indicate that hemolysis is not the exclusive mechanism of anemia and that diminished erythrocyte production also plays a role.

The molecular basis for increased cell destruction with lead exposure includes the inhibition by lead of the activities of the enzymes (Na^+, K^+)-ATPase and pyrimidine-5'-nucleotidase. Erythrocyte membrane (Na^+, K^+)-ATPase is a sulfhydryl enzyme and inhibition of its activity by lead has been well documented (Raghavan et al., 1981; Secchi et al., 1968; Hasan et al., 1967; Hernberg et al., 1967b). In the study of Raghavan et al. (1981), enzyme activity was inversely correlated with membrane lead content ($p < 0.001$) in lead workers with or without symptoms of overt lead toxicity, while correlation with whole blood lead was poor. With enzyme inhibition, there is irreversible loss of potassium ion from the cell with undisturbed input of sodium into the cell, resulting in a relative increase in sodium. Since the cells "shrink," there is a net increase in sodium concentration, which likely results in increased mechanical fragility and cell lysis (Moore et al., 1980).

Both with lead exposure and in subjects with a genetic deficiency of the enzyme pyrimidine-5'-nucleotidase, activity is reduced, leading to impaired phosphorolysis of the nucleotides cytidine and uridine phosphate, which are then retained in the cell, causing interference with the conservation of the purine nucleotides necessary for cellular energetics (Angle and McIntire, 1982; Valentine and Paglia, 1980). A more detailed discussion of lead's interaction with this enzyme is presented in Section 12.3.2.3.

In a series of studies dealing with the hemolytic relationship of lead and vitamin E deficiency in animals, Levander et al. (1980) observed that lead exposure exacerbates the experimental hemolytic anemia associated with vitamin E deficiency by enhancing mechanical fragility, i.e., retarded cell deformability. These workers note that vitamin E deficiency is seen with children having elevated blood lead levels, especially subjects having glucose-6-phosphate dehydrogenase (G-6-PD) deficiency, indicating that the synergistic relationship seen in animals may exist in humans.

Glutathione is a necessary factor in erythrocyte function and structure. In workers exposed to lead, Roels et al. (1975a) found that there is a moderate but significant decrease in red cell glutathione compared with controls. This appears to reflect lead-induced impairment of glutathione synthesis.

12.3.2.3 Effects of Lead on Pyrimidine-5'-Nucleotidase Activity and Erythropoietic Pyrimidine Metabolism. The presence in lead intoxication of basophilic stippling and an anemia of hemolytic nature is similar to what is seen in subjects having a congenital deficiency of pyrimidine-5'-nucleotidase (Py-5-N), an enzyme mediating the phosphorolysis of the pyrimidine nucleotides, cytidine and uridine phosphates. With inhibition these nucleotides accumulate in the red cell or reticulocyte, there is a retardation of ribonuclease-mediated ribosomal RNA catabolism in maturing cells, and the resulting accumulation of aggregates of incompletely degraded ribosomal fragments accounts for the phenomenon of basophilic stippling.

In characterizing the enzyme Py-5-N, Paglia and Valentine (1975) observed that its activity was particularly sensitive to inhibition by certain metals, particularly lead, prompting further investigation of the interplay between lead intoxication and disturbances of erythropoietic pyrimidine metabolism. Paglia et al. (1975) observed that in subjects occupationally exposed to lead but having no evidence of basophilic stippling or significant frequency of anemia, the activity of Py-5-N was reduced to about 50 percent of control subjects and was most impaired in one worker with anemia, about 11 percent of normal. There was a general inverse correlation between enzyme activity and blood lead level. In this report, normal erythrocytes incubated with varying levels of lead showed detectable inhibition at levels as low as 0.1-1.0 μM, with consistent 50 percent inhibition at about 10 μM. Subsequently, these investigators (Valentine et al., 1976) observed that an individual with severe

lead intoxication had an 85 percent decrease in Py-5-N activity, basophilic stippling, and accumulation of pyrimidine nucleotides, mainly cytidine triphosphate. Since these parameters approached values seen in the congenital deficiency of Py-5-N, the data suggest a common etiology for the hemolytic anemia and stippling in both lead poisoning and the congenital disorder.

Several other reports of investigations of Py-5-N activity and pyrimidine nucleotide levels in lead workers have been published (Paglia et al., 1977; Buc and Kaplan, 1978). In nine workers having overt lead intoxication and blood lead values of 80-160 μg/dl, Py-5-N activity was significantly inhibited while the pyrimidine nucleotides comprised 70-80 percent of the total nucleotide pool, in contrast to trace levels in unexposed individuals (Paglia et al., 1977). In the study of Buc and Kaplan (1978), lead workers with or without overt lead intoxication all showed reduced activity of Py-5-N, which was inversely correlated with blood lead when the activity was expressed as a ratio with G-6-PD activity to accommodate an enhanced population of young cells due to hemolytic anemia. Enzyme inhibition was observed even when other indicators of lead exposure were negative.

Angle and McIntire (1978) observed that in 21 children 2-5 years old, with blood lead levels of 7-80 μg/dl, there was a negative linear correlation between Py-5-N activity and blood lead ($r = -0.60$, $p < 0.01$). Basophilic stippling was only seen in the child with the highest blood lead value and only two subjects had reticulocytosis. While adults tended to show a threshold for inhibition of Py-5-N at a blood lead level of 44 μg/dl or higher, there was no clear response threshold in these children. In a related investigation with 42 children 1-5 years old having blood lead levels of <10 to 72 μg/dl, Angle et al. (1982) noted that there was: (1) an inverse correlation ($r = -0.64$, $p < 0.001$) between the logarithm of Py-5-N activity and blood lead; (2) a positive log-log correlation between cytidine phosphates and blood lead in 15 of these children ($r = 0.89$, $p < 0.001$); and (3) an inverse relationship in 12 subjects between log of enzyme activity and cytidine phosphates ($r = -0.796$, $p < 0.001$). Study of the various relationships at low levels was made possible by the use of anion-exchange high performance liquid chromatography. In these studies, there was no threshold of effects of lead on either enzyme activity or cell nucleotide content even below 10 μg/dl. Finally, there was a significant positive correlation of pyrimidine nucleotide accumulation and the accumulation of ZPP.

In subjects undergoing therapeutic chelation with EDTA, Py-5-N activity increased, while there was no effect on pyrimidine nucleotides (Swanson et al., 1982), indicating that the pyrimidine accumulation is associated with the reticulocyte.

The metabolic significance of Py-5-N activity inhibition and nucleotide accumulation with lead exposure is derived from its effects on red cell membrane stability and survival by al-

teration of cellular energetics (Angle and McIntire, 1982), leading to cell lysis. A further consequence may be feedback inhibition of mRNA and protein synthesis, in that denatured mRNA may alter globin mRNA or globin chain synthesis. It was noted earlier that disturbances in heme production also affect the translation of globin mRNA (Freedman and Rosman, 1976). Hence, these two lead-associated disturbances of erythroid tissue function potentiate the effects of each other.

12.3.3 Effects of Alkyl Lead on Heme Synthesis and Erythropoiesis

In the Section 10.7 discussion of alkyl lead metabolism, it was noted that transformations of tetraethyl and tetramethyl lead *in vivo* result in generation not only of neurotoxic trialkyl lead metabolites but also of products of further dealkylation, including inorganic lead. One would therefore expect alkyl lead exposure to be associated with, in addition to other effects, some of those effects classically related to inorganic lead exposure.

Chronic gasoline sniffing has been recognized as a problem habit among children in rural or remote areas (Boeckx et al., 1977; Kaufman, 1973). When such practice involves leaded gasoline, the potential exists for lead intoxication. Boeckx et al. (1977) conducted surveys of children in remote Canadian communities in regard to the prevalence of gasoline sniffing and indications of chronic lead exposure. In one group of 43 children, all of whom sniffed gasoline, mean ALA-D activity was only 30 percent that of control subjects, with a significant correlation between the decrease in enzyme activity and the frequency of sniffing. A second survey of 50 children revealed similar results. Two children having acute lead intoxication associated with gasoline sniffing showed markedly lowered hemoglobin, elevated urinary ALA, and elevated urinary coproporphyrin. The authors estimated that more than half of disadvantaged children residing in rural or remote areas of Canada may have chronic lead exposure via this habit, consistent with the estimate of Kaufman (1973) of 62 percent for children in rural American Indian communities in the Southwest.

Robinson (1978) described two cases of pediatric lead poisoning due to habitual gasoline sniffing, one of which showed basophilic stippling. Hansen and Sharp (1978) reported that a young adult with acute lead poisioning due to chronic gasoline sniffing not only had basophilic stippling, but a 6-fold increase in urinary ALA, elevated urinary coproporphyrin, and an EP level about 4-fold above normal. In the reports of Boeckx et al. (1977) and Robinson (1978), increased lead levels were measured in urine in the course of chelation therapy, indicating that significant amounts of inorganic lead were present.

12.3.4 The Interrelationship of Lead Effects on Heme Synthesis and the Nervous System

Lead-associated disturbances in heme biosynthesis as a possible factor in the neurological effects of lead have been studied because of (1) the recognized similarity between

classic signs of lead neurotoxicity and many, but not all, of the neurological components of the congenital disorder, acute intermittent porphyria, and (2) some unusual aspects of lead neurotoxicity. Both acute attack porphyria and lead intoxication with neurological symptoms are variably accompanied by abdominal pain, constipation, vomiting, paralysis or paresis, demyelination, and psychiatric disturbances (Dagg et al., 1965; Moore et al., 1980; Silbergeld and Lamon, 1980). According to Angle and McIntire (1982), some of the unusual features of lead neurotoxicity are consistent with deranged hematopoiesis: (1) a lag in production of neurological symptoms; (2) the incongruity of early deficits in affective and cognitive function with the regional distribution of lead in the brain; and (3) a better correlation of neurobehavioral deficits with erythrocyte protoporphyrin than with blood lead. Item 3, it should be noted, is not universally the case (Hammond et al., 1980; Spivey et al., 1979).

While the nature and pattern of the derangements in heme biosynthesis in acute attack porphyria and lead intoxication differ in many respects, both involve excessive systemic accumulation and excretion of ALA, and this common feature has stimulated numerous studies of a connection between hemato- and neurotoxicity. In vitro data (Whetsell et al., 1978) have shown that the nervous system is capable of heme biosynthesis in the chick dorsal root ganglion. Sassa et al. (1979) found that the presence of lead in these preparations increases production of porphyrinic material, i.e., there is disturbed heme biosynthesis with accumulation of one or more porphyrins and, presumably, ALA. Millar et al. (1970) reported inhibited brain ALA-D activity in suckling rats exposed to lead, while Silbergeld et al. (1982) observed similar inhibition in brains of adult rats acutely exposed to lead. In the latter study, chronic lead exposure was also associated with a moderate increase in brain ALA without inhibition of ALA-D, suggesting an extra-neural source of the heme precursor. Moore and Meredith (1976) administered ALA to rats and observed that exogenous ALA can penetrate the blood-brain barrier. These reports suggest that ALA can either be generated in situ in the nervous system or can enter the nervous system from elsewhere.

Neurochemical investigations of ALA action in the nervous system have evaluated interactions with the neurotransmitter gamma-aminobutyric acid (GABA). Interference with GABAergic function by exposure to lead is compatible with such clinical and experimental signs of lead neurotoxicity as excitability, hyperactivity, hyperreactivity, and, in severe lead intoxication, convulsions (Silbergeld and Lamon, 1980). Of particular interest is the similarity in chemical structure between ALA and GABA, which differ only in that ALA has a carbonyl group on the alpha carbons, and GABA has a carbonyl group on the beta carbon.

While chronic lead exposure appears to alter neural pathways involving GABA function (Piepho et al., 1976; Silbergeld et al., 1979), this effect cannot be duplicated in vitro

using various levels of lead (Silbergeld et al., 1980). This suggests that lead does not impart the effect by direct interaction or an intact multi-pathway system is required. In vitro studies (Silbergeld et al., 1980a; Nicoll, 1976) demonstrate that ALA can displace GABA from synaptosomal membranes associated with synaptic function of the neurotransmitter on the GABA receptor, but that it is less potent than GABA by a factor of 10^3-10^4, suggesting that levels of ALA achieved even with severe intoxication may not be effectively competitive.

A more significant role for ALA in lead neurotoxicity may well be related to the observation that GABA release is subject to negative feedback control through presynaptic receptors on GABAergic terminals (Snodgrass, 1978; Mitchell and Martin, 1978). Brennan and Cantrill (1979) found that ALA inhibits K^+-stimulated release of GABA from pre-loaded synaptosomes by functioning as an agonist at the presynaptic receptors. The effect is evident at 21.0 μM ALA, while the inhibiting effect is abolished by the GABA antagonists bicuculline and picrotoxin. Of interest also is the demonstration (Silbergeld et al., 1980a) that synaptosomal release of preloaded ^{3}H-GABA, both resting and K^+-stimulated, is also inhibited in animals chronically treated with lead, paralleling the in vitro data of Brennan and Cantrill (1979) using ALA.

Silbergeld et al. (1982) described the comparative effects of lead and the agent succinylacetone, given acutely or chronically to adult rats, in terms of disturbances in heme synthesis and neurochemical indices. Succinylacetone, a metabolite that can be isolated from the urine of patients with hereditary tyrosinemia (Lindblad et al., 1977) is a potent inhibitor of heme synthesis, exerting its effect by ALA-D inhibition and derepression of ALA synthetase (Tschudy et al., 1980, 1983). Both agents, in vivo, showed significant inhibition of high affinity Na^+-dependent uptake of ^{14}C-GABA by cortex, caudate, and substantia nigra. However, neither agent affected GABA uptake in vitro. Similarly, both chronic or acute lead treatment and chronically administered succinylacetone reduced the seizure threshold to the GABA antagonist, picrotoxin. While these agents may involve entirely different mechanisms of toxicity to the GABAergic pathway, the fact remains that two distinct potent inhibitors of the heme biosynthetic pathway and ALA-D, which do not impart a common neurochemical effect by direct action on a neurotransmitter function, have a common neurochemical action in vivo.

Human data relating the hemato- and neurotoxicity of lead to each other are limited. Hammond et al. (1980) reported that the best correlates of the frequency of neurological symptoms in 28 lead workers were urinary and plasma ALA, which showed a higher correlation than EP. These data support a connection between heme biosynthesis impairment and neurological effects of ALA. Of interest here is the clinical report of Lamon et al. (1979) describing the effect of hematin [Fe(III)-heme] given parenterally to a subject with lead intoxication. Over the course of treatment (16 days), urinary coproporphyrin and ALA significantly dropped

and such neurological symptoms as lower extremity numbness and aching diminished. Blood lead levels were not altered during the treatment. Although remission of symptoms in this subject may have been spontaneous, the outcome parallels that observed in hematin treatment of subjects with acute porphyria in terms of similar reduction of heme indicators and relief of symptoms (Lamon et al., 1979).

Taken collectively, all of the available data strongly suggest that ALA, formed in situ or entering the brain, is neurotoxic to GABAergic function in particular. It inhibits K^+-stimulated GABA release by interaction with presynaptic receptors, where ALA appears to be particularly potent at very low levels, based on in vitro results. As described in the section on heme biosynthesis, lead can affect both cellular respiration and cytochrome C levels in the nervous system of the developing rat, which may contribute to manisfestation of some symptoms of lead neurotoxicity. Hence, more than the issue of ALA neurotoxicity should be considered in assessing connections between lead-induced hemato- and neurotoxicity.

12.3.5 Summary and Overview

12.3.5.1 Lead Effects on Heme Biosynthesis. Lead effects on heme biosynthesis are well known because of both their prominence and numerous studies of such effects in humans and experimental animals. The process of heme biosynthesis starts with glycine and succinyl-coenzyme A, proceeds through formation of protoporphyrin IX, and culminates with the insertion of divalent iron into the porphyrin ring, thus forming heme. In addition to being a constituent of hemoglobin, heme is the prosthetic group of many tissue hemoproteins having variable functions, such as myoglobin, the P-450 component of the mixed function oxygenase system, and the cytochromes of cellular energetics. Hence, disturbance of heme biosynthesis by lead poses the potential for multi-organ toxicity.

At present, steps in the heme synthesis pathway that have been best studied in regard to lead effects involve three enzymes: (1) stimulation of mitochondrial delta-aminolevulinic acid synthetase (ALA-S), which mediates formation of delta-aminolevulinic acid (ALA); (2) direct inhibition of the cytosolic enzyme, delta-aminolevulinic acid dehydrase (ALA-D), which catalyzes formation of porphobilinogen from two units of ALA; and (3) inhibition of insertion of iron (II) into protoporphyrin IX to form heme, a process mediated by ferrochelatase.

Increased ALA-S activity has been found in lead workers as well as lead-exposed animals, although the converse, an actual decrease in enzyme activity, has also been observed in several experimental studies using different exposure methods. It appears, then, that enzyme activity increase via feedback derepression or activity inhibition may depend on the nature of the exposure. Using rat liver cells in culture, ALA-S activity was stimulated in vitro at levels as low as 5.0 μM or 1.0 μg Pb/g preparation. The increased activity was seen to be due

to biosynthesis of more enzyme. The threshold for lead stimulation of ALA-S activity in humans, based on a study using leukocytes from lead workers, appears to be about 40 μg Pb/dl. The generality of this apparent threshold to other tissues depends on how well the sensitivity of leukocyte mitochondria mirrors that in other systems. The relative impact of ALA-S activity stimulation on ALA accumulation at lower lead exposure levels appears to be much less than the effect of ALA-D activity inhibition, to the extent that at ALA-D activity is significantly depressed at 40 μg/dl blood lead, where ALA-S activity only begins to be affected.

Erythrocyte ALA-D activity is very sensitive to lead inhibition, which is reversed by reactivation of the sulfhydryl group with agents such as dithiothreitol, zinc, or zinc plus glutathione. Zinc levels that achieve reactivation, however, are well above physiological levels. Although zinc appears to offset inhibitory effects of lead observed in human erythrocytes _in vitro_ and in animal studies, lead workers exposed to both zinc and lead do not show significant changes in the relationship of ALA-D activity to blood lead compared with just lead exposure; nor does the range of physiological zinc in non-exposed subjects affect the activity. In contrast zinc deficiency in animals significantly inhibits activity, with concomitant accumulation of ALA in urine. Since zinc deficiency has also been demonstrated to increase lead absorption, the possibility exists for dual effects of such deficiency on ALA-D activity: (1) a direct effect on activity due to reduced zinc availability; and (2) increased lead absorption leading to further inhibition of activity.

Erythrocyte ALA-D activity appears to be inhibited at virtually all blood lead levels measured so far, and any threshold for this effect in either adults or children remains to be determined. A further measure of this enzyme's sensitivity to lead is a report that rat bone marrow suspensions show inhibition of ALA-D activity by lead at a level of 0.1 μg/g suspension. Inhibition of ALA-D activity in erythrocytes apparently reflects a similar effect in other tissues. Hepatic ALA-D activity was inversely correlated in lead workers with both erythrocyte activity as well as blood lead levels. Of significance are experimental animal data showing that (1) brain ALA-D activity is inhibited with lead exposure and (2) this inhibition appears to occur to a greater extent in developing vs. adult animals, presumably reflecting greater retention of lead in developing animals. In the avian brain, cerebellar ALA-D activity is affected to a greater extent than that of the cerebrum and, relative to lead concentration, shows inhibition approaching that occurring in erythrocytes.

Lead inhibition of ALA-D activity is reflected by elevated levels of its substrate, ALA, in blood, urine, and soft tissues. In one study, increases in urinary ALA were preceded by a rise in circulating levels of the metabolite. Blood ALA was elevated at all corresponding blood lead values down to the lowest determined (18 μg/dl), while urinary ALA increased exponentially with blood ALA.

Urinary ALA is employed extensively as an indicator of excessive lead exposure in lead workers. The value of this measurement in pediatric screening, however, is diagnostically limited if only spot urine collection is done, more satisfactory data being obtainable with 24-hour collections. Numerous independent studies document a direct correlation between blood lead and the logarithm of urinary ALA in human adults and children; the threshold for urinary ALA increases is commonly accepted as being 40 μg/dl. However, several studies of lead workers indicate that the correlation of urinary ALA with blood lead continues below this value, and one study found that the slope of the dose-effect curve in lead workers is dependent upon level of exposure.

The health significance of lead-inhibited ALA-D activity and accumulation of ALA at lower lead exposure levels is controversial, to the extent that the "reserve capacity" of ALA-D activity is such that only the level of inhibition associated with marked accumulation of the enzyme's substrate, ALA, in accessible indicator media may be significant. However, it is not possible to quantify, at lower levels of lead exposure, the relationship of urinary ALA to target tissue levels nor to relate the potential neurotoxicity of ALA at any accumulation level to levels in indicator media; i.e., the blood lead threshold for neurotoxicity of ALA may be different from that associated with increased urinary excretion of ALA.

Accumulation of protoporphyrin in erythrocytes of lead-intoxicated individuals has been recognized since the 1930s, but it has only recently been possible to quantitatively assess the nature of this effect via development of sensitive, specific microanalysis methods. Accumulation of protoporphyrin IX in erythrocytes results from impaired placement of iron (II) in the porphyrin moiety to form heme, an intramitochondrial process mediated by ferrochelatase. In lead exposure, the porphyrin acquires a zinc ion in lieu of native iron, thus forming zinc protoporphyrin (ZPP), and is tightly bound in available heme pockets for the life of the erythrocytes. This tight sequestration contrasts with the relatively mobile non-metal, or free, protoporphyrin (FEP) accumulated in the congenital disorder erythropoietic protoporphyria.

Elevation of erythrocyte ZPP has been extensively documented as being exponentially correlated with blood lead in children and adult lead workers and is presently considered one of the best indicators of undue lead exposure. Accumulation of ZPP only occurs in erythrocytes formed during lead's presence in erythroid tissue, resulting in a lag of at least several weeks before such build-up can be measured. The level of such accumulation in erythrocytes of newly employed lead workers continues to increase when blood lead has already reached a plateau. This influences the relative correlation of ZPP and blood lead in workers with short exposure histories. In individuals removed from occupational exposure, the ZPP level in blood declines much more slowly than blood lead, even years after removal from exposure or after a drop in blood lead. Hence, ZPP level appears to be a more reliable indicator of continuing intoxication from lead resorbed from bone.

The threshold for detection of lead-induced ZPP accumulation is affected by the relative spread of blood lead and corresponding ZPP values measured. In young children (< 4 yr old), the ZPP elevation associated with iron-deficiency anemia must also be considered. In adults, numerous studies indicate that the blood lead threshold for ZPP elevation is about 25-30 µg/dl. In children 10-15 years old, the threshold is about 16 µg/dl; in this age group, iron deficiency is not a factor. In one study, children over 4 years old showed the same threshold, 15.5 µg/dl, as a second group under 4 years old, indicating that iron deficiency was not a factor in the study. Fifty percent of the children had significantly elevated EP levels (2 standard deviations above reference mean EP) at 25 µg/dl blood lead.

At blood lead levels below 30-40 µg/dl, any assessment of the ZPP-blood lead relationship is strongly influenced by the relative analytical proficiency for measurement of both blood lead and EP. The types of statistical analyses used are also important. In a recent detailed statistical study involving 2004 children, 1852 of whom had blood lead values below 30 µg/dl, segmental line and probit analysis techniques were employed to assess the dose-effect threshold and dose-response relationship. An average blood lead threshold for the effect using both statistical techniques yielded a value of 16.5 µg/dl for either the full group or those subjects with blood lead below 30 µg/dl. The effect of iron deficiency was tested for and removed. Of particular interest was the finding that the blood lead values corresponding to EP elevations more than 1 or 2 standard deviations above the reference mean in 50 percent of the children were 28.6 or 35.7 µg Pb/dl, respectively. Hence, fully half of the children had significant elevations of EP at blood lead levels around 30 µg/dl, the currently accepted cut-off value for undue lead exposure. From various reports, children and adult females appear to be more sensitive to lead effects on EP accumulation at any given blood lead level, with children being somewhat more sensitive than adult females.

Lead effects on heme formation are not restricted to the erythropoietic system. Recent studies show that the reduction of serum 1,25-$(OH)_2D$ seen with even low level lead exposure is apparently the result of lead inhibition of the activity of renal 1-hydroxylase, a cytochrome P-450 mediated enzyme. This heme-containing protein, cytochrome P-450 (an integral part of the hepatic mixed function oxygenase system), is affected in humans and animals by lead exposure, especially acute intoxication. Reduced P-450 content correlates with impaired activity of detoxifying enzyme systems such as aniline hydroxylase and aminopyrine demethylase.

Studies of organotypic chick dorsal root ganglion in culture show that the nervous system not only has heme biosynthetic capability but such preparations elaborate porphyrinic material in the presence of lead. In the neonatal rat, chronic lead exposure, resulting in moderately elevated blood lead, is associated with retarded increases in the hemoprotein, cytochrome C, and disturbed electron transport in the developing cerebral cortex. These data parallel effects of lead on ALA-D activity and ALA accumulation in neural tissue. When both these

effects are viewed in the toxicokinetic context of increased retention of lead in both developing animals and children, there is an obvious, serious potential for impaired heme-based metabolic function in the nervous system of lead-exposed children.

As can be concluded from the above discussion, the health significance of ZPP accumulation rests with the fact that it is evidence of impaired heme and hemoprotein formation in many tissues, arising from entry of lead into mitochondria. Such evidence for reduced heme synthesis is consistent with much data documenting lead-associated effects on mitochondria. The relative value of the lead-ZPP relationship in erythropoietic tissue as an index of this effect in other tissues hinges on the relative sensitivity of the erythropoietic system compared with other organ systems. One study of rats exposed to low levels of lead over their lifetime demonstrated that protoporphyrin accumulation in renal tissue was already significant at levels of lead exposure where little change was seen in erythrocyte porphyrin levels.

Other steps in the heme biosynthesis pathway are also known to be affected by lead, although these have not been as well studied on a biochemical or molecular level. Coproporphyrin levels are increased in urine, reflecting active lead intoxication. Lead also affects the activity of the enzyme uroporphyrinogen-I-synthetase, resulting in an accumulation of its substrate, porphobilinogen. The erythrocyte enzyme has been reported to be much more sensitive to lead than the hepatic species, presumably accounting for much of the accumulated substrate. Ferrochelatase is an intramitochondrial enzyme, and impairment of its activity, either directly by lead or via impairment of iron transport to the enzyme, is evidence of the presence of lead in mitochondria.

12.3.5.2 Lead Effects on Erythropoiesis and Erythrocyte Physiology. Anemia is a manifestation of chronic lead intoxication, being characterized as mildly hypochromic and usually normocytic. It is associated with reticulocytosis, owing to shortened cell survival, and the variable presence of basophilic stippling. Its occurrence is due to both decreased production and increased rate of destruction of erythrocytes. In young children (< 4 yr old) iron deficiency anemia is exacerbated by lead effects, and vice versa. Hemoglobin production is negatively correlated with blood lead in young children, where iron deficiency may be a confounding factor, as well as in lead workers. In one study, blood lead values that were usually below 80 μg/dl were inversely correlated with hemoglobin content. In these subjects, no iron deficiency was found. The blood lead threshold for reduced hemoglobin content is about 50 μg/dl in adult lead workers and somewhat lower (40 μg/dl) in children.

The mechanism of lead-associated anemia appears to be a combination of reduced hemoglobin production and shortened erythrocyte survival because of direct cell injury. Lead effects on hemoglobin production involve disturbances of both heme and globin biosynthesis. The hemolytic component to lead-induced anemia appears to be due to increased cell fragility and increased osmotic resistance. In one study using rats, the hemolysis associated with vitamin E

deficiency, via reduced cell deformability, was exacerbated by lead exposure. The molecular basis for increased cell destruction rests with inhibition of (Na^+, K^+)-ATPase and pyrimidine-5'-nucleotidase. Inhibition of the former enzyme leads to cell "shrinkage" and inhibition of the latter results in impaired pyrimidine nucleotide phosphorolysis and disturbance of the activity of the purine nucleotides necessary for cellular energetics.

12.3.5.3 Effects of Alkyl Lead Compounds on Heme Biosynthesis and Erythropoiesis. Tetraethyl lead and tetramethyl lead, components of leaded gasoline, undergo transformation *in vivo* to neurotoxic trialkyl metabolites as well as further conversion to inorganic lead. Hence, one might anticipate that exposure to such agents may show effects commonly associated with inorganic lead in terms of heme synthesis and erythropoiesis. Various surveys and case reports show that the habit of sniffing leaded gasoline is associated with chronic lead intoxication in children from socially deprived backgrounds in rural or remote areas. Notable in these subjects is evidence of impaired heme biosynthesis as indexed by significantly reduced ALA-D activity. In several case reports of frank lead toxicity from habitual leaded gasoline sniffing, effects such as basophilic stippling in erythrocytes and significantly reduced hemoglobin have also been noted.

12.3.5.4 Relationships of Lead Effects on Heme Synthesis to Neurotoxicity. The role of lead-associated disturbances of heme biosynthesis as a possible factor in neurological effects of lead is of considerable interest because of: (1) similarities between classical signs of lead neurotoxicity and several neurological components of the congenital disorder, acute intermittent porphyria; and (2) some of the unusual aspects of lead neurotoxicity. There are two possible points of connection between lead effects on heme biosynthesis and the nervous system. Associated with both lead neurotoxicity and acute intermittent porphyria is the common feature of excessive systemic accumulation and excretion of ALA. Secondly, lead neurotoxicity reflects, to some degree, impaired synthesis of heme and hemoproteins involved in crucial cellular functions. Available information indicates that ALA levels are elevated in the brain of lead-exposed animals, arising via *in situ* inhibition of brain ALA-D activity or via transport to the brain after formation in other tissues. ALA is known to traverse the blood-brain barrier. Hence, ALA is accessible to, or formed within, the brain during lead exposure and may express its neurotoxic potential.

Based on various *in vitro* and *in vivo* data obtained in the context of neurochemical studies of lead neurotoxicity, it appears that ALA can readily play a role in GABAergic function, particularly inhibiting release of the neurotransmitter GABA from presynaptic receptors, where ALA appears to be very potent even at low levels. In an *in vitro* study, agonist behavior by ALA was demonstrated at levels as low as 1.0 μM ALA. This *in vitro* observation supports results of a study using lead-exposed rats in which there was reported inhibition of

both resting and K^+-stimulated release of preloaded ^{3}H-GABA from nerve terminals. Further evidence for an effect of some agent other than lead acting directly is the observation that in vivo effects of lead on neurotransmitter function cannot be duplicated with in vitro preparations to which lead is added. Human data on lead-induced associations between disturbed heme synthesis and neurotoxicity, while limited, also suggest that ALA may function as a neurotoxicant.

The connection of impaired heme and hemoprotein synthesis in the neonatal rat brain was noted earlier, in terms of reduced cytochrome C production and impaired operation of the cytochrome C respiratory chain. Hence, one might expect that such impairment would be most prominent in areas of relatively greater cellularization, such as the hippocampus. As noted in Chapter 10, these are also regions where selective lead accumulation occurs.

12.4 NEUROTOXIC EFFECTS OF LEAD

12.4.1 Introduction

Historically, neurotoxic effects have long been recognized as being among the more severe consequences of human lead exposure (Tanqueral Des Planches, 1839; Stewart, 1895; Prendergast, 1910; Oliver, 1911; Blackfan, 1917). Since the early 1900's, extensive research has focused on the elucidation of lead exposure levels associated with the induction of various types of neurotoxic effects and related issues, e.g. critical exposure periods for their induction and their persistence or reversibility. Such research, spanning more than 50 years, has provided expanding evidence indicating that progressively lower lead exposure levels, previously accepted as "safe," are actually sufficient to cause notable neurotoxic effects of lead.

The neurotoxic effects of extremely high exposures resulting in blood lead levels in excess of 80-100 μg/dl, have been well documented--especially in regard to increased risk for fulminant lead encephalopathy (a well-known clinical syndrome characterized by overt symptoms such as gross ataxia, persistent vomiting, lethargy, stupor, convulsions, and coma of such severity that immediate medical attention is required). The persistence of neurological sequelae in cases of non-fatal lead encephalopathy has also been well established. The neurotoxic effects of subencephalopathic lead exposures in both human adults and children, however, continues to represent a major area of controversy and interest. Reflecting this, much research during the past 10-15 years has focussed on the delineation of exposure-effect relationships for: (1) the occurrence of overt signs and symptoms of neurotoxicity in relation to other indicators of subencephalopathic overt lead intoxication; and (2) the manifestation of more subtle, often difficult-to-detect indications of altered neurological functions in apparently asymptomatic (i.e., not overtly lead-poisoned) individuals.

The present assessment critically reviews the available scientific literature on the neurotoxic effects of lead, first evaluating the results of human studies bearing on the subject and then focusing on pertinent animal toxicology studies. The discussion of human studies is divided into two major subsections focusing on neurotoxic effects of lead exposure in (1) adults and (2) children. Both lead effects on the central nervous system (CNS) and the peripheral nervous system (PNS) are discussed in each case. In general, only relatively brief overview summaries are provided in regard to findings bearing on the effects of extremely high level exposures resulting in encephalopathy or other frank signs or symptoms of overt lead intoxication. Studies concerning the effects of lower level lead exposures are assessed in more detail, especially those dealing with non-overtly lead intoxicated children. As for the animal toxicology studies, particular emphasis is placed on the review of studies that help to address certain important issues raised by the human research findings, rather than attempting an exhaustive review of all animal toxicology studies concerning the neurotoxic effects of lead.

12.4.2 Human Studies

Defining exposure-effect or dose-response relationships between lead and particular neurotoxic responses in humans involves two basic steps. First, there must be an assessment of the internal lead burden resulting from external doses of lead received via various routes of exposure (such as air, water, food, occupational hazards, house dust, etc.). Internal lead burdens may be indexed by lead concentrations in blood, teeth, or other tissue, or by other biological indicators. The second step involves an assessment of the relationship of internal exposure indices to behavioral or other types of neurophysiological responses. The difficulty of this task is reflected by current controversies over existing data. Studies vary greatly in the quality of design, precision of assessment instruments, care in data collection, and appropriateness of statistical analyses employed. Many of these methodological problems are broadly common to research on toxic agents in general and not just to lead alone.

Although epidemiological studies of lead effects have immediate environmental relevance at the human level, difficult problems are often associated with the interpretation of the findings, as noted in several reviews (Bornschein et al., 1980; Cowan and Leviton, 1980; Rutter, 1980; Valciukas and Lilis, 1980; Neddleman and Landrigan, 1981. The main problems are: (1) inadequate markers of exposure to lead; (2) insensitive measures of performance; (3) bias in selection of subjects; (4) inadequate handling of confounding covariates; (5) inappropriate statistical analyses; (6) inappropriate generalization and interpretation of results; and (7) the need for "blind" evaluations by experimenters and technicians. Each of these problems are briefly discussed below.

Each major exposure route--food, water, air, dust, and soil--contributes to a person's total daily intake of lead (see Chapters 7 and 11 of this document). The relative contribution of each exposure route, however, is difficult to ascertain; neurotoxic endpoint measurements, therefore, are most typically evaluated in relation to one or another indicator of overall internal lead body burden. Subjects in epidemiological studies may be misclassified as to exposure level unless careful choices of exposure indices are made based upon the hypotheses to be tested, the accuracy and precision of the biological media assays, and the collection and assay procedures employed. Chapter 9 of this document evaluates different measures of internal exposure to lead and their respective advantages and disadvantages. The most commonly used measure of internal dose is blood lead concentration, which varies as a function of age, sex, race, geographic location, and exposure. The blood lead level is a useful marker of current exposure but generally does not reflect cumulative body lead burdens as well as lead levels in teeth. Hair lead levels, measured in some human studies, are not viewed as reliable indicators of internal body burdens at this time. Future research may identify a more standard exposure index, but it appears that a risk classification similar to

that of the U.S. Centers for Disease Control (1978) in terms of blood lead and FEP levels will continue in the foreseeable future to be the standard approach most often used for lead exposure screening and evaluation. Much of the discussion below is, therefore, focused on defining dose-effect relationships for human neurotoxic effects in terms of blood lead levels; some ancillary information on pertinent teeth lead levels is also discussed.

The frequency and timing of sampling for internal lead burdens represent another important factor in evaluating studies of lead effects on neurological and behavioral functions. For example, epidemiological studies often rely on blood lead and/or erythrocyte protoporphyrin (EP) levels determined at a single point in time to retrospectively estimate or characterize internal exposure histories of study populations that may have been exposed in the past to higher levels of lead than those indicated by a single current blood sample. Relatively few prospective studies exist that provide highly reliable estimates of critical lead exposure levels associated with observed neurotoxic effects in human adults or children, especially in regard to the effects of subencephalopathic lead exposures. Some prospective longitudinal studies on the effects of lead on early development of infants and young children (e.g., Bornschein, 1983) are currently in progress, but the results of these studies are not yet available. The present assessment of the neurotoxic effects of lead in humans must, therefore, rely heavily on published epidemiological studies which typically provide exposure history information of only limited value in defining exposure-effect relationships.

Key variables that have emerged in determining effects of lead on the nervous system include (1) duration and intensity of exposure and (2) age at exposure. Evidence suggest that young organisms with developing nervous systems are more vulnerable than adults with fully matured nervous systems. Particular attention is, therefore, accorded below to discussion of neurotoxic effects of lead in children as a special group at risk.

Precision of measurement is a critical methodological issue, especially when research on neurotoxicity leaves the laboratory setting. Neurotoxicity is often measured indirectly with psychometric or neurometric techniques in epidemiological studies (Valciukas and Lilis, 1980). The accuracy with which these tests reflect what they purport to measure (validity) and the degree to which they are reproducible (reliability) are issues central to the science of measurement theory. Many cross-sectional population studies make use of instruments that are only brief samples of behavior thought to be representative of some relatively constant underlying traits, such as intelligence. Standardization of tests is the subject of much research in psychometrics. The quality and precision of specific test batteries have been particularly controversial issues in evaluating possible effect levels for neurotoxic effects of lead exposure in children. Table 12B (Appendix 12B) lists some of the major tests used, together with their advantages and weaknesses. The following review places most weight on

results obtained with age-normed, standardized psychometric test instruments and well-controlled, standardized nerve conduction velocity (NCV) tests. Other measures, such as reaction time, finger tapping, and certain electrophysiological measures (e.g., cortical evoked and slow-wave potentials) are potentially more sensitive indices, but are still experimental measures whose clinical utility and psychometric properties with respect to the neurobehavioral toxicity of lead remain to be more fully explored.

Selection bias is a critical issue in epidemiological studies in which attempts are made to generalize from a small sample to a large population. Volunteering to participate in a study and attendance at special clinics or schools are common forms of selection bias that often limit how far the results of such studies can be generalized. These factors may need to be balanced in lead neurotoxicity research since reference groups are often difficult to find because of the pervasiveness of lead in the environment and the many non-lead covariates that also affect performance. Selection bias and the effects of confounding can be reduced by choosing a more homogeneous stratified sample, but the generalizability of the results of such cohort studies is thereby limited.

Perhaps the greatest methodological concern in epidemiological studies is controlling for confounding covariates, so that residual effects can be more confidently attributed to lead. Among adults, the most important covariates are age, sex, race, educational level, exposure history, alcohol intake, total food intake, dietary calcium and iron intake, and urban vs. rural styles of living (Valciukas and Lilis, 1980). Among children, a number of developmental covariates are additionally important: parental socioeconomic status (Needleman et al., 1979); maternal IQ (Perino and Ernhart, 1974); pica (Barltrop, 1966); quality of the caregiving environment (Hunt et al., 1982; Milar et al., 1980); dietary iron and calcium intake, vitamin D levels, body fat and nutrition (Mahaffey and Michaelson, 1980; Mahaffey, 1981); and age at exposure. Preschool children below the age of 3-5 years appear to be particularly vulnerable, in that the rate of accumulation of even a low body-lead burden is higher for them than for adults (National Academy of Sciences, Committee on Lead in the Human Environment, 1980). Potential confounding effects of covariates become particularly important when trying to interpret threshold effects of lead exposure. Each covariate alone may not be significant, but, when combined, may interact to pose a cumulative risk which could result in under- or overestimation of a small effect of lead.

Statistical considerations important not only to lead but to all epidemiological studies include adequate sample size (Hill, 1966), the use of multiple comparisons (Cohen and Cohen, 1975), and the use of multivariate analyses (Cooley and Lohnes, 1971). Regarding sample size, false negative conclusions are at times drawn from small studies with low statistical power. It is often difficult and expensive to use large sample sizes in complex research such as that

on lead neurotoxicity. This fact makes it all the more important to use sensitive assessment instruments which have a high level of discriminating power and can be combined into factors for multivariate analysis. Multiple statistical comparisons can then be made while reducing the likelihood of finding a certain number of significant differences by chance alone. This is a serious problem, because near-threshold effects are often small and variable.

A final crucial issue in this and other research revolves around the care taken to assure that investigators are isolated from information that might identify subjects in terms of their lead exposure levels at the time of assessment and data recording. Unconscious biases, nonrandom errors, and arbitrary data correction and exclusion can be ruled out only if a study is performed under blind conditions or, preferably, double-blind conditions.

With the above methodological considerations in mind, the following sections evaluate pertinent human studies, including an overview of lead exposure effects in adults, followed by a more detailed assessment of neurotoxic effects of lead exposures in children.

12.4.2.1 Neurotoxic Effects of Lead Exposures in Adults.

12.4.2.1.1 Overt lead intoxication in adults. Severe neurotoxic effects of extreme exposures to high levels of lead, especially for prolonged periods that produce overt signs of acute lead intoxication, are well documented in regard to both adults and children. The most profound (CNS) effects in adults have been referred to for many years as the clinical syndrome of lead encephalopathy, described in detail by Aub et al. (1926), Cantarow and Trumper (1944), Cumings (1959), and Teisinger and Stýblová (1961). Early features of the syndrome that may develop within weeks of initial exposure include dullness, restlessness, irritability, poor attention span, headaches, muscular tremor, hallucinations, and loss of memory. These symptoms may progress to delirium, mania, convulsions, paralysis, coma, and death. The onset of such symptoms can often be quite abrupt, with convulsions, coma, and even death occurring very rapidly in patients who shortly before appeared to exhibit much less severe or no symptoms of acute lead intoxication (Cumings, 1959; Smith et al., 1938). Symptoms of lead encephalopathy indicative of severe CNS damage and posing a threat to life are generally not seen in adults except at blood lead levels well in excess of 120 μg/dl (Kehoe, 1961a,b,c). Other data (Smith et al., 1938) suggest that acute lead intoxication, including severe gastrointestinal symptoms and/or signs of encephalopathy can occur in some adults at blood lead levels around 100 μg/dl, but ambiguities make this data difficult to interpret.

In addition to the above CNS effects, lead also clearly damages peripheral nerves at toxic, high exposure levels that predominantly affect large myelinated nerve fibers (Vasilescu, 1973; Feldman et al., 1977; Englert, 1980). Pathologic changes in peripheral nerves, as shown in animal studies, can include both segmental demyelination and, in some fibers, axonal degeneration (Fullerton, 1966). The former types of changes appear to reflect lead effects on

Schwann cells, with concomitant endoneurial edema and disruption of myelin membranes (Windebank and Dyck, 1981). Apparently lead induces a breakdown in the blood-nerve barrier which allows lead-rich edema fluid to enter the endoneurium (Dyck et al., 1980; Windebank et al., 1980). Remyelination observed in animal studies suggests either that such lead effects may be reversible or that not all Schwann cells are affected equally (Lampert and Schochet, 1968; Ohnishi and Dyck, 1981). Reports of plantar arch deformities due to old peripheral neuropathies (Emmerson, 1968), however, suggest that lead-induced neuropathies of sufficient severity in human adults could result in permanent peripheral nerve damage. Morphologically, peripheral neuropathies are usually detectable only after prolonged high exposure to lead, with distinctly different sensitivities and histological differences existing among mammalian species. In regard to man, as an example, Buchthal and Behse (1979, 1981), using nerve biopsies from a worker with frank lead neuropathy (blood lead = 150 μg/dl), found histological changes indicative of axonal degeneration in association in NCV reductions that corresponded to loss of large fibers and decreased amplitude of sensory potentials.

Data from certain studies provide a basis by which to estimate lead exposure levels at which adults exhibit overt signs or symptoms of neurotoxicity and to compare such levels with those associated with other types of signs and symptoms indicative of overt lead intoxication (Lilis et al., 1977; Irwig et al., 1978; Dahlgren et al., 1978; Baker et al., 1979; Hänninen et al., 1979; Spivey et al., 1979; Fischbein et al., 1980; Hammond et al., 1980). These studies evaluated the incidence of various clinical signs and symptoms of lead intoxication across a wide range of lead exposures among occupationally exposed smelter and battery plant workers. The reported incidences of particular types of signs and symptoms, both neurological and otherwise, and associated lead exposure levels varied considerably from study to study, but they collectively provide evidence indicating that overt neurological, gastrointestinal, and other lead-related symptoms can occur among adults starting at blood lead levels as low as 40-60 μg/dl. Considerable individual biological variability is apparent, however, among various study populations and individual workers in terms of observed lead levels associated with overt signs and symptoms of lead intoxication, based on comparisons of exposure-effect and dose-response data from the above studies. Irwig et al (1978), for example, report data for black South African lead workers indicative of clearly increased prevalence of both neurological and gastrointestinal symptoms at blood lead levels over 80 μg/dl. Analogously, Hammond et al. (1980) reported significant increases in neurological (both CNS and PNS) and gastrointestinal symptoms among American smelter workers with blood lead levels often exceeding 80 μg/dl, but not among workers whose exposure histories did not include levels above 80 μg/dl--findings in contrast to the results of several other studies. Lilis et al. (1977), for instance, found that CNS symptoms (tiredness, sleeplessness, irritability, headaches) were

reported by 55 percent and muscle or joint pain by 39 percent of a group of lead smelter workers whose blood lead levels had never been found to exceed 80 μg/dl. Low hemoglobin levels (<14g/dl) were found in more than 33 percent of these workers. Also, Spivey et al. (1977) reported significantly increased neurological (mainly CNS, but some PNS) symptoms and joint pain among a group of 69 lead workers with mean ± standard deviation blood lead levels of 61.3 ± 12.8 μg/dl in comparison to a control group with 22.0 ± 5.9 μg/dl blood lead values. Hänninen et al. (1979) similarly reported finding significantly increased neurological (both CNS and PNS) and gastrointestinal symptoms among 25 lead workers with maximum observed blood lead levels of 50-69 μg/dl and significantly increased CNS symptoms among 20 lower exposure workers with maximum blood lead values below 50 μg/dl, compared in each case against a referent control group (N = 23) with blood lead values of 11.9 ± 4.3 μg/dl (mean ± standard deviation).

Additional studies provide evidence of overt signs or symptoms of neurotoxicity occurring at still lower lead exposure levels than those indicated above. Baker et al. (1979) studied dose-response relationships between clinical signs and symptoms of lead intoxication among lead workers in two smelters. No toxicity was observed at blood lead levels below 40 μg/dl. However, 13 percent of those workers with blood lead values in the range 40-79 μg/dl had extensor muscle weakness or gastrointestinal symptoms; and anemia occurred in 5 percent of the workers with 40-59 μg/dl blood lead levels, in 14 percent with levels of 60-79 μg/dl, and in 36 percent with blood lead levels exceeding 80 μg/dl. Also, Fischbein et al. (1980), in a study of 90 cable splicers intermittently exposed to lead, found higher zinc protoporphyrin levels (an indicator of impaired heme synthesis associated with lead exposure) among workers reporting CNS or gastrointestinal symptoms than among other cable splicers not reporting such symptoms. Only 5 percent of these workers had blood lead levels in excess of 40 μg/dl, and the mean ± standard deviation blood lead levels for the 26 reporting CNS symptoms were 28.4 ±7.6 μg/dl and 30 ±9.4 μg/dl for the 19 reporting gastrointestinal symptoms. Caution must be exercised in accepting these blood levels as being representative of average or maximum lead exposures of this worker population, however, in view of the highly intermittent nature of their exposure and probable much higher resulting peaks in their blood lead levels than those coincidentally measured at the time of their blood sampling.

Overall, the above results appear to support the following conclusions: (1) overt signs and symptoms of neurotoxicity in adults are manifested at roughly comparable lead exposure levels as other types of overt signs and symptoms of lead intoxication, such as gastrointestinal complaints; (2) the neurological signs and symptoms are indicative of both central and peripheral nervous system effects; (3) such overt signs and symptoms, both neurological and otherwise, occur at markedly lower blood lead levels than the 60 or 80 μg/dl criteria levels

previously established or recently discussed as being "safe" for occupationally exposed adults; and (4) lowest observed effect levels for the neurological signs and symptoms can most credibly be stated to be in the 40 to 60 μg/dl range. Insufficient information exists presently by which to estimate with confidence to what extent or for how long such overt signs and symptoms persist in adults after termination of precipitating external lead exposures, but at least one study (Dahlgren, 1978) reports evidence of abdominal pain persisting for as long as 29 months after exposure termination among 15 smelter workers, including four whose blood lead levels were between 40 and 60 μg/dl while working.

12.4.2.1.2 Non-Overt lead intoxication in adults. Of special importance for establishing standards for exposure to lead is the question of whether exposures lower than those producing overt signs or symptoms of lead intoxication result in less obvious neurotoxic effects in otherwise apparently healthy individuals. Attention has focused in particular on whether exposures leading to blood lead levels below 80-100 μg/dl may lead to behavioral deficits or other neurotoxic effects in the absence of classical signs of overt lead intoxication.

In adults, if such neurobehavioral deficits occurred with great frequency, one might expect this to be reflected by performance measures in the workplace, such as higher rates of absences or reduced psychomotor performances among occupationally exposed lead workers. Some epidemiological studies have investigated possible relationships between elevated blood lead and general health as indexed by records of sick absences certified by physicians (Araki et al., 1982; Robinson, 1976; Shannon et al., 1976; Tola and Nordman, 1977). However, sickness absence rates are generally poor epidemiologic outcome measures that may be confounded by many variables and are difficult to relate specifically to lead exposure levels. Much more useful are studies discussed below which evaluate lead exposures in relation to direct measurements of CNS or peripheral neurological functions.

Only a few studies have employed sensitive psychometric and/or neurological testing procedures in an effort to demonstrate specific lead-induced neurobehavioral effects in adults. For example, Morgan and Repko (1974) reported deficits in hand-eye coordination and reaction time in an extensive study of behavioral functions in 190 lead-exposed workers (mean blood lead level = 60.5 ± 17.0 μg/dl). The majority of the subjects were exposed between 5 and 20 years. In a similar study, Milburn et al. (1976) found no differences between control and lead-exposed workers on numerous psychometric and other performance tests. On the other hand, several recent studies (Arnvig et al., 1980; Grandjean et al., 1978; Hänninen et al., 1978; Mantere et al., 1982; Valciukas et al., 1978) have found disturbances in visual motor performance, IQ test performance, hand dexterity, mood, nervousness, and coping in lead workers with blood lead levels of 50-80 μg/dl. A graded dose-effect relationship for non-overt CNS lead effects in otherwise apparently asymptomatic adults is indicated by such studies.

In addition to the above studies indicative of CNS dysfunctions in non-overtly lead intoxicated adults, numerous investigations have provided electrophysiological data indicating that peripheral nerve dysfunction in apparently asymptomatic adults can be associated with blood lead values below 80 μg/dl. Such peripheral nerve deficits, i.e. slowed nerve conduction veolocity (NCV), were established by Seppäläinen et al. (1975) for lead workers whose blood lead levels were as low as 50 μg/dl and had never exceeded 70 μg/dl during their entire exposure period (mean = 4.6 years), as determined by regular monitoring. Similar results were obtained in a study by Melgaard et al. (1976) on automobile mechanics exposed to TEL and other lead compounds in lubricating and high-pressure oils. Results of an analysis of the workers' blood for lead, chromium, copper, nickel, and manganese indicated a clear association between lead exposure and peripheral nerve dysfunction. Half of the workers (10 to 20) had elevated blood lead levels (60-120 μg/dl) and showed definite electromyographic deficits. The mean blood lead level for the control group was 18.6 μg/dl. Melgaard et al. (1976) reported additional results on associating lead exposures with polyneuropathy of unknown etiology in 10 cases from the general population. Another study reported by Araki and Honma (1976) provided further confirmation of the Seppäläinen et al. (1975) and Melgaard et al. (1976) findings in that evidence for peripheral neuropathy effects were reported for lead industry workers with blood lead values of 29 to 70 μg/dl.

More recent studies by Araki et al (1980), Ashby (1980), Bordo et al. (1982), Johnson et al. (1980), Seppäläinen et al. (1979), and Seppäläinen and Hernberg (1980, 1982) have confirmed a dose-dependent slowing of NCV in lead workers with blood lead levels below 70 to 80 μg/dl. Seppäläinen et al. (1979) observed NCV slowing in workers with blood lead levels across a range of 29 to 70 μg/dl (Figure 12-2); and Seppäläinen and Hernberg (1980, 1982) found NCV slowing in workers with maximum blood lead levels of 30 to 48 μg/dl, but not among workers with levels below 30 μg/dl. Buchthal and Behse (1979), Lilis et al. (1977), and Paulev et al. (1979), in contrast, found no signs of neuropathy below 80 μg/dl. Reports of low blood lead levels (below 50 μg/dl) in some of the above studies should be viewed with caution until further confirmatory data are reported for larger samples using well verified blood assay results. Nonetheless, these studies are consistent with a continuous dose-response relationship between blood lead concentration and extent and degree of peripheral nerve dysfunction in non-overtly lead intoxicated adults.

The above studies on nerve conduction velocity provide convergent evidence for peripheral nerve dysfunctions occurring in adults with blood lead levels in the 30-70 μg/dl range but not exhibiting overt signs of lead intoxication. Furthermore, although it might be argued that peak levels of lead may have been significant and that substantially higher lead body burdens existing before the time of some of the studies were actually responsible for producing the dysfunctions, it appears that in several cases (Seppäläinen et al., 1975; Seppäläinen and

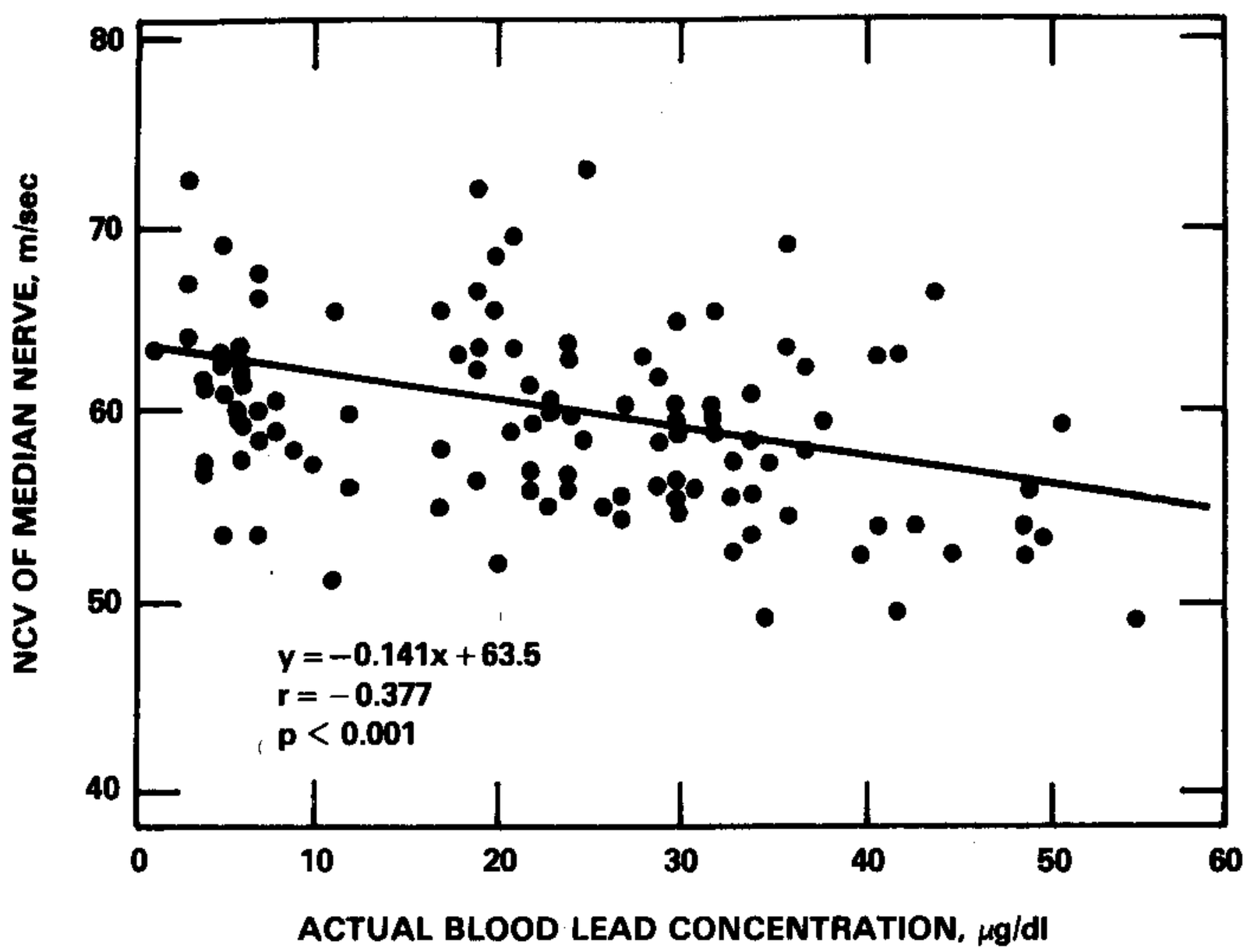

Figure 12-2. Maximal motor nerve conduction velocity (NCV) of the median nerve plotted against the actual blood lead level for 78 workers occupationally exposed to lead and for 34 control subjects.

Source: Seppäläinen et al. (1979).

Hernberg, 1980) blood levels that had never exceeded 70 µg/dl were related to increased peripheral nerve dysfunction; and, in the Seppäläinen and Hernberg (1982) study, NCV slowing was associated with maximum levels of 30-48 µg/dl. The studies by Seppäläinen and her co-workers are generally methodologically sound, having been well controlled for the possible effects of extraneous factors such as history, length, and type of exposure, multiple assessments of different nerves, temperature differences at the NCV assessment sites, plus relevant confounding covariates. Thus, when the Seppäläinen et al. (1975) results are viewed collectively with the data from other studies reviewed here, substantial evidence can be stated to exist for peripheral nerve dysfunctions occurring in adults at blood lead levels of as low as 30 to 50 µg/dl. The question as to whether these reflect mild, reversible effects (Buchthal and Behse, 1981) or are true early warning signals of progressively more serious peripheral neuropathies important in the diagnosis of otherwise unrecognized toxic effects of lead (Feldman et al., 1977; Seppäläinen and Hernberg, 1980) is still a matter of some dispute. Nevertheless, it is clear that these effects represent departures from normal neurologic functioning and their potential relationship to other extremely serious effects (see, for example, the next paragraph) argues for prudence in interpreting their potential health significance.

There are several reports of previous overexposure to heavy metals in amyotrophic lateral sclerosis (ALS) patients and patients dying of motor neuron disease (MND). Conradi et al. (1976, 1978a,b, 1980) found elevated cerebrospinal fluid lead levels in ALS patients as compared with controls. Thus, the possible pathogenic significance of lead in ALS needs to be further explored. In addition, Kurlander and Patten (1978) found that lead levels in spinal cord anterior horn cells of MND patients were nearly three times that of control subjects and that lead levels correlated with illness durations. Despite chelation therapy for about a year, high lead levels remained in their tissue.

12.4.2.2 Neurotoxic Effects of Lead Exposure in Children.

12.4.2.2.1 Overt lead intoxication in children. Symptoms of encephalopathy similar to those that occur in adults have been reported to occur in infants and young children (Prendergast, 1910; Oliver and Vogt, 1911; Blackfan, 1917; McKahann and Vogt, 1926; Giannattasio et al., 1952; Cumings, 1959; Tepper, 1963; Chisolm, 1968), with a markedly higher incidence of severe encephalopathic symptoms and deaths occurring among them than in adults. This may reflect the greater difficulty in recognizing early symptoms in young children, thereby allowing intoxication to proceed to a more severe level before treatment is initiated (Lin-Fu, 1973). In regard to the risk of death in children, the mortality rate for encephalopathy cases was approximately 65 percent prior to the introduction of chelation therapy as standard medical practice (Greengard et al., 1965; National Academy of Sciences, 1972; Niklowitz, 1975; Niklowitz and Mandybur, 1975). The following mortality rates have been reported for children

experiencing lead encephalopathy since the inception of chelation therapy as the standard treatment approach: 39 percent (Ennis and Harrison, 1950); 20 to 30 percent (Agerty, 1952); 24 percent (Mellins and Jenkins, 1955); 18 percent (Tanis, 1955); and 5 percent (Lewis et al., 1955). These data, and those tabulated more recently (National Academy of Sciences, 1972), indicate that once lead poisoning has progressed to the point of encephalopathy, a life-threatening situation clearly exists and, even with medical intervention, is apt to result in a fatal outcome. Historically there have been three stages of chelation therapy. Between 1946 and 1950, dimercaprol (BAL) was used. From 1950 to 1960, calcium disodium ethylenediaminetetraacetate (CaEDTA) completely replaced BAL. Beginning in 1960, combined therapy with BAL and CaEDTA (Chisolm, 1968) resulted in a very substantial reduction in mortality.

Determining precise values for lead exposures necessary to produce acute symptoms, such as lethargy, vomiting, irritability, loss of appetite, dizziness, etc., or later neurotoxic sequelae in humans is difficult in view of the usual sparsity of data on environmental lead exposure levels, period(s) of exposure, or body burdens of lead existing prior to manifestation of symptoms. Nevertheless, enough information is available to permit reasonable estimates to be made regarding the range of blood lead levels associated with acute encephalopathic symptoms or death. Available data indicate that lower blood lead levels among children than among adults are associated with acute encephalopathy symptoms. The most extensive compilation of information on a pediatric population is a summarization (National Academy of Sciences, 1972) of data from Chisolm (1962, 1965) and Chisolm and Harrison (1956). This data compilation relates occurrence of acute encephalopathy and death in children in Baltimore to blood lead levels determined by the Baltimore City Health Department (using the dithizone method) between 1930 and 1970. Blood lead levels formerly regarded as "asymptomatic" and other signs of acute lead poisoning were also tabulated. Increased lead absorption in the absence of detected symptoms was observed at blood lead levels ranging from 60 to 300 μg/dl (mean = 105 μg/dl). Acute lead poisoning symptoms other than signs of encephalopathy were observed from approximately 60 to 450 μg/dl (mean = 178 μg/dl). Signs of encephalopathy (hyperirritability, ataxia, convulsions, stupor, and coma) were associated with blood lead levels of approximately 90 to 700 or 800 μg/dl (mean = 330 μg/dl). The distribution of blood lead levels associated with death (mean = 327 μg/dl) was essentially the same as for levels yielding encephalopathy. These data suggest that blood lead levels capable of producing death in children are essentially identical to those associated with acute encephalopathy and that such effects are usually manifested in children starting at blood lead levels of approximately 100 μg/dl. Certain other evidence from scattered medical reports (Gant, 1938; Smith et al., 1938; Bradley et al., 1956; Bradley and Baumgartner, 1958; Cumings, 1959; Rummo et al., 1979), however, suggests that acute encephalopathy in the most highly susceptible children may be associated with blood lead levels in the range of 80-100 μg/dl. These latter reports are evaluated in detail in the 1977 EPA document Air Quality Criteria for Lead (U.S. EPA, 1977).

From the preceding discussion, it can be seen that severity of symptoms varies widely for different adults or children as a function of increasing blood lead levels. Some show irreversible CNS damage or death at blood lead levels around 100 μg/dl, whereas others may not show any of the usual clinical signs of lead intoxication even at blood lead levels in the 100 to 200 μg/dl or higher range. This diversity of response may be due to: (1) individual biological variation in lead uptake or susceptibility to lead effects; (2) changes in blood lead values from the time of initial damaging intoxication; (3) greater tolerance for a gradually accumulating lead burden; (4) other interacting or confounding factors, such as nutritional state or inaccurate determinations of blood lead; or (5) lack of use of blind evaluation procedures on the part of the evaluators. It should also be noted that a continuous gradation of frequency and severity of neurotoxic symptoms extends into the lower ranges of lead exposure.

Morphological findings vary in cases of fatal lead encephalopathy among children (Blackman, 1937; Pentschew, 1965; Popoff et al., 1963). Reported neuropathologic findings are essentially the same for adults and children. On macroscopic examination the brains are often edematous and congested. Microscopically, cerebral edema, altered capillaries (endothelial hypertrophy and hyperplasia), and perivascular glial proliferation often occur. Neuronal damage is variable and may be caused by anoxia. However, in some cases gross and microscopic changes are minimal (Pentschew, 1965). Pentschew (1965) described neuropathology findings for 20 cases of acute lead encephalopathy in infants and young children. The most common finding was activation of intracerebral capillaries characterized by dilation of the capillaries, with swelling of endothelial cells. Diffuse astrocytic proliferation, an early morphological response to increased permeability of the blood-brain barrier, was often present. Concurrent with such alterations, especially evident in the cerebellum, were changes that Pentschew (1965) attributed to hemodynamic disorders, i.e., ischemic changes manifested as cell necrosis, perineuronal incrustations, and loss of neurons, especially in isocortex and basal ganglia.

Attempts have been made to understand better brain changes associated with encephalopathy by studying animal models. Studies of lead intoxication in the CNS of developing rats have shown vasculopathic changes (Pentschew and Garro, 1966), reduced cerebral cortical thickness and reduced number of synapses per neuron (Krigman et al., 1974a), and reduced cerebral axonal size (Krigman et al., 1974b). Biochemical changes in the CNS of lead-treated neonatal rats have also demonstrated reduced lipid brain content but no alterations of neural lipid composition (Krigman et al., 1974a) and a reduced cerebellar DNA content (Michaelson, 1973). In cases of lower level lead exposure, subjectively recognizable neuropathologic features may not occur (Krigman, 1978). Instead there may be subtle changes at the level of the synapse (Silbergeld et al., 1980a) or dendritic field, myelin-axon relations, and organization of

synaptic patterns (Krigman, 1978). Since the nervous system is a dynamic structure rather than a static one, it undergoes compensatory changes (Norton and Culver, 1977), maturation and aging (Sotelo and Palay, 1971), and structural changes in response to environmental stimuli (Coss and Glohus, 1978). Thus, whereas massive structural damage in many cases of acute encephalopathy would be expected to lead to lasting neurotoxic sequalae, some other CNS effects due to severe early lead insult might be reversible or compensated for, depending upon age and duration of toxic exposure. This raises the question of whether effects of early overt lead intoxication are reversible beyond the initial intoxication or continue to persist.

In cases of severe or prolonged nonfatal episodes of lead encephalopathy, there occur neurological sequelae qualitatively similar to those often seen following traumatic or infectious cerebral injury, with permanent sequelae being more common in children than in adults (Mellins and Jenkins, 1955; Chisolm, 1962, 1968). The most severe sequelae in children are cortical atrophy, hydrocephalus, convulsive seizures, and severe mental retardation (Mellins and Jenkins, 1955; Perlstein and Attala, 1966; Chisolm, 1968). Children who recover from acute lead encephalopathy but are re-exposed to lead almost invariably show evidence of permanent central nervous system damage (Chisolm and Harrison, 1956). Even if further lead exposure is minimized, 25 to 50 percent show severe permanent sequelae, such as seizure disorders, blindness, and hemiparesis (Chisolm and Barltrop, 1979).

Lasting neurotoxic sequelae of overt lead intoxication in children in the absence of acute encephalopathy have also been reported. Byers and Lord (1943), for example, reported that 19 out of 20 children with previous lead poisoning later made unsatisfactory progress in school, presumably due to sensorimotor deficits, short attention span, and behavioral disorders. These latter types of effects have since been confirmed in children with known high exposures to lead, but without a history of life-threatening forms of acute encephalopathy (Chisolm and Harrison, 1956; Cohen and Ahrens, 1959; Kline, 1960). Perlstein and Attala (1966) also reported neurological sequelae in 140 of 386 children (37 percent) following lead poisoning without encephalopathy. Such sequelae included mental retardation, seizures, cerebral palsy, optic atrophy, and visual-perceptual problems in some children with minimal intellectual impairment. The severity of sequelae was related to severity of earlier observed symptoms. For 9 percent of those children who appeared to be without severe symptoms at the time of diagnosis of overt lead poisoning, mental retardation was observed upon later follow-up. The conclusion of the neurological effects observed by Perlstein and Attala (1966) being persisting effects of earlier overt lead intoxication without encephalopathy might be questioned in view of no control group having been included in the study; however, it is extremely unlikely that 37 percent of any randomly selected control group from the general pediatric population would exhibit the types of neurological problems observed in that proportion of the cohort of children with earlier lead intoxication studied by Perlstein and Attala (1966).

Numerous studies (Cohen et al., 1976; Fejerman et al., 1973; Pueschel et al., 1972; Sachs et al., 1978, 1979, 1982) suggest that, in the absence of encephalopathy, chelation therapy may ameliorate the persistence of neurotoxic effects of overt lead poisoning (especially cognitive, perceptual, and behavioral deficits). On the other hand, one recent study found a residual effect on fine motor performance even after chelation (Kirkconnell and Hicks, 1980).

In summary, pertinent literature definitively demonstrates that lead poisoning with encephalopathy results in a greatly increased incidence of permanent neurological and cognitive impairments. Also, several studies further indicate that children with symptomatic lead poisoning in the absence of encephalopathy also show a later increased incidence of neurological and behavioral impairments.

12.4.2.2.2 Non-Overt lead intoxication in children

In addition to neurotoxic effects associated with overt lead intoxication in children, growing evidence indicates that lead exposures not leading to overt lead intoxication in children can induce neurological dysfunctions. This issue has attracted much attention and generated considerable controversy during the past 10 to 15 years. However, the evidence for and against the occurrence of significant neurotoxic deficits at relatively low levels of lead exposure is quite mixed and largely interpretable only after a thorough critical evaluation of methods employed in the various important studies on the subject. Based on the five criteria listed earlier (i.e., adequate markers of exposure to lead, sensitive measures, appropriate subject selection, control of confounding covariates, and appropriate statistical analysis), the 20 population studies summarized in Table 12-1 were conducted rigorously enough to warrant at least some consideration here. Even so, no epidemiological study is completely flawless and, therefore, overall interpretation of such findings must be based on evaluation of: (1) the internal consistency and quality of each study; (2) the consistency of results obtained across independently conducted studies; and (3) the plausibility of results in view of other available information.

Rutter (1980) has classified studies evaluating neurobehavioral effects of lead exposure in non-overtly lead intoxicated children according to several types, including four categories reviewed below: (1) clinic-type studies of children thought to be at risk because of high lead levels; (2) other studies of children drawn from general (typically urban or suburban) pediatric populations; (3) samples of children living more specifically in close proximity to lead emitting smelters; and (4) studies of mentally retarded or behaviorally deviant children. Major attention is accorded here to studies falling under the first three categories. As will be seen, quite mixed results have emerged from the studies reviewed.

12.4.2.2.2.1 Clinic-type studies of children with high lead levels. The clinic-type studies are typified by evaluation of children with relatively high lead body burdens as identified through lead screening programs or other large-scale programs focussing on mother-infant health relationships and early childhood development.

TABLE 12-1. SUMMARY OF PUBLISHED RESULTS FROM STUDIES OF LEAD EFFECTS ON NEUROBEHAVIORAL FUNCTIONS OF NON-OVERTLY LEAD INTOXICATED CHILDREN

Reference	Population studied	N/group	Age at testing, yr	Blood lead, μg/dl	Psychometric tests employed	Summary of results (C=control; Pb=lead)[a]	Levels of significance[b]
Clinic-type Studies of Children with High Lead Levels							
De la Burde and Choate (1972)	Inner city (Richmond, VA)	Control = 72 Lead = 70	4 4	Not assayed[c] 40-100[d]	Stanford-Binet IQ Other measures	C = 94 Pb = 89 C > Pb on 3/4 tests	p <0.05 N.S.-p <0.01
De la Burde and Choate (1975)	Follow-up same subjects	Control = 67 Lead = 70	7 7	See above[e] See above[e]	WISC Full Scale IQ Neurologic exam Other measures	C = 90 Pb = 87 C better than Pb C > Pb on 9/10 tests	p <0.01 p <0.01 N.S.-p <0.001
Rummo et al. (1974, 1979)	Inner city (Providence, RI)	Control Ss = 45	4-8 ($\bar{x}$ = 5.8)	$\bar{x}$ = 23 ± 8	McCarthy General Cognitive	C = 93; S = 94; L = 88; P = 77	N.S.-p <0.01 (P vs C)
		Short Pb Ss = 15	4-8 ($\bar{x}$ = 5.6)	$\bar{x}$ = 61 ± 7	McCarthy Subscales	C+S > L > P on 5/5 tests	N.S.-p <0.01 (P vs C)
		Long Pb Ss = 20	4-8 ($\bar{x}$ = 5.6)	$\bar{x}$ = 68 ± 13	Neurologic exam rating	C+S > L > P on ratings	N.S.
		Post enceph Pb = 10	4-8 ($\bar{x}$ = 5.3)	$\bar{x}$ = 88 ± 40	Objective neurologic tests	C+S > L > P on 3/12 tests	N.S.-p <0.01 (P vs C)
Kotok (1972)	Inner city (New Haven, CT)	Control = 25 Lead = 24	1.1 -5 .5 ($\bar{x}$ = 2.7) 1.0 - 5.8 (x = 2.8)	20-55 58-137	Denver Developmental Scale	C > Pb on 1/3 Subscales	N.S.
Kotok et al. (1977)	Inner city (Rochester, NY)	Control = 36 Lead = 31	1.9 - 5.6 ($\bar{x}$ = 3.6) 1.7 - 5.4 (- = 3.6)	11-40 61-200	IQ Equivalent for six ability classes: Social maturity; Spatial relations; Spoken vocab; Info. comprehension; Visual attention; Auditory memory	IQ Equivalent for each: C = 126 Pb = 124 C = 101 Pb = 92; C = 93 Pb = 92; C = 96 Pb = 95; C = 93 Pb = 90 C = 100 Pb = 93	p <0.10 for spatial p >0.10 for all other ability classes
Perino and Ernhart (1974)	Inner city (New York, NY)	Control = 50	3-6	10-30	McCarthy General Cognitive	C = 90 Pb = 80	p <0.01
		Lead = 30	3-6	40-70	McCarthy Subscales	C > Pb on 5/5 scales	N.S.-p <0.01
Ernhart et al. (1981)	Follow-up same subjects	Control = 31 Lead = 32	8-13	21.3±3.7[i] 32.4±5.3	McCarthy General Cognitive Index	Shared Variance = 7.7	p <0.05
					McCarthy Subscales	(2/5) 8.0, 7.4	p <0.05
					Reading Tests	1.3	N.S.
					Exploratory Tests (Bender Gestalt, Draw-A-Child)	?	N.S.
					Conners Teachers Rating Scale	?	N.S.

PRELIMINARY DRAFT

TABLE 12-1. (continued)

Reference	Population studied	N/group	Age at testing, yr	Blood lead, µg/dl	Psychometric tests employed	Summary of results (C=control; Pb=lead)[a]	Levels of significance[b]
General Population Studies							
Needleman et al. (1979)	General population (Boston, MA area)	Control = 100	7	PbT < 10 ppm[h]	WISC Full scale IQ	C = 106.6 Pb = 102.1	p < 0.03
		Lead = 58	7	PbT > 20 ppm	WISC Verbal IQ	C = 103.9 Pb = 99.3	p <0.03
					WISC Performance IQ	C = 108.7 Pb = 104.9	N.S.
					Seashore Rhythm Test	C = 21.6 Pb = 19.4	p <0.002
					Token Test	C = 24.8 Pb = 23.6	N.S.
					Sentence Repetition Test	C = 12.6 Pb = 11.3	p <0.04
					Delayed Reaction Time	C > Pb on 3/4 blocks	p <0.01
					Teacher's Behavior Rating	C = 9.5 Pb = 8.2	p <0.02
McBride et al. (1982)	Urban and suburban (Sydney, Australia)	Moderate = C.100	4,5	19-30 µg/dl	Peabody Picture Voc. Test	C = 105 Pb = 104	N.S.
		Low = C.100	4,5	0.5-9 µg/dl	Fine Motor Tracking	C > Pb 1/4 comparisons	p <0.05
					Pegboard	C = 20 Pb = 20	N.S.
					Tapping Test	C = 30 Pb = 31	N.S.
					Beam Walk	C = 5 Pb = 4	N.S.
					Standing Balance	C > Pb 1/4 comparisons	p <0.05
					Rutter Activity Scale	C = 1.9 Pb - 2.1	N.S.
Yule et al. (1981)	Urban (London, England)	Group 1 = 20	9	8.8[j]	WISC-R Full Scale IQ	Gp1 < Gp2 > Gp3 > Gp4	p <0.029
		Group 2 = 29	9	11.6	Verbal IQ	Gp1 < Gp2 > Gp3 > Gp4	p <0.04
		Group 3 = 29	8	14.5	Performance IQ	Gp1 < Gp2 > Gp3 > Gp4	N.S.
		Group 4 = 21	8	19.6	Vernon Spelling Test[k]	Gp1 > Gp2 > Gp3 > Gp4	p <0.001
					Vernon Arithmetic Test[k]	Gp1 < Gp2 > Gp3 > Gp4	N.S.
					Neale Reading Test[k]	Gp1 < Gp2 > Gp3 > Gp4	p <0.001
Yule et al. (1983)	Same subjects	Same	Same	Same	Needleman Teacher's Behavior Ratings	Linear Trend 3/4 items	p <0.05
					Conners Teachers Questionnaire Factors 1,2,4,5	Gp1 < Gps2-4	p <0.05
					Rutter Teacher Rating Scale for Activity	Linear Trend 25/36 items	N.S.

PRELIMINARY DRAFT

Table 12-1. (continued)

Reference	Population studied	N/group	Age at testing, yr	Blood lead, µg/dl	Psychometric tests employed	Summary of results (C=control; Pb=lead)[a]			Levels of significance[b]
						HIGH	MED	LOW	
Smith et al. (1983)	Urban (London, UK)	Hi = 155	6,7	PbT ≥ 8.0	WISC-R Full Scale	105	105	107	N.S.
		Med = 103	6,7	PbT = 5-5.5	Verbal IQ	103	103	105	N.S.
		Low = 145	6,7	PbT < 2.5	Performance IQ	106	106	108	N.S.
				(All in µg/g)					
				$\bar{x}$ PbB = 13.1 µg/dl	Word Reading Test	40	42	45	N.S.
					Seashore Rhythm Test	20	20	21	N.S.
					Visual Sequential Memory	20	19	20	N.S.
					Sentence Memory	9	9	9	N.S.
					Shape Copying	14	14	14	N.S.
					Mathematics	15	15	16	N.S.
					Mean Visual RT (secs)	.39	.37	.37	N.S.
					Conners Teachers Ratings	13	11	11	N.S.
						Low		Hi	
Yule and Lansdown (1983)	Urban (London, UK)	80	9	7-12	WISC-R Full Scale	107		105	N.S.
		82	9	13-24	Verbal IQ	104		103	N.S.
					Performance IQ	108		106	N.S.
					Neale Reading Acc.	114		111	N.S.
					Neale Reading Comp.	113		109	N.S.
					Vernon Spelling	101		99	N.S.
					Vernon Math	100		99	N.S.
Harvey et al. (1983)	Urban (Birmingham, UK)	189	2.5	15.5	British Ability Scales	Regression F Ratio			N.S.
					Naming		<1		N.S.
					Recall		1.26		N.S.
					Comprehension		<1		N.S.
					Recognition		<1		N.S.
					IQ		<1		N.S.
					Stanford-Binet Items				
					Shapes		<1		N.S.
					Blocks		2.34		N.S.
					Beads		2.46		N.S.
					Playroom Activity		?		N.S.

PRELIMINARY DRAFT

Table 12-1. (continued)

Reference	Population studied	N/group	Age at testing, yr	Blood lead, µg/dl	Psychometric tests employed	Summary of results (C=control; Pb=lead)[a]	Levels of significance[b]
Smelter Area Studies							
Landrigan et al. (1975)	Smelter area (El Paso, TX)	Control = 46 Lead = 78	3-15 ($\bar{x}$ = 9.3) 3-15 (x = 8.3)	<40 40-68	WISC Full Scale IQ[f]	C = 93 Pb = 87	N.S.
					WPPSI Full Scale IQ[g]	C = 91 Pb = 86	N.S.
					WISC + WPPSI Combined	C = 93 Pb = 88	p <0.01
					WISC + WPPSI Subscales	C > Pb on 13/14 scales	N.S.-p <0.01
					Neurologic testing	C > Pb on 4/4 tests	N.S.-p <0.001
McNeil and Ptasnik (1975)	Smelter area (El Paso, TX)	Control = 61-152 Lead = 23-161	1.5 - 18(Mdn = 9) 1.5 - 18(Mdn = 9)	<40 >40	McCarthy General Cognitive	C = 82 Pb = 81	N.S.
					WISC-WAIS Full Scale IQ	C = 89 Pb = 87	N.S.
					Oseretsky Motor Level	C = 101 Pb = 97	N.S.
					California Personality	C > Pb, 6/10 items	p <0.05
					Frostig Perceptual Quotient	C = 100 Pb = 103	N.S.
					Finger-Thumb Apposition	C = 27 Pb = 29	N.S.
Ratcliffe (1977)	Smelter area Manchester, Eng.	Control = 23 Lead = 24	4-7 4-8	28.2 44.4	Griffiths Mental Dev.	C = 101-111 Pb = 97-107	N.S.
					Frostig Visual Perception	C = 14.3 Pb = 11.8	N.S.
					Pegboard Test	C = 17.5 Pb = 17.3	N.S.
						C = 19.5 Pb = 19.8	N.S.
Winneke et al. (1982a)	Smelter area (Duisburg, FRG)	Control = 26 Lead = 26	8 8	PbT = 2.4 ppm[h] PbT = 9.2 ppm No PbB	German WISC Full Scale	C = 122 Pb = 117	N.S.
					Verbal IQ	C = 130 Pb = 124	N.S.
					Performance IQ	C = 130 Pb = 123	N.S.
					Bender Gestalt Test	C = 17.2 Pb = 19.6	p <0.05
					Standard Neurological Tests	C = 2.7 Pb = 7.2	N.S.
					Conners Teachers Rating Scale	C = ? Pb = ?	N.S.
Winneke et al. (1982b)	Smelter area (Stolburg, FRG)	89	9.4	PbT = 6.16 ppm[h] PbB = 14.3 µg/dl	German WISC Full Scale IQ	Prop. of Variance=-0.0	N.S.
					Verbal 0	-0.5	N.S.
					Performance IQ	+0.6	N.S.
					Bender Gestalt Test	+2.1	p <0.05
					Standard Neurological Tests	+1.2	N.S.
					Conners Teachers Rating Scale	0.4-1.3	N.S.
					Wiener Reaction Performance	+2.0	N.S.

[a]Mean test scores for control children indicated by C = $\bar{x}$; mean scores for respective lead-exposed groups indicated by Pb = $\bar{x}$, except for Rummo (1979) study where C = control, S = short-term lead-exposed subjects, L = long-term lead-exposed group, and P = post-encephalopathy lead group. [b]N.S. = non-significant, i.e. p >0.05. Note exception of p <0.10 listed for spacial ability results in Kotok et al. (1977) study. Significance levels are those found after partialing out confounding covariates. [c]Urinary coproporphyrin levels were not elevated. [d]Or ≥30 µg/dl with positive radiologic findings, suggesting earlier exposure in excess of 50-60 µg/dl. [e]Assays for lead in teeth showed the Pb-exposed group to be approximately twice as high as controls (202 µg/g vs. 112 µg/g, respectively). [f]Used for children over 5 years of age. [g]Used for children under 5 years of age. [h]Main measure was dentine lead (PbT). [i]Dentine levels not reported for statistical reasons. [j]Blood lead levels taken 9-12 months prior to testing; none above 33 µg/dl. [k]Data not corrected for age.

PRELIMINARY DRAFT

Of the several pediatric studies presenting evidence for CNS deficits being associated with lead exposure in asymptomatic children, most all are either retrospective or cross-sectional studies except the work of De la Burde and Choate (1972, 1975). De la Burde and Choate (1972) observed neurological dysfunctions, fine motor dysfunction, impaired concept formation, and altered behavioral profiles in 70 preschool children exhibiting pica and elevated blood lead levels (in all cases above 30 μg/dl; mean = 59 μg/dl) in comparison with matched control subjects not engaging in pica. Subjects were drawn from the Collaborative Study of Cerebral Palsy, Mental Retardation, and Other Neurologic Disorders of Infancy and Childhood (Broman et al., 1975), which was conducted in Richmond, Virginia, and had a total population of 3400 mothers. The De la Burde and Choate study population was drawn from this group, in which all mothers were followed throughout pregnancy and all children were postnatally evaluated by regular pediatric neurologic examinations, psychological testing, and medical interviews. All children subject to prenatal, perinatal, and early postnatal insults were excluded from the study, and all had to have normal neurologic examinations and Bayley tests at eight to nine months of age. These are important points which add value to the study. It is unfortunate that blood lead data were not regularly obtained; however, at the time of the study in the late 1960s, 10 to 20 ml of venous blood was required for a blood lead determination and such samples usually had to be obtained by either jugular or femoral puncture. The other control features (housing location and repeated urinary coproporphyrin tests) would be considered the state of the art for such a study at the time that it was carried out.

In a follow-up study on the same children (at 7 to 8 years old), De la Burde and Choate (1975) reported continuing CNS impairment in the lead-exposed group as assessed by a variety of psychological and neurological tests. In addition, seven times as many lead-exposed children were repeating grades in school or being referred to the school psychologist, despite many of their blood lead levels having by then dropped significantly from the initial study. In general, the De la Burde and Choate (1972, 1975) studies appear to be methodologically sound, having many features that strengthen the case for the validity of their findings. For example, there were appreciable numbers of children (67 lead-exposed and 70 controls) whose blood lead values were obtained in preschool years and who were old enough (7 years) during the follow-up study to cooperate adequately for reliable psychological testing. The psychometric tests employed were well standardized and acceptable as sensitive indicators of neurobehavioral dysfunction, and the testing was carried out in a blind fashion (i.e., without the evaluators knowing which were control or lead-exposed subjects).

The De la Burde and Choate (1972, 1975) studies might be criticized on several points, but none provide sufficient grounds for rejecting their results. One difficulty is that blood lead values were not determined for control subjects in the initial study; but the lack of history of pica, as well as tooth lead analyses done later for the follow-up study, render it improbable that appreciable numbers of lead-exposed subjects might have been wrongly assigned to the control group. Subjects in the control group did have a history of pica, but not for paint. Also, results indicating no measurable coproporphyrins in the urine of control subjects at the time of initial testing further confirm proper assignment of those children to the nonexposed control group. A second point of criticism is the use of multiple chi-square statistical analyses, but the fact that the control subjects did significantly better on virtually every measure makes it unlikely that all of the observed effects were due to chance alone. One last problem concerns ambiguities in subject selection which complicate interpretation of the results obtained. Because the lead-exposed group included children with blood lead levels of 40 to 100 μg/dl, or of at least 30 μg/dl with "positive radiographic findings of lead lines in the long bones, metallic deposits in the intestines, or both," observed deficits might be attributed to blood lead levels as low as 30 μg/dl. Other evidence (Betts et al., 1973), however, suggests that such a simple interpretation is probably not accurate. That is, the Betts et al. (1973) study indicates that lead lines are usually seen only if blood levels exceed 60 μg/dl for most children at some time during exposure, although some (about 25 percent) may show lead lines at blood lead levels of 40 to 60 μg/dl. In view of this, the de la Burde and Choate results can probably be most reasonably interpreted as showing persisting neurobehavioral deficits at blood lead levels of 40 to 60 μg/dl or higher.

In another clinic-type child study, Rummo et al. (1974, 1979), found significant neurobehavioral deficits (hyperactivity, lower scores on McCarthy scales of cognitive function, etc.) among Providence, Rhode Island, inner-city children who had previously experienced high levels of lead exposure that had produced acute lead encephalopathy. Mean maximum blood lead levels recorded for those children at the time of encephalopathy were 88 ± 40 μg/dl. However, children with moderate blood lead elevation but not manifesting symptoms of encephalopathy were not significantly different (at $p < 0.05$) from controls on any measure of cognitive functioning, psychomotor performance, or hyperactivity. Still, when the data from the Rummo et al. (1979) study for performance on the McCarthy General Cognitive Index or several McCarthy Subscales are compared (see Table 12-1), the scores for long-term moderate-exposure subjects consistently fall below those for control subjects and lie between the latter and the encephalopathy group scores. Thus, it appears that long-term moderate lead exposure may have, in fact, exerted dose-related neurobehavioral effects. The overall dose-response trend might have been shown to be statistically significant if other types of analyses were used or if

larger samples were assessed. However, control for confounding variables in the different exposure groups would also have to be considered. Note that (1) the maximum blood lead levels for the short-term and long-term exposure subjects were all greater than 40 μg/dl (means = 61 ± 7 and 68 ± 13 μg/dl, respectively), whereas control subjects all had blood lead levels below 40 μg/dl (mean = 23 ± 8 μg/dl), and (2) the control and lead-exposed subjects were inner-city children well matched for socioeconomic background, parental education levels, incidence of pica, and other pertinent factors, but not parental IQ.

A somewhat similar pattern of results emerged from a study by Kotok et al. (1977) in which 36 Rochester, New York, control-group children with blood lead levels less than 40 μg/dl were compared with 31 children having distinctly elevated blood lead levels (61 to 200 μg/dl) but no classical lead intoxication symptoms. Both groups were well matched on important background factors, notably including their propensity to exhibit pica. Again, no clearly statistically significant differences between the two groups were found on numerous tests of cognitive and sensory functions. However, mean scores of control-group children were consistently higher than those of the lead-exposed group for all six of the ability classes listed. Kotok (1972) had reported earlier that developmental deficiencies (using the comparatively insensitive Denver Development Screening test) in a group of children having elevated lead levels (58 to 137 μg/dl) were identical to those in a control group similar in age, sex, race, environment, neonatal condition, and presence of pica, but whose blood lead levels were lower (20 to 55 μg/dl). Children in the lead-exposed group, however, had blood lead levels as high as 137 μg/dl, whereas some control children had blood lead levels as high as 55 μg/dl. Thus, the study essentially compared two groups with different degrees of markedly elevated lead exposure rather than one of lead-exposed vs. nonexposed control children.

Perino and Ernhart (1974) reported a relationship between neurobehavioral deficits and blood lead levels ranging from 40 to 70 μg/dl in a group of 80 inner-city preschool black children, based on the results of a cross-sectional study including children detected as having elevated lead levels via the New York City lead screening program. One key result reported was that the high-lead children had McCarthy Scale IQ scores markedly lowe than those of the low-lead group (mean IQ = 90 vs 80, respectively). Also, the normal correlation of 0.52 between parents' intelligence and that of their offspring was found to be reduced to only 0.10 in the lead-exposed group, presumably because of the influence of another factor (lead) that interfered with the normal intellectual development of the lead-exposed children. One obvious possible alternative explanation for the reported results, however, might be differences in the educational backgrounds of parents of the control subjects when compared with lead-exposed subjects, because parental education level was found to be significantly negatively related to blood lead levels of the children participating in the Perino and Ernhart

(1974) study. The importance of this point lies in the fact that several other studies (McCall et al., 1972; Elardo et al., 1975; Ivanans, 1975) have demonstrated that higher parental education levels are associated with more rapid development and higher intelligence quotients (IQs) for their children.

Ernhart et al. (1981) were able to follow up 63 of the 80 preschool children of the Perino and Ernhart (1974) study once they reached school age, using the McCarthy IQ scales, various reading achievement tests, the Bender-Gestalt test, the Draw-A-Child test, and the Conners Teacher's Questionnaire for hyperactivity. The children's blood lead levels correlated significantly with FEP ($r = 0.51$) and dentine lead levels ($r = 0.43$), but mean blood lead levels of the moderately elevated group had decreased after five years. When control variables of sex and parent IQ were extracted by multivariate analyses, the observed differences were reported to be greatly reduced but remained statistically significant for three of seven tests on the McCarthy scales in relation to concurrently measured blood lead levels but not in relation to the earlier blood lead levels for the same children. This led Ernhart et al. (1981) to reinterpret their 1974 (Perino and Ernhart, 1974) IQ results (in which they had not controlled for parental education) as either not likely being due to lead or, if due to lead, then representing only minimal effects on intelligence.

The Perino and Ernhart (1974) and Ernhart et al. (1981) studies were intensively reviewed by an expert committee convened by EPA in March, 1983 (see Appendix 12-C). The committee found that blood lead measurements used in the Perino and Ernhart (1974) study were of acceptable reliability and the psychometric measures for children were acceptable. However, the IQ measure used for their parents was of questionable utility, other confounding variables may not have been adequately measured, and the statistical analyses did not deal adequately with confounding variables. As for the Ernhart et al. (1981) follow-up study, the committee found the psychometric measures to be acceptable, but the blood lead sampling method raised questions about the reliability of the reported blood lead levels and the statistical analyses did not adequately control for confounding factors. The committee concluded, therefore, that the Perino and Ernhart (1974) and Ernhart et al. (1981) study results, as published, neither confirm nor refute the hypothesis of associations between neuropsychologic deficits and low-level lead exposures in children. It was also recommended that the entire Ernhart data set be reanalyzed, using statistical analyses that better control for confounding factors and including longitudinal analyses of data for subjects that were evaluated in both the Perino and Ernhart (1974) and the Ernhart et al. (1981) studies. A sample longitudinal analysis provided by one committee member, using uncorrected blood lead values and unadjusted psychometric scores from such subjects, suggested that an association may exist between changes in blood lead levels and changes in IQ scores from the first to the second sampling point.

Two recent reports of a study of 193 children from the Philadelphia cohort of the Collaborative Perinatal Project at age seven years examined the persistence of lead-related neuropsychological deficits using circumpulpal rather than primary dentine lead assays at ages 10-14 years (Shapiro and Marecek, 1983, Marecek et al., 1983). Performance differences on several subtests of the Wechsler Intelligence Scale for Children (WISC) and Bender-Gestalt Test were found to persist after four years, these effects being more evident when related to circumpulpal than to primary dentine lead levels. Methodologically, this study suffers from sampling bias, subject ascertainment bias, poor control of covarying social factors, and use of different testers at different testing periods, with no notation as to their blindess.

Odenbro et al. (1983) studied psychological development of children (aged 3-6 yr) seen in Chicago Department of Health Clinics (August, 1976 - February, 1977), evaluating Denver Development Screening test (DDST) and Wechsler IQ scales (WPPSI) scores in relation to blood lead levels obtained by repeated sampling during the three previous years. A significant correlation ($r = -0.435$, $p < 0.001$) was reported between perceptual-visual-motor ability and mean blood levels measured. Statistically significant ($p < 0.005$) deficits in verbal productivity and perceptual visual motor performance (measured by the WPPSI) were found for children with mean blood lead levels of 30-40 µg/dl versus control children with mean blood lead levels <25 µg/dl, using two-tailed Student's t-tests. These results are highly suggestive of neuropsychologic deficits being associated with blood lead levels of 30-60 µg/dl in preschool children. However, questions can be raised regarding the adequacy of the statistical analyses employed, especially in regard to sufficient control for confounding covariates, e.g., parental IQ, education, and socioeconomic status.

The above studies generally found higher lead-exposure groups to do more poorly on IQ or other types of psychometric tests. However, many studies did not control for importtant confounding variables or, when such were taken into account, differences between lead exposed and control subjects were often no longer statistically significant. Still, the consistency of finding lower IQ values among at-risk higher lead children across the studies lends credence to cognitive deficits occurring in apparently asymptomatic children with relatively high blood lead levels. The De la Burde studies in particular point to 40-60 µg/dl as likely lowest observed effect levels among such children.

12.4.2.2.2.2 General population studies. These studies evaluated samples of non-overtly lead intoxicated children drawn from and thought to be representative of the general pediatric population. They generally aimed to evaluate asymptomatic children with lower lead body burdens than those of children in most of the above clinic-type studies.

A pioneering, general population study was reported by Needleman et al. (1979), who used shed deciduous teeth to index lead exposure. Teeth were donated from 70 percent of a total population of 3329 first and second grade children from two towns near Boston. Almost all

children who donated teeth (2146) were rated by their teachers on an eleven-item classroom behavior scale devised by the authors to assess attention disorders. An apparent dose-response function was reported for ratings on the behavior scale not taking potentially confounding variables into account. After excluding various subjects for control reasons, two groups (<10th and >90th percentiles of primary dentine lead levels) were provisionally selected for further in-depth neuropsychologic testing. Later, some provisionally eligible children were also excluded for various reasons, leaving 100 low-lead (<10 ppm dentine lead) children for comparison with 58 high-lead (>20 ppm dentine lead) children in statistical analyses reported by Needleman et al. (1979). A preliminary analysis on 39 non-lead variables showed significant differences between the low- high-lead groups for age, maternal IQ and education, maternal age at time of birth, paternal SES, and paternal education. Some of these variables were entered as covariates into an analysis of covariance along with lead. Significant effects ($p < 0.05$) were reported for full-scale WISC-R IQ scores, WISC-R verbal scales scores, for 9 of 11 classroom behavior scale items, and several experimental measures of perceptual-motor behavior.

Additional papers published by Needleman and coworkers report on results of the same or further analyses of the data discussed in the initial paper by Needleman et al. (1979). For example, a paper by Needleman (1982) provided a summary overview of findings from the Needleman et al. (1979) study and findings reported by Burchfiel et al. (1980) that are discussed later in this section concerning EEG patterns for a subset of children included in the 1979 study. Needleman (1982) summarized results of an additional analysis of the 1979 data set reported elsewhere by Needleman, Levitan and Bellinger (1982). More specifically, cumulative frequency distributions of verbal IQ scores for low- and high-lead subjects from the 1979 study were reported by Needleman et al. (1982), and the key point made was that the average IQ deficit of four points demonstrated by the 1979 study did not just reflect children with already low IQs having their cognitive abilities further impaired. Rather the entire distribution of IQ scores across all IQ levels was shifted downward in the high-lead group, with none of the children in that group having verbal IQs over 125. Another paper, by Bellinger and Needleman (1983), provided still further follow-up analyses of the 1979 N. Eng. J. Med. data set, focusing mainly on comparison of the low- and high-lead children's observed versus expected IQs based on their mother's IQ. Bellinger and Needleman reported that regression analyses showed that IQs of children with elevated levels of dentine-lead (>20 ppm) fell below those expected based on their mothers' IQs and the amount by which a child's IQ falls below the expected value increases with increasing dentine-lead levels in a nonlinear fashion. Scatter plots of IQ residuals by dentine-lead levels, as illustrated and discussed by Bellinger and Needleman (1983), indicated that regressions for children with 20-29.9 ppm dentine lead in the high-lead

group did not reveal significant associations between increasing lead levels in that range and IQ residuals, in contrast to statistically significant ($p < 0.05$) correlations between IQ residuals and dentine-lead for high-lead group children with 30-39.9 ppm dentine lead levels.

The Needleman et al. (1979) study and spin-off analyses published later by Needleman and coworkers were critically evaluated by the same expert committee noted above that was convened by EPA in March, 1983, and which evaluated the Perino and Ernhart (1974) and Ernhart et al. (1981) studies (see Appendix 12-C). In regard to the original study reported by Needleman et al. (1979), the expert committee found that dentine-lead was adequately determined as a measure of cumulative lead exposure and the psychometric data for the subject children generally appeared to be adequately collected and of acceptable reliability. However, the committee concluded that the reported dose-response relationship between dentine-lead levels and teachers' ratings of classroom behavior cannot be accepted as valid, due to: (1) serious reservations regarding the adequacy of classification of subjects into lead exposure categories using only the first dentine-lead level obtained for each child and (2) failure to control for effects of confounding variables. The committee also found that the reported statistically significant effects of lead on IQ and other behavioral neuropsychologic abilities measured for the low- and high-lead groups could not be accepted as valid, due to: (1) errors made in calculations of certain parental IQ scores entered as a control variable in analyses of covariance; (2) failure to take age and father's education into account adequately in the analyses of covariance; (3) use of a forward elimination approach rather than a backwards elimination strategy in statistical analyses; (4) concerns regarding the basis for classification of children in terms of dentine-lead levels; and (5) questions about possible bias due to exclusion of data for large numbers of provisionally eligible subjects from statistical analyses. The committee concluded, therefore, that the study results, as published by Needleman et al. (1979), neither confirm nor refute the hypothesis of associations between neuropsychologic deficits and low-level lead exposure in non-overtly lead intoxicated children. In regard to the publications by Needleman (1982), Needleman et al. (1982), and Bellinger and Needleman (1983) describing further analyses of the same data set reported on by Needleman et al. (1979), the committee concluded that the findings reported in these later papers also cannot be accepted as valid, in view of the above reservations regarding the basic analyses reported by Needleman et al. (1979) and additional problems with the later "spin-off" analyses. The committee also recommended that the entire Needleman data set be reanalyzed, correcting for errors in data calculation and entry, using better Pb exposure classification, and appropriately adjusting for confounding factors.

A recent study of urban children in Sydney, Australia (McBride et al., 1982) involved 454 preschoolers (aged 4-5 yr) with blood lead levels of 2 to 29 μg/dl. Children born at the Women's Hospital in Sydney were recruited via personal letter. No blood lead measures were

available on non-participants. Blood levels were evaluated after neurobehavioral testing, but earlier exposure history was apparently not assessed. Using a multiple statistical comparison procedure and Bonferroni correction to protect against study-wise error, no statistically significant differences were found between two groups with blood lead levels more than one standard deviation above and below the mean (>19 μg/dl vs. <9 μg/dl) on the Peabody Picture Vocabulary IQ Test, on a parent rating scale of hyperactivity devised by Rutter, or on three tests of motor ability (pegboard, standing balance, and finger tapping). In one test of fine motor coordination (tracking), five-year old boys in the higher lead group performed worse than boys in the lower lead group. In one test of gross motor skill (walking balance), results for the two age groups were conflicting. This study suffers from many methodological weaknesses and cannot be regarded as providing evidence for or against an effect of low-level lead exposures in non-overtly lead intoxicated children. For example, a comparison of socioeconomic status (father's occupation and mother's education) of the study sample with the general population showed that it was higher than Bureau of Census statistics for the Australian work force as a whole. There was apparently some self-selection bias due to a high proportion of professionals living near the hospital. Also, other demographic variables such as mother's IQ, pica, and caregiving environment were not evaluated.

Another recent large scale study (Smith et al., 1983) of tooth lead, behavior, intelligence and a variety of other psychological skills was carried out in a general population sample of over 4000 children aged 6 to 7 years in three London boroughs, 2663 of whom donated shed teeth for analysis. Of these, 403 children were selected to form six groups, one each of high (8 μg/g or more), intermediate (5-5.5 μg/g), and low (2.5 μg/g or less) tooth lead levels for two socioeconomic groups (manual vs. non-manual workers). Parents were intensively interviewed at home regarding parental interest and attitudes toward education and family characteristics and relationships. The early history of the child was then studied in school using tests of intelligence (WISC-R), educational attainment, attention, and other cognitive tasks. Teachers and parents completed the Conners behavior questionnaires. Results showed that intelligence and other psychological measures were strongly related to social factors, especially social grouping. Lead level was linked to a variety of factors in the home, especially the level of cleanliness, and to a lesser extent, maternal smoking. There was no statistically significant link between lead level and IQ or academic performance. However, when rated by teachers (but not by parents), there were small, reasonably consistent (but not statistically significant) tendencies for high-lead children to show more behavioral problems after the different social covariables were taken into account statistically. The Smith et al. (1983) study has much to recommend it: (1) a well-drawn sample of adequate size; (2) three tooth lead groupings based on well-defined classifications minimizing possible overlaps of

exposure groupings using whole tooth lead values, including quality-controlled replicate analyses comparisons for the same tooth and duplicate analyses comparisons across multiple teeth from the same child; (3) blood lead levels on a subset of 92 children (averaging 13.1 μg/dl), which correlated reasonably well with tooth lead levels (r = 0.45); (4) cross-stratified design of social groups; (5) extensive information on social covariates and exposure sources; and (6) statistical control for potentially confounding covariates in the analsyes of study results. However, one possible source of selection bias was that tooth donors had a significantly higher social status than non-donors. Thus, the reported results may be less generalizable to the lower socioeconomic working classes, where one might expect the effects of lead exposure to be greater (Yule and Lansdown, 1983).

Harvey et al. (1983) also recently reported that blood lead made no significant contribution to IQ decrements after appropriate allowance had been made for social factors. This study involved 189 children, average age 2.5 years and 15.5 μg/dl blood lead, of middle class workers from the inner city of Birmingham, England. The investigators utilized a wide range of behavioral measures of activity level and psychomotor performance. Strengths of this study are: (1) a well-drawn sample, (2) extensive evaluation of 15 confounding social factors, (3) a wide range of abilities evaluated, and (4) blind evaluations. However, evaluation of lead burden was based on only a single venous blood sample, so that exposure history was not documented as well as in the study by Smith et al. (1983). Nevertheless, a stronger correlation between IQ and blood lead levels was found in children of manual workers (r = -0.32) than in children of non-manual workers (r = -0.06), consistent with findings from the Yule and Lansdown (1983) study discussed below.

Yule et al. (1981) carried out a pilot study on the effects of low-level lead exposure on 85 percent of a population of 195 children aged 6-12 years, whose blood lead concentrations had been determined some nine months earlier as part of a European Economic Community survey. The blood lead concentrations ranged from 7 to 32 μg/dl, and the children were assigned to four quartiles encompassing the following values: 7 to 10 μg/dl; 11 to 12 μg/dl; 13 to 16 μg/dl; and 17 to 32 μg/dl. The tests of achievement and intelligence were similar to those used in the Lansdown et al. (1974) and Needleman et al. (1979) studies. There were significant associations between blood lead levels and scores on tests of reading, spelling, and intelligence, but not on mathematics (Yule et al., 1981). These differences in performance largely remained after age, sex, and father's occupation were taken into account. However, other potentially confounding social factors were not controlled in this study. Another paper by Yule et al. (1983) dealt with the results pertinent to attention deficits. While there were few differences between groups on the Rutter Scale, the summed scores on the Needleman questionnaire across the blood lead groupings approached significance (p = 0.096). Three of

the questionnaire items showed a significant dose-response function ("Day Dreamer," "Does not Follow Sequence of Direction," "Low Overall Functioning"). Nine of 11 items were highly correlated with children's IQ. Therefore, the Needleman questionnaire may be tapping IQ-related attention deficits as opposed to measures of conduct disorder and socially maladaptive behavior (Yule et al., 1983). The hyperactivity factors on the Conners and Rutter scales were reported to be related to blood lead levels (7-12 vs. 13-32 μg/dl), but the authors noted that caution is necessary in interpreting their findings in view of the crude measures of social factors available and differences between countries in diagnosing attention deficit disorders. Moreover, since the blood lead values reported were determined only once (nine months before psychological testing), earlier lead exposures may not be fully reflected and the reported blood lead levels cannot be accepted confidently as those with which any behavioral effects might be associated. Also, home environment and parental IQ and education were not evaluated.

Yule and Lansdown (1983) reported a second, better designed study with similar methods and procedures using 194 children living in a predominantly lower-middle-class area of London near a busy roadway. In this study, a lengthy structured interview yielded data on sources of exposure, medical history, and many potentially confounding variables. Parental IQ was also examined. In contrast to the first pilot study, no statistically significant relationships were found even before social class was controlled for in the statistical analyses. Still, the authors stated that there was some evidence of weak associations between lead level and intelligence in working-class groups but whether these are of a causal nature in either direction is unclear.

Two studies by Winneke and colleagues, the first a pilot study (Winneke et al., 1982a) and the second an extended study (Winneke et al., 1982b) discussed later, employed teeth lead analyses analogous to some of the above studies. In the pilot study, incisor teeth were donated by 458 children aged 7 to 10 years in Duisburg, Germany, an industrial city with airborne lead concentrations between 1.5 and 2.0 $\mu g/m^3$. Two extreme exposure groups were formed, a low-lead group with 2.4 μg/g mean tooth lead level ($n = 26$) and another, high-lead group with 7 μg/g mean tooth lead level ($n = 16$), and matched for age, sex, and father's occupational status. The two groups did not differ significantly on confounding covariates, except that the high-lead group showed more perinatal risk factors. Parental IQ and quality of the home environment were not among the 52 covariables examined. The authors found a marginally significant decrease ($p < 0.10$) of 5-7 IQ points and a significant decrease in perceptual-motor integration ($p < 0.05$), but no significant differences in hyperactivity as measured by the Conners Teachers' Questionnaire administered during testing. As with the Yule et al. (1981) study, the inadequacy of the background social measures (e.g., parental IQ, caregiving environment, and pica), and group differences in perinatal factors weaken this study.

None of the general population studies reviewed provide strong evidence for neuropsychologic deficits being associated with relatively low body lead burdens in non-overtly lead intoxicated children representative of general pediatric populations. All of the studies reporting statistically significant associations between cognitive (IQ) or other behavior (e.g., attentional) deficits have methodological weaknesses, especially inadequate control for confounding covariates such as parental IQ or socioeconomic status. On the other hand, in view of the consistent pattern of results from such studies showing relationships between lead and neuropsychologic deficits before major confounding variables are controlled for, one cannot completely rule out the possibility that lead may be contributing to the observed deficits, especially given the cross-sectional design used in such studies (see Appendix 12-C introduction). The findings of no significant associations between lead and cognitive/behavioral deficits in several recently reported studies (generally controlling better for confounding variables) may not be incompatible with this possibility, in view of the latter studies apparently having evaluated children with lead body burdens likely generally lower than the former studies reporting at least suggestive evidence for lead effects on cognitive and behavioral functions.

12.4.2.2.2.3 Smelter area studies. These studies evaluated children with elevated lead exposures associated with residence in close proximity to lead emitting smelters.

For example, Lansdown et al. (1974) reported a relationship between blood lead level in children and the distance they lived from lead-processing facilities, but no relationship between blood lead level and mental functioning. However, only a minority of the lead-exposed cohort had blood lead levels over 40 μg/dl. Furthermore, this study failed to consider adequately social factors such as socioeconomic status.

In another study, Landrigan et al. (1975) found that lead-exposed children living near an El Paso, Texas, smelter scored significantly lower than matched controls on measures of performance IQ and finger-wrist tapping. The control children in this study were, however, not well matched by age or sex to the lead-exposed group, although the results remained statistically significant after adjustments were attempted for age differences. McNeil and Ptasnik (1975) found negative results in another sample of children living near the same lead smelter in El Paso who were generally comparable medically and psychologically to matched controls living elsewhere in the same city, except for the direct effects of lead (blood lead level, free erythrocyte protoporphyrin levels, and X-ray findings). An extensive critique of these two studies made by another expert committee (see Appendix 12-D) found that no reliable conclusions could be based on either of the two El Paso smelter studies in view of various methodological and other problems affecting the conduct of the studies.

A later study by Ratcliffe (1977) of children living near a battery factory in Manchester, England, found no relation between their blood levels taken at two years of age (28 μg/dl vs. 44 μg/dl in low- vs. high-lead groups) and testing done at age five on the Griffiths Mental Development Scales, the Frostig Developmental Test of Visual Perception, a pegboard test, or a behavioral questionnaire. The differences in scores, although small, favored the low-lead exposure children, i.d., they had somewhat better scores than the higher exposure group. The failure to repeat blood lead assays at age five weakens this otherwise adequate study; potentially higher blood lead levels occurring after age two among control children may have lessened exposure differences between the low- and high-lead groups.

Winneke et al. (1982b) carried out a study which involved 115 children aged 9.4 years living in the lead smelter town of Stolberg. Tooth lead ($\bar{X}$ = 6.16 ppm, range = 2.0-38.5 ppm) and blood lead levels ($\bar{X}$ = 13.4 μg/dl; range = 6.8-33.8 μg/dl were significantly correlated ($r = 0.47$; $p < 0.001$) for the children studied. Using stepwise multiple regression analysis, the authors found significant ($p < 0.05$) or marginally significant ($p < 0.10$) associations between tooth lead levels and measures of perceptual-motor integration, reaction time performance, and four behavioral rating dimensions, including distractibility. This was true even after taking into account age, sex, duration of labor at birth, and socio-heredity background as covariates. However, the proportion of explained variance due to lead never exceeded 6 percent for any of these outcomes, and no significant association was found between tooth lead and WISC verbal-IQ after the effects of socio-hereditary background were eliminated.

The above smelter area studies, again, do not provide strong evidence for cognitivie or behavior deficits being associated with lead exposure in nonovertly lead exposed children. At the same time, the possibility of such deficits being associated with lead exposure in apparently asymptomatic children cannot be ruled out, either, given the overall pattern of results obtained with the cross sectional study design typically employed (see Appendix 12-C introduction).

Several studies have also reported significant associations between hair lead levels and behavioral or cognitive testing endpoints (Pihl and Parkes, 1977; Hole et al., 1979; Hansen et al., 1980; Capel et al., 1981; Ely et al., 1981; Thatcher et al., 1982a,b; Marlowe et al., 1982, 1983; Marlowe and Errera, 1982). Measures of hair lead are easily contaminated by external exposure and are generally questionable in terms of accurately reflecting internal body burdens (see Chapter 9). Such data, therefore, cannot be credibly used to evaluate relationships between absorbed lead and nervous system effects and are not discussed further.

12.4.2.2.2.4 Studies of mentally retarded or behaviorally abnormal children. Other studies, of mentally retarded or autistic individuals and infants, have shown such abnormal populations to have somewhat higher lead levels than the control groups (Beattie et al., 1975;

David et al., 1972, 1976, 1979a,b, 1982a,b, 1983; Moore et al., 1977). However, whether disorders such as mental retardation, hyperactivity, autism, etc. are the causes or the effects of lead exposure is a difficult issue to resolve, and most of the studies cited employed study designs not capable of achieving such resolution. Still, results of at least one study (David et al., 1983) indicate that chelation therapy leading to reduced lead levels resulted in some improvement in behavior among a group of retarded individuals, suggesting that lead may contribute to deviant behavior patterns among such behaviorally abnormal populations, even if lead was not the key etiological factor originally causing the retardation or other behavioral abnormalities.

12.4.2.2.2.5 Electrophysiological studies of lead effects in children. In addition to studies using psychometric and behavioral testing approaches, electrophysiological studies of CNS lead neurotoxicity in non-overtly lead-intoxicated children have been conducted.

Burchfiel et al. (1980) used computer-assisted spectral analysis of a standard EEG examination on 41 children from the Needleman et al. (1979) study and reported significant EEG spectrum differences in percentages of low-frequency delta activity and in alpha activity in spontaneous EEGs of the high-lead children. Percentages of alpha and delta frequency EEG activity and results for several psychometric and behavioral testing variables (e.g., WISC-R full-scale IQ and verbal IQ, reaction time under varying delay, etc.) for the same children were then employed as input variables (or "features") in direct and stepwise discriminant analyses. The separation determined by these analyses for combined psychological and EEG variables ($p < 0.005$) was reported to be strikingly better than the separation of low-lead from high-lead children using either psychological ($p < 0.041$) or EEG ($p < 0.079$) variables alone. Unfortunately, no dentine lead or blood lead values were reported for the specific children from the Needleman et al. (1979) study who underwent the EEG evaluations reported by Burchfiel et al. (1980), and making it impossible to estimate lead-exposure levels associated with observed EEG effects. (See also Appendix 12-C).

The relationship between low-level lead exposure and neurobehavioral function (including electrophysiological responses) in children aged 13-75 months was extensively explored in another study, conducted at the University of North Carolina in collaboration with the U.S. Environmental Protection Agency. Psychometric evaluation (Milar et al., 1980, 1981a) revealed lower IQ scores for children with elevated blood lead levels of 30 μg/dl or higher compared with children with levels under 30 μg/dl, but the observed IQ deficits were confounded by poorer home caregiver environment scores in children with elevated blood lead levels (Milar et al., 1980); and no relationship between blood lead and hyperactive behavior (as indexed by standardized playroom measures and parent-teacher rating scales) was observed in these children (Milar et al., 1981a). On the other hand, electrophysiological assessments, including

analyses of slow cortical potentials during sensory conditioning (Otto et al., 1981) and EEG spectra (Benignus et al., 1981), did provide evidence of CNS effects of lead in the same children. In contrast to psychometric and behavioral findings, a significant linear relationship between blood lead (ranging from 6 to 59 μg/dl) and slow wave voltage (SW) was observed (Otto et al., 1981) as depicted in Figure 12-3. Analyses of quadratic and cubic trends in SW voltage, moreover, did not reveal any evidence of a threshold for this effect. The slope of the blood lead x SW voltage function, however, varied systematically with age. No effect of blood lead on EEG power spectra or coherence measures was observed, but the relative amplitude of synchronized EEG between left and right hemispheres (gain spectra) increased relative to blood lead levels (Benignus et al., 1981). A significant cubic trend for gain between the left and right parietal lobes was found with a major inflection point at 15 μg/dl. This finding suggests that EEG gain is altered at blood lead levels as low as 15 μg/dl, although the clinical and functional significance of this measure has not been established. A follow-up study of slow cortical potentials and EEG spectra in a subset (28 children aged 35 to 93 months) of the original sample was carried out two years later (Otto et al., 1982). Slow wave voltage during sensory conditioning again varied as a linear function of blood lead, even though the mean lead level had declined by 11 μg/dl (from 32.5 μg/dl to 21.1 μg/dl). The similarity of SW results obtained at initial and follow-up assessments suggests that the observed alterations in this parameter of CNS function are persistent, despite a significant decrease in the mean blood lead level during the two-year interval.

Results of the neurobehavioral study and two-year follow-up described above are important for several reasons. First, no significant relationship between child IQ and EEG measures was found in the initial (Benignus et al., 1981; Otto et al., 1981) or follow-up study. SW voltage and EEG gain thus appear to provide CNS indices of lead exposure effects that may be both more sensitive than and independent of standardized psychometric measures used in other studies. Electrophysiological measures such as these hold considerable promise as indicators of CNS function that are free of cultural bias and other linguistic and motor constraints attendant to traditional paper-and-pencil or behavioral tests. Observation of a linear relationship between SW voltage and blood lead within a range of 6 to 59 μg/dl, without evidence of any threshold effect level, is also provocative, particularly in view of the apparent persistence of the effect over a two-year interval. The inflection point in the EEG gain function at 15 μg/dl provides additional evidence of the effect of lead exposure of CNS function in young children at levels considerably below those previously considered to be safe (30 μg/dl). Interpretation of these electrophysiological data, however, must be carefully tempered in view of: (1) SW voltage and EEG gain are both experimental measures, the clinical and functional significance of which is presently unknown; (2) estimated effective blood lead levels associated with the EEG effects are somewhat probalematic because the effects might have resulted

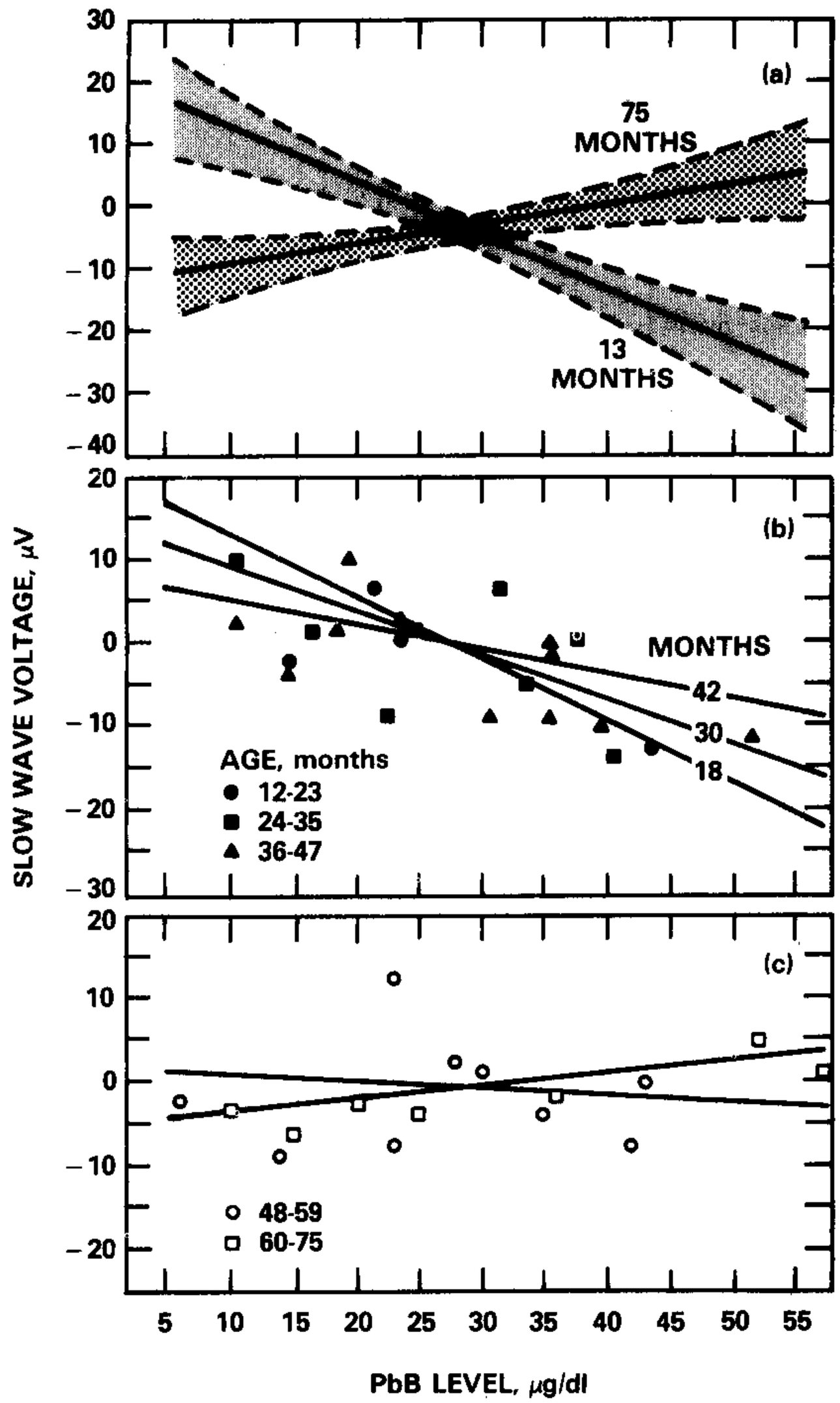

Figure 12-3. (a) Predicted SW voltage and 95% confidence bounds in 13- and 75-month-old children as a function of blood lead level. (b) Scatter plots of SW data from children aged 13-47 months with predicted regression lines for ages 18, 30, and 42 months. (c) Scatter plots for children aged 48-75 months with predicted regression lines for ages 54 and 66 months. These graphs depict the linear interaction of blood lead and age.

Source: Otto et al. (1981).

from higher blood lead levels prior to the reported studies; and (3) the study sample was relatively small (n = 43 for the original and 28 for the follow-up SW analyses). In view of these caveats, these findings need to be replicated in an independent sample. Nevertheless, they provide clear evidence of altered CNS functioning being associated with relatively low level lead exposure of non-overtly lead intoxicated children and at least lead levels likely well below 30 μg/dl.

The adverse effects of lead on peripheral nerve function in children remain to be considered. Lead-induced peripheral neuropathies, although often seen in adults after prolonged exposures, are rare in children. Several articles (Anku and Harris, 1974; Erenberg et al., 1974; Seto and Freeman, 1964), however, describe case histories of children with lead-induced peripheral neuropathies, as indexed by electromyography, assessment of nerve conduction velocity, and observation of other overt neurological signs, such as tremor and wrist or foot drop. Frank neuropathic effects have been observed at blood lead levels of 60 to 80 μg/dl (Erenberg et al., 1974). In other cases, signs indicative of peripheral neuropathy have been reported to be associated with blood lead values of 30 μg/dl. In these latter cases, however, lead lines in long bones suggest probable past exposures leading to peak blood lead levels at least as high as 40 to 60 μg/dl and probably in excess of 60 μg/dl (based on the data of Betts et al., 1973). In each of these case studies, some, if not complete, recovery of affected motor functions was reported after treatment for lead poisoning. A tentative association has also been hypothesized between sickle cell disease and increased risk of peripheral neuropathy as a consequence of childhood lead exposure. Half of the cases reported (10 out of 20) involved inner-city black children, several with sickle cell anemia (Anku and Harris, 1974; Feldman et al., 1973; Lampert and Schochet, 1968; Seto and Freeman, 1964; Imbus et al., 1978). In summary, (1) evidence exists for frank peripheral neuropathy in children, and (2) such neuropathy can be associated with blood lead levels at least as low as 60 μg/dl and, possibly, as low as 40-60 μg/dl.

Further evidence for lead-induced peripheral nerve dysfunction in children is provided by the data from two studies by Feldman et al. (1972, 1977) of inner city children and from a study by Landrigan et al. (1976) of children living in close proximity to a smelter in Idaho. The nerve conduction velocity results from this latter study are presented in Figure 12-4 in the form of a scatter diagram relating peroneal nerve conduction velocities to blood lead levels. No clearly abnormal conduction velocities were observed, although a statistically significant negative correlation was found between peroneal NCV and blood lead levels ($r = -0.38$, $p < 0.02$ by one-tailed t-test). These results, therefore, provide evidence for significant slowing of nerve conduction velocity (and, presumably, for advancing peripheral neuropathy as a function of increased blood lead levels), but do not allow clear statements to be made regarding a threshold for pathologic slowing of NCV.

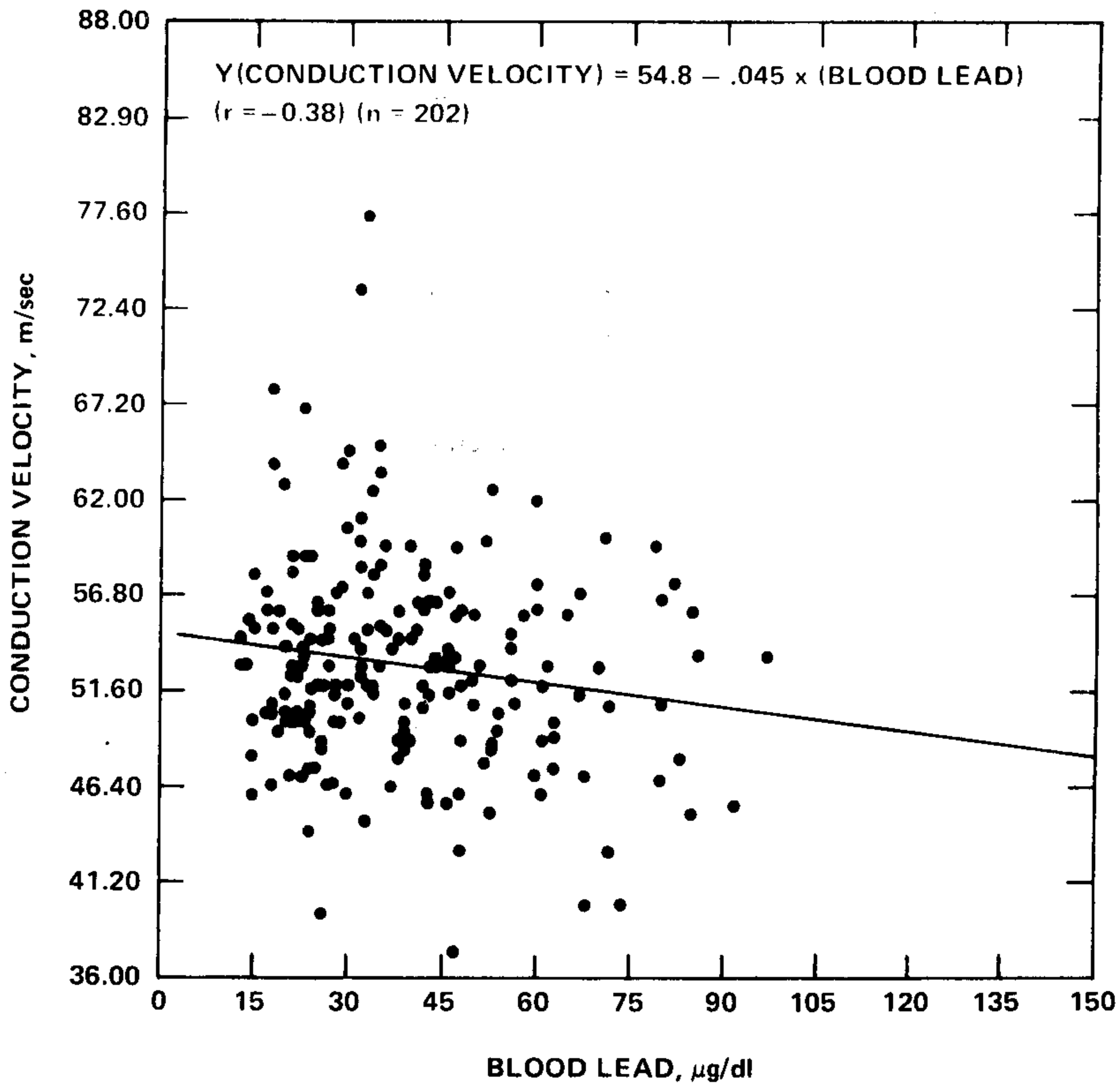

Figure 12-4. Peroneal nerve conduction velocity versus blood lead level, Idaho, 1974.

Source: Landrigan et al. (1976).

12.4.3 Animal Studies

The following sections focus on recent experimental studies of lead effects on behavioral, morphological, physiological, and biochemical parameters of nervous system development and function in laboratory animals. Several basic areas or issues are addressed: (1) behaviorial toxicity, including the question of critical exposure periods for concurrent induction or later expression of behavioral dysfunction in motor development, learning performance, and social interactions; (2) alterations in morphology, including synaptogenesis, dendritic development, myelination, and fiber tract formation; (3) perturbations in various electrophysiological parameters, e.g., ionic mechanisms of neurotransmission or conduction velocities in various tracts; (4) disruptions of biochemical processes such as energy metabolism and chemical neurotransmission; (5) the persistence or reversibility of the above types of effects beyond the cessation of external lead exposure; and (6) the issue of "threshold" for neurotoxic effects of lead.

Since the initial description of lead encephalopathy in the developing rat (Pentschew and Garro, 1966), considerable effort has been made to define more closely the extent of nervous system involvement at subencephalopathic levels of lead exposure. This experimental effort has focused primarily on exposure of the developing organism. The interpretation of a large number of studies dealing with early in vivo exposure to lead has, however, been made difficult by variations in certain important experimental design factors across available studies.

One of the more notable of the experimental shortcomings of some studies has been the occurrence of undernutrition in experimental animals (U.S. Environmental Protection Agency, 1977). Conversely, certain other studies of lead neurotoxicity in experimental animals have been confounded by the use of nutritionally fortified diets, i.e., most commercial rodent feeds (Michaelson, 1980). In general, deficiencies of certain minerals result in increased absorption of lead, whereas excesses of these minerals result in decreased uptake (see Chapter 10). Commercial feeds may also be contaminated by variable amounts of heavy metals, including as much as 1.7 ppm of lead (Michaelson, 1980). Questions have also been raised about possible nutritional confounding due to the acetate radical in lead acetate solutions, which are often used as the source of lead exposure in experimental animal studies (Barrett and Livesey, 1982).

Another important factor that varies among many studies is the route of exposure to lead. Exposure of the suckling animal via the dam would appear to be the most "natural" method, yet may be confounded by lead-induced chemical changes in milk composition. On the other hand, intragastric gavage allows one to determine precisely the dose and chemical form of administered lead, but the procedure is quite stressful to the animal and does not necessarily reflect the actual amount of lead absorbed by the gut. Injections of lead salts (usually

performed intraperitoneally) do not mimic natural exposure routes and can be complicated by local tissue calcinosis at the site of repeated injections.

Another variable in experimental animal studies that merits attention concerns species and strains of experimental subjects used. Reports by Mykkänen et al. (1980) and Overmann et al. (1981) have suggested that hooded rats and albino rats may differ in their sensitivity to the toxic effects of lead, possibly because of differences in their rates of maturation and/or rates of lead absorption. Such differences may account for variability of lead effects and exposure-response relationships between different species as well.

Each of the above factors may lead to widely variable internal lead burdens in the same or different species exposed to roughly comparable amounts of lead, making comparison and interpretation of results across studies difficult. The force of this discussion, then, is to emphasize the importance of measurements of blood and tissue concentrations of lead in experimental studies. Without such measures, attempts to formulate dose-response relationships are futile. This problem is particularly evident in later sections dealing with the morphological, biochemical, and electrophysiological aspects of lead neurotoxicity. In vitro studies accorded attention in those sections, in contrast to in vivo studies, are of limited relevance in dose-response terms. The in vitro studies, however, provide valuable information on basic mechanisms underlying the neurotoxic effects of lead.

The following sections discuss and evaluate the most recent studies of nervous system involvement at subencephalopathic exposures to lead. Older studies reviewed in the previous Air Quality Criteria Document for Lead (U.S. Environmental Protection Agency, 1977) are cited as needed to illustrate particular points but, in general, the discussion below focuses on more recent work.

12.4.3.1 Behavioral Toxicity: Critical Periods for Induction and Expression of Effects. The 1977 EPA review (U.S. Environmental Protection Agency, 1977) of animal behavioral studies and a number of articles since then (e.g., Shigeta et al., 1977; Zenick et al., 1979; Crofton et al., 1980; Kimmel, 1983) have pointed to the perinatal period of ontogeny as a particularly critical time for the induction of behavioral effects due to lead exposure. Such findings are consistent with the general pattern of development of the nervous system in the experimental animals that have been investigated (see Reiter, 1982) and are reviewed in some detail in the ensuing sections of this chapter.

Alterations in the behavior of rats exposed after weaning or after maturation have also been reported (Angell and Weiss, 1982; Cory-Slechta and Thompson, 1979; Cory-Slechta et al., 1981; Donald et al., 1981; Geist and Mattes, 1979; Lanthorn and Isaacson, 1978; Nation et al., 1982; Shapiro et al., 1973). These findings stand in contrast to results from other studies showing some effects in rats as being produced only by early perinatal exposure (e.g., Brown,

1975; Brown et al., 1971; Padich and Zenick, 1977; Shigeta et al., 1977; Snowdon, 1973). Nevertheless, behavioral effects of relatively low-level exposure to lead have also been noted in adult subjects of other species, including pigeons (Barthalmus et al., 1977; Dietz et al., 1979) and fish (Weir and Hine, 1970), and the effects of lead exposure during adulthood are not to be dismissed as inconsequential, although the present evaluation focuses mainly on the effects of lead exposure early in development.

12.4.3.1.1 Development of motor function and reflexes. A variety of methods have been used to assess the effect of lead on the ability of experimental animals to respond appropriately, either by well defined motor responses or gross movements, to a defined stimulus. Such responses have been variously described as reflexes, kineses, taxes, and "species-specific" behavior patterns. The air righting reflex, which refers to the ability to orient properly with respect to gravity while falling through the air and to land on one's feet, is only one of several commonly used developmental markers of neurobehavioral function (Tilson and Harry, 1982). Overmann et al. (1979) found that development of this particular reflex was slowed in rat pups exposed to lead via their dams (0.02 or 0.1 percent lead as lead acetate in the dams' drinking water). However, neither the auditory startle reflex nor the ability to hang suspended by the front paws was affected.

Grant et al. (1980) exposed rats indirectly to lead in utero and during lactation through the mothers' milk and, after weaning, directly through drinking water containing the same lead concentrations their respective dams had been given. In addition to morphological and physical effects [see Sections 12.5, 12.6, and 12.11 for discussions of this work as reported by Kimmel et al. (1980), Fowler et al. (1980), Faith et al. (1979), and Luster et al. (1978)], there were delays in the development of surface righting and air righting reflexes in subjects exposed under the 0.005 and 0.025 percent lead conditions; other reflexive patterns showed no effect. The median blood lead concentration for the 0.005-percent subjects at postnatal day (PND) 11 was 35 μg/dl; the median brain lead concentration was 0.07 μg/g. Locomotor development generally showed no significant alteration due to lead exposure. Body weight was significantly depressed for the most part in the 0.005- and 0.025-percent pups.

The ontogeny of motor function was also investigated by Overmann et al. (1981). Exposure of pups to lead was limited to the period from parturition to weaning and occurred through adulteration of the dams' drinking water with lead acetate (0.01 or 0.1 percent lead acetate). The development of swimming performance was assessed on alternate days from PND 6 to 24. No alterations in swimming ability were found. Rotorod performance was also tested at PND 21, 30, 60, 90, 150, and 440. Overall, the ability to remain on a rotating rod was significantly impaired ($p < 0.01$) at 0.1 percent and tended to be impaired ($0.10 > p > 0.05$) at 0.1 percent (blood lead values were not reported). However, data for individual days were statistically

significant only on PND-60 and 150. An adverse effect of lead exposure on rotorod performance at PND 30-70 was also found in an earlier study by Overmann (1977) at a higher exposure level of 30 mg/kg lead acetate by intubation (average PbB value at PND 21 was 173.5 ± 32.0 μg/dl). At blood lead concentrations averaging 33.2 ± 1.4 μg/dl, however, performance was not impaired. Moreover, other studies using rotorods at average blood lead concentrations of approximately 61 μg/dl (Zenick et al., 1979) and 30 to 48 μg/dl (Grant et al., 1980) have not found significant effects of lead on such performance when tested at PND 21 and 45, respectively. Comparisons between studies are confounded by differences in body weight and age at time of testing and by differences in speed and size of the rotorod apparatus (Zenick et al., 1979).

Delays in the development of gross activity in rat pups have been reported by Crofton et al. (1980) and by Jason and Kellogg (1981). It should be noted that very few studies have been designed to measure the rate of development of activity. Ideally, subjects should be assessed daily over the entire period of development in order to detect any changes in the rate at which a behavior pattern occurs and matures. In the study by Crofton et al. (1980), photocell interruptions by pups as they moved through small passageways into an "exploratory cage" adjacent to the home cage were automatically counted on PND 5 to 21. Pups exposed in utero through the dams' drinking water (0.01 percent solution of lead as lead chloride) lagged controls by approximately one day in regard to characteristic changes in daily activity count levels starting at PND 16. (Blood lead concentrations at PND 21 averaged 14.5 ± 6.8 μg/dl for representative pups exposed to lead in utero and 4.8 ± 1.5 μg/dl for controls.) Another form of developmental lag in gross activity around PND 15-18, as measured in an automated activity chamber, was reported by Jason and Kellogg (1981). Rats were intubated on PND 2-14 with lead at 25 mg/kg (PbB = 50.07 ± 5.33 μg/dl) and 75 mg/kg (PbB = 98.64 ± 9.89 μg/dl). In this case, the observed developmental lag was in the characteristic decrease in activity that normally occurs in pups at that age (Campbell et al., 1969; Melberg et al., 1976); thus, lead-exposed pups were significantly more active than control subjects at PND 18.

One question that arises when ontogenetic effects are discovered concerns the possible contribution of the lead-exposed dam to her offsprings' slowed development through, for example, reduced or impaired maternal care giving behavior. A detailed assessment of various aspects of maternal behavior in chronically lead-exposed rat dams by Zenick et al. (1979), discussed more fully in Section 12.4.3.1.4, and other studies using cross-fostering techniques (Crofton et al., 1980; Mykkänen et al., 1980) suggest that the deleterious effects observed in young rats exposed to lead via their mothers' milk are not ascribable to alterations in the dams' behavior toward their offspring. Chronically lead-exposed dams may, if anything, tend to respond adaptively to their developmentally retarded pups by, for example, more quickly retrieving them to the nest (Davis, 1982).

12.4.3.1.2 Locomotor activity. The spontaneous activity of laboratory animals has been measured frequently and in various ways as a behavioral assay in pharmacology and toxicology (Reiter and MacPhail, 1982). Such activity is sometimes described as gross motor activity or exploratory behavior, and is distinguished from the motor function tests noted in the previous section by the lack of a defined eliciting stimulus for the activity. With reports of hyperactivity in lead-exposed children (see Section 12.4.2), there has naturally been considerable interest in the spontaneous activity of laboratory animals as a model for human neurotoxic effects of lead (see Table 12-2). As the 1977 review (U.S. Environmental Protection Agency, 1977) of this material demonstrated, however, and as other reviews (e.g., Jason and Kellogg, 1980; Michaelson, 1980; Mullenix, 1980) have since confirmed, the use of activity measures as an index of the neurotoxic effects of lead has been fraught with difficulties.

First, there is no unitary behavioral endpoint that can be labeled "activity." Activity is, quite obviously, a composite of many different motor actions and can comprise diverse behavior patterns including (in rodents) ambulation, rearing, sniffing, grooming, and, depending on one's operational definition, almost anything an animal does. These various behavior patterns may vary independently, so that any gross measure of activity which fails to differentiate these components will be susceptible to confounding. Thus, different investigators' definitions of activity are critical to interpreting and comparing these findings. When these definitions are sufficiently explicit operationally (e.g., activity as measured by rotations of an "activity wheel"), they are frequently not comparable with other operational definitions of activity (e.g., movement in an open field as detected by photocell interruptions). Moreover, empirical comparisons show that different measures of activity do not necessarily correlate with one another quantitatively (e.g., Copobianco and Hamilton, 1976; Tapp, 1969).

In addition to these rather basic difficulties, activity levels are influenced greatly by numerous variables such as age, sex, estrous cycle, time of day, novelty of environment, and food deprivation. If not controlled properly, any of these variables can confound measurements of activity levels. Also, nutritional status has been a frequent confounding variable in experiments examining the neurotoxic effects of lead on activity (see the review by U.S. Environmental Protection Agency, 1977; Jason and Kellogg, 1980; Michaelson, 1980). In general, it appears that rodents exposed neonatally to sufficient concentrations of lead experience undernutrition and subsequent retardation in growth; and, as Loch et al. (1978) have shown, retarded growth per se can induce increased activity of the same types that were attributed to lead alone in some earlier studies.

In view of the various problems associated with the use of activity measures as a behavioral assay of the neurotoxic effects of lead, the discrepant findings summarized in Table 12-2 should come as no surprise. Until the measurement of "activity" can be better

TABLE 12-2. EFFECTS OF LEAD ON ACTIVITY IN RATS AND MICE

Increased	Decreased	Age-dependent, qualitative, mixed or no change
Driscoll and Stegner (1978)	Driscoll and Stegner (1976)	Barrett and Livesey (1982)
Golter and Michaelson (1975)	Flynn et al. (1979)	Brown (1975)
Kostas et al. (1976)	Gray and Reiter (1977)	Crofton et al. (1980)
Overmann (1977)	Reiter et al. (1975)	Cutler (1977)
Petit and Alfano (1979)	Verlangieri (1979)	Dolinsky et al. (1981)
Sauerhoff and Michaelson (1973)		Dubas and Hrdina (1978)
Silbergeld and Goldberg (1973, 1974a,b)		Geist and Balko (1980)
Weinreich et al. (1977)		Geist and Praed (1982)
Winneke et al. (1977)		Grant et al. (1980)
		Gross-Selbeck and Gross-Selbeck (1981)
		Hastings et al. (1977)
		Jason and Kellogg (1981)
		Kostas et al. (1978)
		Krehbiel et al. (1976)
		Loch et al. (1978)
		Minsker et al. (1982)
		Mullenix (1980)
		Ogilvie and Martin (1982)
		Rafales et al. (1979)
		Schlipköter and Winneke (1980)
		Sobotka and Cook (1974)
		Sobotka et al. (1975)
		Zimering et al. (1982)

standardized, there appears to be little basis for comparing or utility in further discussing the results of studies listed in Table 12-2.

12.4.3.1.3 Learning ability. When animal learning studies related to the neurotoxic effects of lead were reviewed in 1977 (U.S. Environmental Protection Agency, 1977), a number of criticisms of existing studies were noted. A major limitation of early work in this field was the lack of adequate information on the exposure regimen (dosage of lead, how precisely administered, timing of exposure) and the resulting body burdens of lead in experimental subjects (concentrations of lead in blood, brain, or other tissue; time course of blood or tissue lead values, etc.). A review of studies appearing since 1977 reveals a notable improvement in this regard. A number of more recent studies have also attempted to control for the confounding factors of litter effects and undernutrition--variables that were generally not controlled in earlier studies.

Unfortunately, other criticisms are still valid today. The reliability and validity of behavioral assays remain to be established adequately, although progress is being made. The reliability of a number of common behavioral assays for neurotoxicity is currently being determined by several independent U.S. laboratories (Kimmel et al., 1982). The results of this program should help standardize some behavioral testing procedures and perhaps create some reference methods in behavioral toxicology. Also, as well-described studies are replicated within and between laboratories, the reliability of certain experimental paradigms for demonstrating neurotoxic effects is effectively established.

Some progress is also being made in dealing with the issue of the validity of animal behavioral assays. As the neurological and biochemical mechanisms underlying reliable behavioral effects become better understood, the basis for extrapolating from one species to another becomes stronger and more meaningful. An awareness of different species' phylogenetic, evolutionary, and ecological relationships can also help elucidate the basis for comparing behavioral effects in one species with those observed in another (Davis, 1982).

Tables 12-3 and 12-4 summarize exposure conditions, testing conditions, and results of a number of recent studies of animal learning (see U.S. Environmental Protection Agency, 1977, for a summary of earlier studies). Some general issues emerge from an examination of these studies. One point of obvious interest is the lowest level of exposure at which behavioral effects are clearly evident. Such a determination is best done on a species-by-species basis. Rats seem to be the species of choice for the great majority of the behavioral studies, despite the concerns that have repeatedly been expressed concerning the appropriateness of this species as a subject for behavioral investigation (e.g., Lockard, 1968, 1971; Zeigler, 1973). Of the studies not obviously confounded by nutritional or litter effects, those by Winneke et al. (1977, 1982c) and by Cory-Slechta and Thompson (1979) report alterations in

TABLE 12-3. RECENT ANIMAL TOXICOLOGY STUDIES OF LEAD EFFECTS ON LEARNING IN RODENT SPECIES

Reference	Experimental animal (species or strain)	Lead exposure: Pb conc. (medium)	Lead exposure: period (route)	Treatment groups (n)	Litters per group	Tissue lead (age measured)	Age at testing	Testing paradigm (task)	Non-behavioral effects	Behavioral results
Hastings et al. (1977)	Rat (L-E)	0.01 or 0.05% (water)	PND 0-21 (dam's milk)	C (12) Pb_1 (12) Pb_2 (12)	Random selection from 9 litters	PbB (20 d): C: 11 μg/dl Pb_1: 29 Pb_2: 42 (60 d): C: 4 Pb_1: 5 Pb_2: 9	~90-186 d	Operant (successive brightness discrim.)	None	No sig. differences between Pb-Ss and C-Ss in learning original or reversed discrim. task.
Overmann (1977)	Rat (L-E)	10, 30, or 90 mg/kg (gavage)	PND 3-21 (direct)	C Pb_1 12- Pb_2 25 Pb_3 ea.	?	PbB (21 d) C: 15 μg/dl Pb_1: 33.2 Pb_2: 173.5 Pb_3: 226.1	26-29 d 67-89 d 79-101 d 83-105 d 95-117 d	Aversive conditioning (1) active 2) passive) Operant (inhibit response) E-maze (discrim.: 1) spatial 2) tactile 3) visual)	None	Pb_3-Ss sig. slower in acquisition and extinction of active avoidance response; no sig. diffs. for passive avoidance. All Pb groups failed to inhibit responses as well as C-Ss. No sig. diffs. on E-maze tasks, except $Pb_{2,3}$-Ss sig. worse than C-Ss when on tactile discrim.
Padich and Zenick (1977)	Rat (CD)	375 mg/kg (water)	Preconception to a) Weaning (via dam) or b) termination (via dam and direct); or Weaning to termination (direct only)	0-0 (10) 0-Pb (10) Pb-0 (10) Pb-Pb (10)	5 5 5 5	?	42-? d	Operant (FR 20)	Body wt. of Pb-Ss < 0-Ss from birth to weaning.	Pb-Pb group had sig. fewer rewarded responses even though responding at sig. higher rate.
Winneke et al. (1977)	Rat (W)	372 mg/kg (food)	Preconception - Testing (via dam and direct)	C (20) Pb (20)	? (random selection from 110 male pups)	PbB (~16 d): C: 1.7 μg/dl Pb: 26.6 (~190 d) Pb: 28.5	100-200 d	Lashley jumping stand (visual discrim. of stimulus: 1) orientation 2) size)	Body wt. of Pb-Ss > C-Ss; however, size of Pb-Ss litters < C-S litters.	Pb-Ss sig. slower to learn size discrimination; no diff. between Pb and C groups on orientation discrim. (a relatively easy task).

PRELIMINARY DRAFT

TABLE 12-3. (continued)

Reference	Experimental animal (species or strain)	Lead exposure: Pb conc. (medium)	Lead exposure: period (route)	Treatment groups (n)	Litters per group	Tissue lead (age measured)	Age at testing	Testing paradigm (task)	Non-behavioral effects	Behavioral results
Dietz et al. (1978)	Rat Expt. 1(L-E)	200 mg/kg (gavage)	PND 3-30 (direct)	C (6) Pb (7)	2, split	?	3 mo or 21 mo	Operant (minimum 20-sec pd. between bar-presses)	None	Short IRTs (≤4 sec) more prevalent in Pb-Ss than in C-Ss, but did not result in different reward rates; Pb-Ss showed higher variability in response-rate under d-amphetamine treatment.
	Expt. 2(CD)	0.01% (water)	Preconception to termination (via dam until weaning, then direct)	C (4)* Pb (4)	?	?	8 mo		Pb-body wt. lower 1 wk. prior to test; C reduced to same wt. at test.	
Lanthorn & Isaacson (1978)	Rat (L-E)	0.27% (water)	Adult (direct)	C (4) Pb (6)	?	?	Adult	T-maze (1) spontan. alternation 2) light discrim. 3) spatial discrim.)	C-Ss pair-fed to control for loss of body wt.	Pb-Ss had sig. lower rate of spontaneous alternation; Pb-Ss sig. slower than C-Ss only on 1st spatial discrim. task.
Cory-Schlecta & Thompson (1979)	Rat (S-D)	(1) 0.005, (2) 0.03, or (3) 0.1% (water)	PND 20- (a) 70 or (b) 150 (direct)	1a: C (4)* Pb (5) 1b: C (4)* Pb (6) 2: C (3)* Pb (4) 3: C (4)* Pb (5)	random assignment	PbB (150 d): C: ~6 μg/dl 1a: ~3 1b: ~7 2: ~27 3: ~42	55-140 d	Operant (FI-30 sec)	None	Increased response rate and inter-S variability in groups $Pb_{1a,b}$ and Pb_2; decreased response rate in group Pb_3; effects in Pb_{1a} reversed after exposure terminated.
Cory-Schlecta et al. (1981)	Rat (S-D)	(1) 0.01 or (2) 0.03% (water)	PND 21-?	C (4) Pb_1 (5) Pb_2 (5)	random assignment	Brain-Pb (post-test): C: 14-26 ng/g Pb_1: 40-142 Pb_2: 320-1080	55-? d	Operant (minimum duration bar-press)	None	Pb groups impaired: decreased response durations; increased response latencies; failure to improve performance by external stimulus control.

*Weight-matched controls

PRELIMINARY DRAFT

TABLE 12-3. (continued)

Reference	Experimental animal (species or strain)	Lead exposure: Pb conc. (medium)	Lead exposure: period (route)	Treatment groups (n)	Litters per group	Tissue Lead (age measured)	Age at testing	Testing paradigm (task)	Non-behavioral effects	Behavioral results
Geist & Mattes (1979)	Rat (S-D)	0.001 or 0.0025% (water)	PND 23-termination (direct)	C (7) Pb_1 (7) Pb_2 (7)	?	?	58-? d	Hebb-Williams maze (find way to goal box)	None	Pb_1- and Pb_2-Ss made sig. more errors than C-Ss; Pb_2-Ss slower than C-Ss to traverse maze.
Flynn et al. (1979)	Rat (L-E) Expt. 1	0.25% (water)	Preconception - PND 22 (via dam)	C (8) Pb (10)	8 10	Brain-Pb (3 d): C: ~0 Pb: 0.174 µg/g (30-34 d): no sig. diffs.	?	Radial arm maze (spontaneous alternation)	Brain wts. of Pb-Ss < C-Ss; no other differences.	No sig. difference between Pb-Ss and C-Ss.
	Expt. 2	0.1% (water), 225 mg/kg (gavage), 0.25% (water)	Preconception - Birth (via dam), Birth - Weaning (direct), Weaning - termination (direct)	C (12) Pb (12)	6 6	(75-76 d): C: 0.13 µg/g Pb: 1.85	49-58 d	Passive avoidance (remain in 1 of 2 compartments to avoid elect. shock)	None	No sig. difference in trials to criterion, but Pb-Ss made sig. fewer partial excursions from "safe" compartment.
	Expt. 3	same as above except 90 mg/kg (gavage)	same as above except stopped at PND 33	C (10) Pb (10)	4	see above	58-60 d	Shuttle-box signalled avoidance (move from one compartment to other to avoid elect. shock)	None	No sig. difference between Pb-Ss and C-Ss.
Petit & Alfano (1979)	Rat (L-E)	0.2 or 2% (food)	PND 1-25	C_i (22) C_e (22) Pb_{1_i} (22) Pb_{1_e} (22) Pb_{2_i} (22) Pb_{2_e} (22)	~7 each; split for "i" (isolation and "e" (enrichment) conditions	PbB (25 d): C: 2 µg/dl Pb_1: 331 Pb_2: 1297	66-115 d	Hebb-Williams maze (find way to goal box) Passive avoidance (remain in compartment to avoid shock)	Body wts. of Pb_2-Ss < C-Ss, Pb_1-Ss > C-Ss; gross toxicity in Pb_2-Ss; lower brain wts. in Pb-Ss	No sig. diff. between Pb- and C-Ss in maze learning; Isolation-reared Pb-Ss less successful than C_i-Ss on passive avoidance task; enrichment-reared Pb_1-Ss = C_e-Ss but Pb_{2_e}-Ss sig. worse on passive avoidance.

PRELIMINARY DRAFT

TABLE 12-3 (continued)

Reference	Experimental animal (species or strain)	Lead exposure: Pb conc. (medium)	Lead exposure: period (route)	Treatment groups (n)	Litters per group	Tissue lead (age measured)	Age at testing	Testing paradigm (task)	Non-behavioral effects	Behavioral results
Zenick et al. (1978)	Rat (CD)	500 mg/kg (water)	Preconception - Weaning (via dam)	C (10) Pb (10)	5 5	?	30-40 d 55-63 d	Water T-maze 1) black-white discrim. 2) shape discrim.	Body wt. of Pb-Ss < C-Ss from birth to 50 d.	On both discrim. tasks, Pb-Ss made sig. more errors with sig. shorter response
Zenick et al. (1979)	Rat (CD)	375 mg/kg (water)	Preconception to a) Weaning (via dam) or b) termination (via dam and direct)	0-0 (?) Pb-0 (?) Pb-Pb (?)	5 5 5	?	42-? d	Operant (FI-1 min)	Body wt. of Pb-Ss < 0-Ss from birth to weaning.	Pb-Pb group had sig. fewer rewarded responses across sessions than Pb-0 or 0-0 groups.
Hastings et al. (1979)	Rat (L-E)	0.01 or 0.1% (water)	PND 0 - 21 (dam's milk)	C (23) Pb_1 (11) Pb_2 (13)	Random selection from 15 litters	PbB (20 d): C: 11 µg/dl Pb_1: 29 Pb_2: 65 Brain-Pb (20 d): C: 12.5 µg% Pb_1: 29 Pb_2: 65	120 d 270 d 330 d	(1) Operant (simult. vis. discrim.) (2) T-maze (success. vis. discrim.) (3) Operant (go/no-go task)	None	Pb_2-Ss sig. slower to reach criterion than C-Ss on simultaneous visual discrimination task; no sig. differences on successive and go/no-go discrim. tasks.
Schlipköter & Winneke (1980)	Rat (?) Expt. 1	0.23% (food)	Preconception - PND 120 (via dam and direct)	C (?) Pb_1 (18)	?	PbB all C: <5 µg/dl Pb_1 (120 d): 39.5 (8 mo): 12.0	7 mo	Lashley jumping stand (cue - size discrim.)	?	Sig. increase in error repetition by Pb_1-Ss.
	Expt. 2	0.075% (food)	a) Prenatal-7 mo (via dam and direct) b) Prenatal-Weaning (via dam)	C (10) Pb_{2a} (10) Pb_{2b} (10)	?	Pb_{2a}: (21 d) 29.2 (7 mo) 27.0 Pb_{2b}: (21 d) 29.2 (7 mo) 5.2	"	"	?	Non-sig. ($p < 0.10$) increase in error repetition by Pb_2-Ss.
	Expt. 3	------Same as Expt. 2------		C (14) Pb_{3a} (14) Pb_{3b} (14)	?	Pb_{3a}: (21 d) 29.9 (7 mo) 30.8 Pb_{3b}: (21 d) 29.9 (7 mo) 1.8	"	"	?	No sig. diffs. between Pb_3-Ss and C-Ss.
	Expt. 4	0.025 or 0.075% (food)	Prenatal - 7 mo (via dam and direct)	C (10) Pb_{4a} (10) Pb_{4b} (10)	?	(120 d) Pb_{4a}: 17.8 Pb_{4b}: 28.6	"	Water maze (spatial discrim.)	?	35% of Pb_4-Ss failed to reach criterion (vs. 10% C-Ss); 35% also failed retest after 1 wk (vs. 0% C-Ss).

PRELIMINARY DRAFT

TABLE 12-3. (continued)

Reference	Experimental animal (species or strain)	Lead exposure: Pb conc. (medium)	Lead exposure: period (route)	Treatment groups (n)	Litters per group	Tissue lead (age measured)	Age at testing	Testing paradigm (task)	Non-behavioral effects	Behavioral results
Gross-Selbeck & Gross-Selbeck (1981)	Rat F_1 (W)	0.5 g/kg (food)	Postweaning - termination (direct)	C (6) Pb (6)	?	PbB (~180 d): C: 6.2 µg/dl Pb: 22.7	Adult	Operant (DRH)	None	Both F_1 and F_2 (especially F_2) Pb-Ss had greater % rewarded responses than C-Ss, i.e., Pb-Ss bar-pressed at higher rate than C-Ss.
	F_2	"	Preconception - Weaning (via dam)	C (6) Pb (6)	?	(~110 d): C: 3.7 Pb: 4.6	3-4 mo	"	"	
Angell & Weiss (1982)	Rat (L-E)	0.1% (water)	PND 3-21 (dam's milk) and/or 21-130 (direct)	0-0 (20) 0-Pb (20) Pb-0 (24) Pb-Pb (24)	5, split 6, split	PbB(130d): 0-0: 2 µg/dl 0-Pb: 66 Pb-0: 9 Pb-Pb: 64	58-130 d	Operant (Mult FI-TO-FR-TO)	Pb-Pb Ss sig. lower body wt. postweaning	Groups exposed post-weaning (0-Pb, Pb-Pb) had longer Inter-Response Times; group exposed preweaning (Pb-0) had shorter IRTs.
Milar et al. (1981b)	Rat (L-E)	25, 100, or 200 mg/kg	PND 4-31 (direct)	C (10) Pb_1 (5) Pb_2 (4)	3 4 4	PbB (32 d) C: 5 µg/dl Pb_1: 26 Pb_2: 123	50 d	Operant (spatial alternation levers)	Pb_2-Ss sig. slower rate	No sig. differences between C-Ss and Pb-Ss.
Nation et al. (1982)	Rat (S-D)	10 mg/kg (food)	PND 100-termination (direct)	C (8) Pb (8)	?	?	156 d	Operant (conditioned suppression of responding on mult. VI schedule)	None	Presentation of tone associated with electrical shock disrupted steady-state responding more in PB-Ss than in C-Ss.
Winneke et al. (1982c)	Rat (W) Expt. 1	0.004, 0.012, or 0.037% (food)	Preconception - Testing (via dam and direct)	C(16) Pb_1 (16) Pb_2 (16) Pb_3 (16)	Random selection from 5-6 litters per condition	?	70-100 d	Shuttle-box signalled avoidance (move from one compartment to avoid elect. shock)	ALA-D at 90 d: C: 7.05 U/l Pb_1: 4.26 Pb_2: 1.92 Pb_3: 1.18	Expt. 1 Pb-Ss sig. faster than C-Ss to learn avoidance response.
	Expt. 2	-Continuation of Expt. 1-		C (10) Pb_2 (10) Pb_3 (10)	(females dropped; no Pb_1 group for Expt. 2)	?	190-250 d	Lashley jumping stand (size discrim.)		Expt. 2 Pb-Ss sig. slower than C-Ss to learn size discrim.

PRELIMINARY DRAFT

TABLE 12-3 (continued)

Reference	Experimental animal (species or strain)	Lead exposure: Pb conc. (medium)	Lead exposure: period (route)	Treatment groups (n)	Litters per group	Tissue lead (age measured)	Age at testing	Testing paradigm (task)	Non-behavioral effects	Behavioral results
Taylor et al. (1982)	Rat (CD)	0.01 or 0.02% (water)	Preconception - Weaning (via dam)	C (12) Pb_1 (16) C_2 (4) Pb_2 (4)	6* 8* 2* 2*	PbB (21 d) C: 3.7 µg/dl Pb_1: 38.2 Pb_2: 49.9	11 d	Runway (traverse alley to reach dam and dry suckle)	None	No sig. diffs. in acquisition of response, but both Pb groups sig. slower to extinguish when response no longer rewarded.
Kowalski et al. (1982)	Mouse (Wistar)	0.0002% (water)	Adult (direct)	C (16) Pb (16)	?	?	(13 d after start of exposure)	Water T-maze (spatial discrim.)	None	Pb-Ss made more errors than C-Ss; food deprivation exacerbated effect.
McLean et al. (1982)	Mouse (Swiss)	0.002 or 0.2% (water)	Adult (direct)	C (16) Pb_1 (16) Pb_2 (16)	?	?	(10 d after start of expos.)	Water T-maze (spatial discrim.)	None	Pb-Ss showed no improvement in performance compared to C-Ss.

*Inferred from information in report.

Abbreviations:

?	information not given in report
ALA-D	delta Aminolevulinic Acid Dehydrase
C	Control group
CD	substrain of Sprague Dawley
DRH	Differential Reinforcement of High response rates
F_1	1st Filial generation
F_2	2nd Filial generation
FI	Fixed Interval
FR	Fixed Ratio
IRT	Inter Response Time
L-E	Long Evans
N/A	Not Applicable
NaAc	sodium acetate
Pb	lead-exposed group
$Pb(Ac)_2$	lead acetate
PbB	blood lead
PND	Post-Natal Day
S	Subject
S-D	Sprague Dawley
TO	Time Out
U/l	µmol ALAD/min x liter erythrocytes
VI	Variable Interval
W	Wistar
WGTA	Wisconsin General Testing Apparatus
X	experimental group

PRELIMINARY DRAFT

TABLE 12-4. RECENT ANIMAL TOXICOLOGY STUDIES OF LEAD EFFECTS ON LEARNING IN PRIMATES

Reference	Experimental animal (species or strain)	Lead exposure: Pb conc. (medium)	Lead exposure: period (route)	Treatment groups (n)	Litters per group	Tissue lead (age measured)	Age at testing	Testing paradigm (task)	Non-behavioral effects	Behavioral results
Bushnell & Bowman (1979a)	Monkey (Macaca mulatta)	~0.07 or 0.16% (milk) adjusted to maintain target PbB	Daily for 1st yr (direct)	C (4) Pb_1 (3) Pb_2 (3)	N/A	PbB (1st yr): C: ~5 µg/dl* Pb_1: 37* Pb_2: 58*	5-10 mo	WGTA (form discrim. reversal learning)	None	Both Pb-exposed groups retarded in reversal learning; Pb_2-Ss especially impaired on 1st reversal following overtraining.
	Expt. 1									
	Expt. 2 Test 1	--same as Expt. 1--		C (4) Pb_1 (4) Pb_2 (4)	N/A	PbB (1st yr): C: ~4 µg/dl* Pb_1: 32* Pb_2: 65*	1.5-4.5 mo	2-choice maze (discr. reversal learning) non-food reward	None	Pb_2-Ss sig. retarded on 1st reversal (confirms Expt. 1 using different task and reward to control for possible confounding by motivational or motor factors).
	Test 2	----------Continuation of Expt. 2----------					5-12 mo	WGTA (series of 4 reversal discr. problems)	None	Both Pb groups retarded in reversal learning; Pb_2-Ss impaired on 1st reversals regardless of prior overtraining.
	Test 3	---------Continuation of Expt. 2--------- after exposure terminated at 12 mo				PbB (16 mo): C: ~5 µg/dl Pb_1: 19 Pb_2: 46	15 mo	WGTA (discr. reversal learning, more	None	Pb_2-Ss retarded on 1st reversal.
Bushnell & Bowman (1979b)	Monkey (Macaca mulatta)	--Continuation of Bushnell & Bowman (1979a)--				PbB (56 mo): C: 4 µg/dl Pb_1: 5 Pb_2: 6	49-55 mo	WGTA (spatial discr. reversal learning)	None	Both Pb-exposed groups retarded in reversal learning; 3 Pb_2-Ss failed to retain motor pattern for operating WGTA from 2 yrs earlier.

*Corrected annual averages obtained from Bushnell (1978)

PRELIMINARY DRAFT

TABLE 12-4 (continued)

Reference	Experimental animal (species or strain)	Lead exposure: Pb conc. (medium)	Lead exposure: period (route)	Treatment groups (n)	Litters per group	Tissue lead (age measured)	Age at testing	Testing paradigm (task)	Non-behavioral effects	Behavioral results
Rice & Willes (1979)	Monkey (Macaca fascicularis)	500 µg/kg (milk)	Daily for 1st year (direct)	C (4) Pb (4)	N/A	PbB (200 d): C: <5 µg/dl Pb: 35-70 (400 d): Pb: 20-50	421-714 d	WGTA (form discrim. reversal)	None	Pb-Ss slower to learn successive reversals.
Rice et al. (1979)	----------Continuation of Rice & Willes (1979)----------					PbB (400+ d): 20-30 µg/dl	2.5-3 yr	Operant (mult. FI-TO)	None	Pb-Ss responded at higher rates, had shorter IRTs, and tended to respond more during time-out (unrewarded).

Abbreviations:

?	information not given in report
ALA-D	delta Aminolevulinic Acid Dehydrase
C	Control group
CD	substrain of Sprague Dawley
DRH	Differential Reinforcement of High response rates
F_1	1st Filial generation
F_2	2nd Filial generation
FI	Fixed Interval
FR	Fixed Ratio
IRT	Inter Response Time
L-E	Long Evans
N/A	Not Applicable
NaAc	sodium acetate
Pb	lead-exposed group
$Pb(Ac)_2$	lead acetate
PbB	blood lead
PND	Post-Natal Day
S	Subject
S-D	Sprague Dawley
TO	Time Out
U/l	µmol ALAD/min x liter erythrocytes
VI	Variable Interval
W	Wistar
WGTA	Wisconsin General Testing Apparatus
X	experimental group

PRELIMINARY DRAFT

learning task performances by rats with blood lead levels below 30 μg/dl. Winneke et al. (1977) exposed Wistar rats in utero and postnatally to a diet containing 0.07 percent lead as lead acetate. Between PND 100 and 200 the subjects were tested on two types of visual discrimination learning tasks using either "easy" stimuli (vertical vs. horizontal stripes) or "difficult" stimuli (white circles or differing diameters). Blood lead concentrations were measured at about PND 16 (26.6 μg/dl) and PND 190 (28.5 μg/dl). Although there were no significant differences between lead-exposed and control subjects on the easy discrimination task, the lead-exposed subjects performed significantly ($p < 0.01$) worse than controls on the size discrimination task. The performance of the lead group continued around change level (50 percent correct) essentially throughout the 4-week training period; control subjects began to improve after about 2 weeks of training and reached an error rate of about 15 percent by 3 to 4 weeks. Stated differently, 8 out of 10 control animals reached criterion performance levels within 27 days, whereas only one of the lead-exposed subjects did ($p < 0.01$).

More recently, Winneke et al. (1982c) repeated the size discrimination experiment and added another test involving shock avoidance. As in the earlier study, exposure started in utero and continued through behavioral testing. Different concentrations of lead acetate in the diet were used to yield average blood lead levels of 18.3 and 31.2 μg/dl after 130 days of feeding, compared to 5 μg/dl for control subjects. These values were not determined directly from the subjects in this study but were based on separate work by Schlipköter and Winneke (1980). However, ALA-D activity was measured directly in selected female subjects at PND-90 and was found to be inhibited 73 percent and 83 percent, respectively, for the different levels of lead exposure. Consistent with their earlier findings, Winneke et al. (1982c) found that lead-exposed subjects were significantly slower to reach criterion performance levels on the size discrimination task. However, on the shock avoidance task, the lead-exposed subjects were significantly quicker than control subjects to reach the criterion of successful performance. Although seemingly incongruous with the impairment found in the discrimination task, the latter finding is consistent with results obtained by Driscoll and Stegner (1976), who found performance on a shock avoidance task enhanced by lead exposure at a level high enough (~0.15 percent lead in dams' drinking water) to cause a 20 percent weight reduction in the subjects prior to weaning. Both the size discrimination deficits and the enhanced avoidance performance are indicative of alterations in normal neural functioning consequent to lead exposure.

Cory-Slechta and Thompson (1979) exposed Sprague-Dawley rats to 0.0025, 0.015, or 0.05 percent drinking water solutions of lead as lead acetate starting at PND 20-22. Operant conditioning on a fixed-interval 30-second schedule of reinforcement (food pellet delivered upon the first bar-press occurring at least 30 sec after preceding pellet delivery) began at PND

55-60. Blood lead concentrations measured at approximately PND 150 were reported in graphical form roughly as follows: 0.0025-percent solution group, 5 to 10 μg/dl PbB; 0.015-percent group, 25 to 30 μg/dl PbB; 0.05-percent group, 40 to 45 μg/dl PbB. Subjects exposed to 0.0025 or 0.015 percent lead solutions showed a "significantly" (no probability value reported) higher median response rate than matched controls during the first 30 sessions of training; response rates continued to be significantly higher over the next 60 sessions for the 0.0025-percent group and over the next 30 sessions for the 0.015-percent group (at which points training terminated for each group). Moreover, latencies to the first response in the 30-sec interval (the beginning of the typical "fixed-interval scallop" cumulative response pattern) were significantly shorter in the 0.0025- and 0.015-percent groups. However, response rates for the group exposed to the 0.05 percent solution were significantly lower than the control group's rates for the first 40 sessions; correspondingly, response latencies were longer for the highest exposure group.

Other work by Cory-Slechta et al. (1981) repeated the earlier study's exposure regimen (using 0.005 and 0.015 percent solutions) and examined the effects on another aspect of operant performance. In this study the subjects were required to depress a bar for a specified minimum duration (0.5 to 3.0 sec) before a food pellet could be delivered. Intersubject variability increased greatly in the lead-exposed groups (see also, e.g., Cory-Slechta and Thompson, 1979; Dietz et al., 1978; Hastings et al., 1979). In general, though, treated subjects tended to shorten their response durations ($p = 0.04$ for the 0.005-percent group; $p = 0.03$ for the 0.015-percent group). This tendency would contribute toward a reduced rate of reinforcement, which is associated with (and perhaps accounts for) an observed tendency toward increased response latencies in the lead-exposed subjects ($p = 0.04$ in the 0.015-percent group). Although blood lead values were not reported by Cory-Slechta et al. (1981), brain lead concentrations at approximately PND 200 ranged from 40 to 142 ng/g for the 0.005-percent group and 320 to 1080 ng/g for the 0.015-percent group. Given the same exposure regimens in the two studies, blood lead values should be comparable.

The Gross-Selbeck and Gross-Selbeck (1981) study (partly described below in Section 12.4.3.1.5) also tested Wistar rats exposed post-weaning to a diet containing 0.05 percent lead daily until completion of behavioral testing at ~180 days of age, at which time the average blood lead level was 22.7 μg/dl. Although no differences were apparent in preliminary operant barpress training, differences between lead-treated and control groups did appear when the subjects were required to bar-press at a very high rate (e.g., 2 presses per second). The lead-treated subjects outperformed, i.e., bar-pressed more rapidly than, the control subjects.

Except for monkeys, few other species have recently been studied in sufficient detail to warrant discussion here. One of the primate studies, that by Bushnell and Bowman (1979b),

is discussed under Section 12.4.3.1.5 because it examined learning ability some time after neonatal exposure to lead had terminated. In brief, that study showed impaired discrimination reversal learning performance at 40 months of age, even though lead exposure was limited to the first 12 months and the mean blood lead level was about 32 μg/dl for the "low-lead" group during that period. When measured following behavioral testing, average blood lead concentrations were similar to control levels, i.e., 5-6 μg/dl.

Other studies of nonhuman primates, however, have examined learning ability while lead exposure was ongoing. In a more comprehensive report, to which the above-described study (Bushnell and Bowman, 1979b) was a follow-up, Bushnell and Bowman (1979a) ran a series of tests on discrimination reversal learning in rhesus monkeys over the second through sixteenth months of life. Lead acetate was fed to the subjects during the first 12 months so as to maintain nominal blood lead levels of 50 and 80 μg/dl in the low-lead and high-lead groups (actual blood lead concentrations varied considerably during the first year, particularly for the high-lead groups). Although lead dosing was terminated at 12 months, blood lead levels were still somewhat elevated over control levels at the completion of behavioral testing (18.75 ± 2.87 μg/dl, low-lead group; 46.25 ± 6.74 μg/dl, high-lead group). The basic finding that appeared consistently throughout this series of tests, including two separate experiments involving different groups of subjects (see Table 12-4), was that young rhesus monkeys with blood lead levels on the order of 30 to 50 μg/dl, compared to control groups with levels of approximately 5 μg/dl, were significantly retarded in their ability to learn a visual discrimination task in which the cues were reversed from time to time according to specified criteria. In addition, the higher exposure subjects were especially slow in mastering the first reversal problem, following extended training on the original discrimination task.

Rice and Willes (1979) attempted to replicate the Bushnell and Bowman (1979a) findings. They fed Rhesus monkeys lead acetate from day one of life and obtained blood lead concentrations in their four experimental subjects between 35 and 70 μg/dl around PND 200, which dropped to 20-50 μg/dl by PND 400; the four control subjects' levels were generally 5 μg/dl or lower. At 2-3 years of age, while lead exposure continued, the subjects were trained on a WGTA form-discrimination task similar to that used by Bushnell and Bowman (1979a). Consistent with the latter study, Rice and Willes (1979) used a reversal-learning paradigm in which the correct discriminative cue was reversed once the task was mastered. Although initially the lead-treated monkeys performed better than controls (fewer trials to criterion and fewer errors), over successive reversals (4 through 12) the control subjects made fewer errors and required fewer trials to reach criterion performance in each daily session. This difference disappeared following session 12, which was extended 500 trials beyond the criterion level ("overtraining"). Overall, the lead-treated subjects appeared to make more errors in per-

forming the reversal tasks; analysis of variance yielded a significant main effect ($p = 0.05$), but this applied only to sessions 6 through 12, which would seem to be a somewhat arbitrary selection of data for analysis. The authors did note, however, that the success of the lead-treated monkeys in the first few trials appeared to result from the treated subjects' reluctance to manipulate the novel negative stimulus after 100 pretraining trials in which only the positive stimulus was presented. Thus, the unexpected initial success of the lead-exposed subjects may have been an artifact of the pretraining procedure. By this interpretation, the lead-treated monkeys in Rice and Willes' (1979) study and the high-lead group of monkeys in Bushnell and Bowman's (1979a) study were both showing perturbed behavior, that is, refractoriness to alter their behavior under changed conditions.

Rice and her coworkers studied the same two groups of subjects at 2-3 years of age on an operant conditioning task involving a multiple fixed-interval/time-out schedule of reinforcement (Rice et al., 1979). This schedule alternated a 10- to 90-sec time-out period, during which responses were unrewarded, with an 8-min fixed interval, at the end of which a push on a lighted disk was rewarded. The lead-treated monkeys, whose blood-lead levels had by then stabilized at 20-30 μg/dl, showed a higher response rate than controls during the fixed interval and shorter pauses between responses (lower median interresponse times). The treated monkeys also tended to respond more during the time-out period, even though responses were not rewarded.

In conclusion, it appears that alterations in behavior in rats and monkeys occur as a consequence of chronic exposure to dietary lead resulting in blood lead levels on the order of 30-50 μg/dl. These alterations in behavior are clearly indicative of altered neural functioning, especially in the CNS in view of certain of the tasks employed. Another question that arises, however, is whether such alterations represent impairment in overall functioning of the lead-exposed subjects. As some studies indicate, lead-treated subjects may actually perform better than non-treated control subjects on certain learned tasks. For example, in the Winneke et al. (1982c) study, the task on which lead-exposed rats excelled required the subjects to move from one compartment to the other in a two-compartment shuttle box in order to avoid receiving an electrical shock to the feet. A successful avoidance response had to occur within 5 seconds after the onset of a warning stimulus. Similar findings have been reported by Driscoll and Stegner (1976) for shock-avoidance performance. As previously described, a study by Gross-Selbeck and Gross-Selbeck (1981) required rats to press a bar for food under an operant conditioning schedule that rewarded only high rates of responding. By responding more rapidly, the lead-treated subjects were more successful than untreated control subjects in maximizing their rewards.

Because of the contingencies of reinforcement specified in the just-cited experiments, a tendency to respond with greater alacrity or less hesitation was properly adaptive for the subjects. Other conditions, however, could make the same tendency unadaptive, as, for example, in the study by Cory-Slechta et al. (1981), which required rats to press a bar and hold it down longer than rats are normally inclined to do. In that case the lead-treated subjects were less successful than untreated controls. Thus, success or failure (or enhancement or impairment of performance) may be misleading designations for the behavioral alterations measured under arbitrary experimental conditions (cf. Penzien et al., 1982). Of greater importance may be the underlying tendency to respond more rapidly or "excessively," regardless of whether or not such responding is appropriate for the reinforcement contingencies of an experiment. Such a tendency may be inferred from results of other studies of the neurotoxic effects of lead (e.g., Angell and Weiss, 1982; Overmann, 1977; Rice et al., 1979). Taken together, these reports might be interpreted as suggesting a possible "hyper-reactivity" (cf. Winneke et al., 1982c) in lead-treated animals. They and others (e.g., Petit and Alfano, 1979) have noted the commonality of such types of behavioral deficits with experimental studies of lesions to the hippocampus (see also Sections 12.4.3.2.1 and 12.4.3.5.).

12.4.3.1.4 Effects of lead on social behavior. The social behavior and organization of even phylogenetically closely related species may be widely divergent. For this and other reasons, there is little or no basis to assume that, for example, aggressiveness in a lead-treated Rhesus monkey provides a model of aggressiveness in a lead-exposed human child. However, there are other compelling grounds for including animal social behavior in the present review. As in the case of nonsocial behavior patterns, characteristics of an animal's interactions with conspecifics may reflect neurological (especially CNS) impairment due to toxic exposure. Also, certain aspects of animal social behavior have evolved for the very purpose (in a non-teleological sense) of indicating an individual's physiological state or condition (Davis, 1982). Such behavior could potentially provide a sensitive and convenient indicator of toxicological impairment.

Two early reports (Silbergeld and Goldberg, 1973; Sauerhoff and Michaelson, 1973) suggested that lead exposure produced increased aggressiveness in rodents. Neither report, however, attempted to quantify these observations of increased aggression. Later, Hastings et al. (1977) examined aggressive behavior in rats that had been exposed to lead via their dams' milk. Solutions containing 0, 0.01, or 0.05 percent lead as lead acetate constituted the dams' drinking water from parturition to weaning at PND 21, at which time exposure was terminated. This lead treatment produced no change in growth of the pups. Individual pairs of male offspring (from the same treatment groups) were tested at PND 60 for shock-elicited aggression. Both lead-exposed groups (average blood lead levels of 5 and 9 μg/dl and brain lead levels of

8 and 14 μg/100g) showed significantly less aggressive behavior than the control group. There were no significant differences among the groups in the flinch/jump thresholds to shock, which suggests that the differences seen in shock-elicited aggression were not caused by differences in sensitivity to shock.

A study by Drew et al. (1979) utilized apomorphine to induce aggressive behavior in 90-day-old rats and found that earlier lead exposure attenuated the drug-induced aggressiveness. Lead exposure occurred between birth and weaning primarily through the dams' milk or through food containing 0.05 percent lead as lead acetate. No blood or tissue concentrations of lead were measured. There were no significant differences in the weights of the lead-treated and control animals at PND 10, 20, 30, or 90.

Using laboratory mice exposed as adults, Ogilvie and Martin (1982) also observed reduced levels of aggressive behavior. Since the same subjects showed no differences in vitality or open field activity measures, the reduction in aggressiveness did not appear to be due to a general effect of lead on motor activity. Blood lead levels were estimated from similarly treated groups as being approximately 160 μg/dl after 2 weeks of exposure and as 101 μg/dl after 4 weeks of exposure.

Cutler (1977) used ethological methods to assess the effects of lead exposure on social behavior in laboratory mice. Subjects were exposed from birth (via their dams' milk) and post-weaning to a 0.05 percent solution of lead as lead acetate (average brain lead concentrations were 2.45 nmol/g for controls and 4.38 nmol/g for experimental subjects). At 8 weeks of age social encounters between subjects from the same treatment group were analyzed in terms of numerous specified, identifiable behavioral and postural elements. The frequency and duration of certain social and sexual investigative behavior patterns were significantly lower in lead-treated mice of both sexes than in controls. Lead-exposed males also showed significantly reduced agonistic behavior compared with controls. Overall activity levels (nonsocial as well as social behavior) were not affected by the lead treatment. Average body weights did not differ for the experimental and control subjects at weaning or at the time of testing.

A more recent study by Cutler and coworkers (Donald et al., 1981) used a similar paradigm of exposure and behavioral evaluation, except that exposure occurred either only prenatally or postnatally and testing occurred at two times, 3-4 and 14-16 weeks of age. Statistically significant effects were found only for the postnatal exposure group. Although total activity in postnatally exposed mice did not differ from that of controls at either age of testing, the incidence of various social activities did differ significantly. As juveniles (3-4 weeks old), lead-treated males (and to some extent, females) showed decreased social investigation of a same-sex conspecific. This finding seems to be consistent with Cutler's (1977) earlier observations made at 8 weeks of age. Aggressive behavior, however, was almost

nonexistent in both control and lead-treated subjects in the later study, and so could not be compared meaningfully. Although the authors do not comment on this aspect of their study, it seems likely that differences in the strains of laboratory mice used as subjects could well have been responsible for the lack of aggressive behavior in the Donald et al.'s (1981) study (cf., e.g., Adams and Boice, 1981). Later testing at 14 to 16 weeks revealed that lead-exposed female subjects engaged in significantly more investigative behavior of a social or sexual nature than did control subjects, while males still showed significant reductions in such behavior when encountering another mouse of the same sex. This apparent disparity between male and female mice is one of relatively few reports of gender differences in sensitivity to lead's effects on the nervous system (cf. Cutler, 1977; Verlangieri, 1979). In this case, Donald et al. (1981) hypothesized that the disparity might have been due to differences in brain lead concentrations: 74.7 μmol/kg in males versus 191.6 μmol/kg in females (blood lead concentrations were not measured). The Donald et al. (1981) study, along with the above-mentioned study of Ogilvie and Martin (1982), point out the importance of not focusing exclusively on perinatal exposure in assessing neurotoxic effects of chronic lead exposure.

The social behavior of rhesus monkeys has also been evaluated as a function of early lead exposure. A study by Allen et al. (1974) reported persistent perturbations in various aspects of the social behavior of lead-exposed infant and juvenile monkeys, including increased clinging, reduced social interaction, and increased vocalization. However, exposure conditions varied considerably in the course of this study, with overt toxicity being evident as blood lead levels at times ranged higher than 500 μg/dl.

A more recent study consisting of four experiments (Bushnell and Bowman, 1979c) also examined social behavior in infant Rhesus monkeys, but under more systematically varied exposure conditions. In experiments 1 and 2, daily ingestion of lead acetate during the first year of life resulted in blood lead levels of 30-100 μg/dl, with consequent suppression of play activity, increased clinging, and greater disruption of social behavior when the play environment was altered. Experiment 3, a comparison of chronic and acute lead exposure (the latter resulting in a peak blood lead concentration of 250-300 μg/dl during weeks 6-7 of life), revealed little effect of acute exposure except in the disruption that occurred when the play environment was altered. Otherwise, only the chronically exposed subjects differed significantly from controls in various categories of social behavior. Experiment 4 of the study showed that prenatal exposure alone, with blood lead concentrations of exposed infants ranging between 33 and 98 μg/dl at birth, produced no detectable behavioral effects under the same procedures of evaluation. Overall, neither aggressiveness nor dominance was clearly affected by lead exposure.

Another aspect of social behavior--interaction between mothers and their offspring--was examined in lead-exposed rats by Zenick et al. (1979). Dams chronically received up to 400 mg/kg lead acetate in their drinking water on a restricted daily schedule (blood lead concentrations averaged 96.14 ± 16.54 μg/dl in the high-exposure group at day 1 of gestation). Dams and their litters were videotaped on PND 1-11, and the occurrence of certain behavior patterns (e.g., lying with majority of pups, lying away from pups, feeding) was tabulated by the experimenters. In addition, dams were tested for their propensity to retrieve pups removed from the nest. Neither analysis revealed significant effects of lead exposure on the behavior of the dams. However, restricted access to drinking water (whether lead-treated or not) appeared to confound the measures of maternal behavior.

The above studies suggest that aggressive behavior in particular is, if anything, reduced in laboratory animals as a result of exposure to lead. Certain other aspects of social behavior in laboratory mice, namely components of sexual interaction and social investigation, also appear to be reduced in lead-treated subjects, although there may be gender differences in this regard following chronic post-maturational exposure. Young rhesus monkeys also appear to be sensitive to the disruptive effects of lead on various aspects of social behavior. All of these alterations in social behavior are indicative of altered neural functioning as a consequence of lead exposure in several mammalian species.

12.4.3.1.5 Persistence of neonatal exposure effects. The specific question of persisting, long-term consequences of lead effects on the developing organism has been addressed in a number of studies by carrying out behavioral testing some time after the termination of lead exposure. Such evidence of long-term effects has been reported for rhesus monkeys by Bushnell and Bowman (1979b). Their subjects were fed lead acetate so as to maintain blood lead (PbB) levels of either 50 ± 10 (low-lead) or 80 ± 10 μg/dl (high-lead) throughout the first year of life (actual means and standard errors for the year were reported as 31.71 ± 2.75 and 65.17 ± 6.28 μg/dl). Lead treatment was terminated at 12 months of age, after which blood lead levels declined to around 5 - 6 μg/dl at 56 months. At 49 months of age the subjects were reintroduced to a discrimination reversal training procedure using new discriminative stimuli. Despite their extensive experience with the apparatus (Wisconsin General Test Apparatus) during the first two years of life, most of the high-lead subjects failed to retain the simple motor pattern (pushing aside a small wooden block) required to operate the apparatus. Remedial training largely corrected this deficit. However, both high- and low-lead groups required significantly more trials than the control group ($p < 0.05$) to reach criterion performance levels. This difference was found only on the first discrimination task and nine reversals of it. Successive discrimination problems showed no differential performance effects, which indicates that with continued training the lead-treated subjects were able to achieve the same level of performance as controls.

Studies using rats have also suggested that behavioral perturbations may be evident some time after the termination of exposure to lead. Hastings et al. (1979) exposed rat pups to lead through their mothers' milk by providing the dams 0.01 or 0.1 percent solutions of lead as lead acetate for drinking water. Exposure was stopped at weaning, at which time average blood-lead values were 29 (± 5) and 65 (± 25) μg/dl, respectively. At 120 days of age the subjects were placed on an operant conditioning simultaneous visual discrimination task. Although Hastings et al. (1979) did not actually measure blood lead levels in adult subjects at the time of behavioral testing, they presumed that the levels for control and experimental groups were by then probably quite similar, i.e., on the order of 10 μg/dl, based on prior work (Hastings et al., 1977.) Forty-six percent of the high-lead group and 37 percent of the low-lead group failed to learn the task within 60 days; only 4 percent of the control group failed to reach criterion. In terms of time to reach criterion, controls required a mean of 23 days while the low-lead subjects required 32 days and the high-lead rats 39 days (high-lead vs. controls, $p < 0.01$). Additional testing on a successive discrimination task at 270 days of age and a go/no-go discrimination task at 330 days revealed no significant differences between controls and lead-treated subjects. Since the three tests were not counter-balanced in presentation, there is no way to determine whether the lack of effects in the two latter tests may have been a function of the order of testing or age at the time of testing or, more simply, a function of the latter tests' lack of sensitivity to neurotoxic effects.

Gross-Selbeck and Gross-Selbeck (1981) also found alterations in the operant behavior of adult rats after perinatal exposure to lead via mothers whose blood lead levels averaged 20.5 μg/dl. At the time of testing (3 to 4 months postnatally) the lead-exposed subjects' blood lead levels averaged 4.55 μg/dl, compared to 3.68 μg/dl in control subjects. Although the two groups appeared qualitatively similar in their behavior in an open-field test and in preliminary bar-press training, the lead-exposed subjects tended to respond at a much higher rate than did control subjects when rewarded for responding quickly. Since the schedule differentially reinforced high response rates, the lead-exposed subjects performed more successfully than did the control subjects. This was true for three different variations on the basic schedule examined by the authors. As noted earlier, in this case, the heigtened response rate was adaptive within the context of the particular task used but may not have been under other contingencies. Most importantly here, it is indicative of altered CNS function persisting for months beyond the cessation of lead exposure early in development.

Results from the above studies indicate that behavioral effects may exist as sequelae to past lead exposure early in development of mammalian species, even though blood lead levels at the time of later behavioral assessment are essentially "normal."

12.4.3.2 Morphological Effects

12.4.3.2.1 In vivo studies. Recent key findings on the morphological effects of in vivo lead exposure on the nervous system are summarized in Table 12-5. It would appear that certain types of glial cells are sensitive to lead exposure, as Reyners et al. (1979) found a decreased density of oligodendrocytes in cerebral cortex of young rats exposed from birth to 0.1 percent lead in their food. Higher exposure concentrations (0.2-0.4 percent lead salts), especially during the prenatal period (Bull et al., 1983), can reduce synaptogenesis and retard dendritic development in the cerebral cortex (McCauley and Bull, 1978; McCauley et al., 1979, 1982) and the hippocampus of developing rats (Campbell et al., 1982, and Alfano and Petit, 1982). Some of these effects, e.g. on cerebral cortex appear to be transient (McCauley et al., 1979, 1982). Suckling rats subjected to increasing exposures of lead exhibit more pronounced effects, such as reduction in the number and average diameter of axons in the optic nerve at 0.5 percent lead acetate exposure (Tennekoon et al., 1979), a general retardation of cortical synaptogenesis at 1.0 percent lead carbonate exposure (Averill and Needleman, 1980), or a reduction in cortical thickness at 4.0 percent lead carbonate exposure (Petit and LeBoutillier, 1979). This latter exposure concentration also causes a delay in the onset and peak of Schwann cell division and axonal regrowth in regenerating peripheral nerves in chronically exposed adult rats (Ohnishi and Dyck, 1981). In short, both neuronal and glial components of the nervous system appear to be affected by neonatal or chronic lead exposure.

Organolead compounds have also been demonstrated to have a deleterious effect on the morphological development of the nervous system. Seawright et al. (1980) administered triethyl lead acetate (Et_3Pb) by gavage to weanling (40-50 g) and "young adult" (120-150 g) rats. Single doses of 20 mg Et_3Pb/kg caused impaired balance, convulsions, paralysis, and coma in both groups of treated animals. Peak levels in blood and brain were noted two days after exposure, with extensive neuronal necrosis evident in several brain regions by three days post-treatment. Weekly exposures to 10 mg Et_3Pb/kg for 19 weeks resulted in less severe overt signs of intoxication (from which the animals recovered) and moderate to severe loss of neurons in the hippocampal region only.

12.4.3.2.2 In vitro studies. Björklund et al. (1980) placed tissue grafts of developing nervous tissue in the anterior eye chambers of adult rats. When the host animals were given 1 or 2 percent lead acetate in their drinking water, the growths of substantia nigral and hippocampal, but not cerebellar, grafts were retarded. Grafts of the developing cerebral cortex in host animals receiving 2 percent lead exhibited a permanent 50 percent reduction in size (volume), whereas 1 percent lead produced a slight increase in size in this tissue type. The authors felt that this anomalous result might be explained by a hyperplasia of one particular cell type at lower concentrations of lead exposure.

TABLE 12-5. SUMMARY OF KEY STUDIES OF MORPHOLOGICAL EFFECTS OF IN VIVO LEAD EXPOSURE

Species	Exposure protocol	Peak blood lead level	Observed effect	Reference
Young rats	0.1% Pb^{++} in chow PND 0-90		Decreased density of oligodendrocytes in cerebral cortex	Reyners et al. (1979)
	0.1% $Pb(Ac)_2$ in dams' drinking water PND 0-32		Significant inhibition in myelin deposition and maturation in whole brain	Stephens and Gerber (1981)
	0.2% $PbCl_2$ in dams' drinking water from gestation thru PND0	80 µg/dl at birth	Less mature synoptic profile in cerebral cortex at PND-15	McCauley and Bull (1978) McCauley et al. (1979)
			30% reduction in synoptic density in cerebral cortex at PND15 (returned to normal at PND21)	McCauley et al. (1982)
	0.2% $Pb(Ac)_2$ in dams' drinking water PND 0-25		15-30% reduction in synaptic profiles in hippocampus	Campbell et al. (1982)
	0.4% $PbCO_3$ in dams' drinking water PND 0-30		Retardation in temporal sequence of hippocampal dendritic development	Alfano and Petit (1982)
	0.5% $Pb(Ac)_2$ in dams' drinking water PND 0-21		10-15% reduction in number of axons in optic nerve; skewing of fiber diameters to smaller sizes	Tennekoon et al. (1979)
	1% $PbCO_3$ in chow PND 0-60	385 µg/dl (PND 21)	Retardation of cortical synaptogenesis over and above any nutritional effects	Averill and Needleman (1980)
	4% $PbCO_3$ in dams' chow PND 0-28	258 µg/dl (PND 28)	13% reduction in cortical thickness and total brain weight; reduction in synaptic density	Petit and LeBoutillier (1979)
	4% $PbCO_3$ in dams' chow PND 0-25		Reduction in hippocampal length and width; similar reduction in afferent projection to hippocampus	Alfano et al. (1982)
Adult rats	4% $PbCO_3$ in chow for 3 mos.		Delay in onset and peak of Schwann cell division and axonal regrowth in regenerating nerves	Ohnishi and Dyck (1981)
	4% $PbCO_3$ in chow PND 0-150	300 µg/dl (PND 150)	Demyelination of peripheral nerves beginning PND 20-35	Windebank et al. 1980

PND: post-natal day

$Pb(Ac)_2$: lead acetate

$PbCO_3$: lead carbonate

Organolead compounds have also been demonstrated to affect neuronal growth (Grundt et al., 1981). Cultured cells from embryonic chick brain were exposed to 3.16 μM triethyllead chloride in the incubation medium for 48 hr, resulting in a 50 percent reduction in the number of cells exhibiting processes. There was no observed effect on glial morphology.

Other investigations have focused on morphological aspects of the blood-brain barrier and its possible disruption by lead intoxication (Kolber et al., 1980). Capillary endothelial cells isolated from rat cerebral cortex and exposed to 100 μM lead acetate *in vitro* (Silbergeld et al., 1980b) were examined by electron microscopy and X-ray microprobe analysis. Lead deposits were found to be sequestered preferentially in the mitochondria of these cells in much the same manner as calcium. This affinity may be the basis for lead-induced disruption of transepithelial transport of Ca^{2+} and other ions.

12.4.3.3 Electrophysiological Effects.

12.4.3.3.1 *In vivo studies*. Recent key findings on the electrophysiological effects of *in vivo* lead exposure are summarized below in Table 12-6. The visual system appears to be particularly susceptible to perturbation by neonatal lead exposure. Suckling rats whose dams were given drinking water containing 0.2 percent lead acetate had significant alterations in their visual evoked responses (VER) and decreased visual acuity at PND 21, at which time their blood lead levels were 65 μg/dl (Cooper et al., 1980; Fox et al., 1977; Impelman et al., 1982; Fox and Wright, 1982; Winneke, 1980). Both of these observations are indicative of depressed conduction velocities in the visual pathways. These same exposure levels also increased the severity of the maximal electroshock seizure (MES) response in weanling rats who exhibited blood lead levels of 90 μg/dl (Fox et al., 1978, 1979). The authors speculated that neonatal lead exposure acts to increase the ratio of excitatory to inhibitory systems in the developing cerebrospinal axis. Such exposure can also lead to lasting effects on the adult nervous system, as indicated by persistent decreases in visual acuity and spatial resolution in 90-day old rats exposed only from birth to weaning to 0.2 percent lead acetate (Fox et al., 1982).

The adult nervous system is also vulnerable to lead-induced perturbation at low levels of exposure. Hietanen et al. (1980) found that chronic exposure of adult rabbits to 0.2 percent lead acetate in drinking water resulted in an 85 percent inhibition of motor conduction velocity in the sciatic nerve.

12.4.3.3.2 *In vitro studies*. Palmer et al. (1981) and Olson et al. (1981) looked at intraocular grafts of cerebellar tissue from 14- to 15-day-old rats in host animals treated for 2 months with drinking water containing 1 percent lead acetate, followed by plain water for 4-5 months. They found no alterations in total growth or morphology of grafts in treated vs. control hosts, yet the Purkinje neurons in the lead-exposed grafts had almost no spontaneous activity. Host cerebellar neurons, on the other hand, and both host and graft neurons in

TABLE 12-6. SUMMARY OF KEY STUDIES OF ELECTROPHYSIOLOGICAL EFFECTS OF IN VIVO LEAD EXPOSURE

Species	Exposure protocol	Peak blood lead level	Observed effect	Reference
Suckling rat	0.2% $Pb(Ac)_2$ in dams' drinking water PND 0-20	90 µg/dl (PND 20)	More rapid appearance and increased severity of MES response	Fox et al. (1978, 1979)
	0.2% $Pb(Ac)_2$ in dams' drinking water PND 0-21	65 µg/dl (PND 21)	1) Increased latencies and decreased amplitudes of primary and secondary components of VER; 2) decreased conduction velocities in visual pathways; 3) 25-50% decrease in scotopic visual acuity 4) persistent decreases in visual acuity and spatial resolution at PND 90	Fox et al. (1977); Impelman et al. (1982); Cooper et al. (1980); Winneke (1980); Fox and Wright (1982) Fox et al. (1982)
Young rhesus monkeys	$Pb(Ac)_2$ solutions in food PND 0-365	300 µg/dl (PND 60) 85 µg/dl	Severe impairment of discrimination accuracy; loss of scotopic function	Bushnell et al. (1977)
Adult rabbit	0.2% $Pb(Ac)_2$ in drinking water for 4 weeks		85% reduction in motor conduction velocity of sciatic nerve	Hietanen et al. (1980)

PND: post-natal day
$Pb(Ac)_2$: lead acetate
MES: maximal electroshock seizure
VER: visual evoked response

PRELIMINARY DRAFT

control animals, all exhibited significant levels of spontaneous activity. Taylor et al. (1978) recorded extracellularly from cerebellar Purkinje cells in adult rats both in situ and in intraocular grafts in an effort to determine what effect lead had on the norepinephrine (NE)-induced inhibition of Purkinje cell spontaneous discharge. Application of exogenous NE to both in situ and in oculo cerebellum produced 61 and 49 percent inhibitions of spontaneous activity, respectively. The presence of 5-10 μM lead reduced this inhibition to 28 and 13 percent, respectively. This "disinhibition" was specific for NE, as responses to both cholinergic and parallel fiber stimulation in the same tissue remained the same. Furthermore, application of lead itself did not affect spontaneous activity, but did inhibit adenylate cyclase activity in cerebellar homogenates at the same concentration required to disinhibit the NE-induced reduction of spontaneous activity (3 to 5 μM).

Fox and Sillman (1979) looked at receptor potentials in the isolated, perfused bullfrog retina and found that additions of lead chloride caused a reversible, concentration-dependent depression of rod (but not cone) receptor potentials. Concentrations of 5 μM produced an average 16 percent depression, while 12.5 μM produced an average 23 percent depression.

Evidence that lead does indeed resemble other divalent cations, in that it appears to interfere with chemically mediated synaptic transmission, has been obtained in studies of peripheral nerve function. For example, lead is capable of blocking neural transmission at peripheral adrenergic synapses (Cooper and Steinberg, 1977). Measurements of the contraction force of the rabbit saphenous artery following stimulation of the sympathetic nerve endings indicated that lead blocks muscle contraction by an effect on the nerve terminals rather than by an effect on the muscle. Since the response recovered when the Ca^{2+} concentration was increased in the bathing solution, it was concluded that lead does not deplete transmitter stores in the nerve terminals, but more likely blocks NE release.

It has also been demonstrated that lead depresses synaptic transmission at the peripheral neuromuscular junction by impairing acetylcholine (ACh) release from presynaptic terminals (Kostial and Vouk, 1957; Manalis and Cooper, 1973; Cooper and Manalis, 1974). This depression of neurotransmitter release evoked by nerve stimulation is accompanied by an increase in the spontaneous release of ACh, as evidenced by the increased frequency of spontaneous miniature endplate potentials (MEPPs). Kolton and Yaari (1982) found that this increase in MEPPs in the frog nerve/muscle preparation could be induced by lead concentrations as low as 5 μM.

The effects of lead on neurotransmission within the central nervous system have also been studied. For example, Kim et al. (1980) fed adult rabbits 165 mg lead carbonate per day for five days and looked at Ca^{2+} retention in brain slices. Treated animals showed a 75 percent increase in Ca^{2+} retention time, indicating that lead inhibited the mediated efflux of Ca^{2+} from the incubated brain slice. Investigation of the in vitro effects of lead on Ca^{2+} binding

was carried out by Silbergeld and Adler (1978) on caudate synaptosomes. They determined that 50 μM lead caused an 8-fold increase in $^{45}Ca^{2+}$ binding and that in both control and lead-treated preparations the addition of ATP increased binding, while ruthenium red and Ca^{2+} decreased it. Further findings in this series of experiments demonstrated that lead inhibits the Na^{+}-stimulated loss of Ca^{2+} by mitochondria and that blockade of dopamine (DA) uptake by 5 μM benztropine reversed the lead-stimulated increase in Ca^{2+} uptake by synaptosomes. The authors concluded that lead affects the normal mechanisms of Ca^{2+} binding and uptake, perhaps by chelating with DA in order to enter the nerve terminal. By inhibiting the release of Ca^{2+} bound to mitochondria there, lead essentially causes an increase in the Ca^{2+} concentration gradient across the nerve terminal membrane. As a result, more Ca^{2+} would be expected to enter the nerve terminal during depolarization, thus effectively increasing synaptic neurotransmission at dopaminergic terminals without altering neuronal firing rates.

12.4.3.4 Biochemical Alterations. The majority of previous investigations of biochemical alterations in the nervous system following exposure to lead have focused on perturbations of various neurotransmitter systems, probably because of the documentation extant on the neurophysiological and behavioral roles played by these transmitters. Recently, however, somewhat more attention has been centered on the impact of lead exposure on energy metabolism and other cellular homeostatic mechanisms such as protein synthesis and glucose transport. A significant portion of this work has, however, been conducted *in vitro*.

12.4.3.4.1 In vivo studies. Recent key findings on the biochemical effects of *in vivo* exposure are summarized in Table 12-7. Although the majority of recent work has continued to focus on neurotransmitter function, it appears that the mechanisms of energy metabolism are also particularly vulnerable to perturbation by lead exposure. McCauley, Bull, and coworkers have demonstrated that exposure of prenatal rats to 0.02 percent lead chloride in their dams' drinking water leads to a marked reduction in cytochrome content in cerebral cortex, as well as a possible uncoupling of energy metabolism. Although the reduction in cytochrome content is transient and disappears by PND 30, it occurs at blood lead levels as low as 36 μg/dl (McCauley and Bull, 1978; Bull et al., 1979); delays in the development of energy metabolism may be seen as late as PND 50 (Bull, 1983).

There does not appear to be a selective vulnerability of any particular neurotransmitter system to the effects of lead exposure. Pathways utilizing dopamine (DA), norepinephrine (NE), serotonin (5-HT), and γ-aminobutyric acid (GABA) are all affected in neonatal animals at lead-exposure concentrations of 0.2-2.0 percent lead salts in dams' drinking water. Although the blood lead values reported following exposure to the lower lead concentrations (0.2-0.25 percent lead acetate or lead chloride) range from 47 μg/dl (Goldman et al., 1980) to 87 μg/dl (Govoni et al., 1980), a few general observations can be made:

TABLE 12-7. SUMMARY OF KEY STUDIES ON BIOCHEMICAL EFFECTS OF IN VIVO LEAD EXPOSURE

Species	Exposure protocol	Peak blood lead level	Observed effect	Reference
Suckling rat	0.004% $Pb(Ac)_2$ in dams' drinking water PND 0-35		Decline in synthesis and turnover of striatal DA	Govoni et al. (1979, 1980); Memo et al. (1980a, 1981)
	0.02% $PbCl_2$ in dams' drinking water from gestation thru PND 0-21	80 µg/dl (at birth) 36 µg/dl (PND 21)	1) Transient 30% reduction in cytochrome content of cerebral cortex; 2) possible uncoupling of energy metabolism 3) delays in development of energy metabolism	McCauley and Bull (1978); McCauley et al. (1979); Bull et al. (1979) Bull (1983)
	0.2% $Pb(Ac)_2$ in dams' drinking water PND 0-21	47 µg/dl (PND 21)	1) 23% decrease in NE levels of hypotholamus and striatum; 2) increased turnover of NE in brainstem	Goldman et al. (1980)
	0.25% $Pb(Ac)_2$ in dams' drinking water PND 0-35		Decline in synthesis and turnover of striatal DA	Govoni et al. (1978a)
	0.25% $Pb(Ac)_2$ in dams' drinking water PND 0-35		Increase in DA synthesis in frontal cortex and nuc. accumbens(10-30% and 35-45%, respectively)	Govoni et al. (1979, 1980); Memo et al. (1980a, 1981)
	0.25% $Pb(Ac)_2$ in dams' drinking water PND 0-35		1) 50% increase in DA binding to striatal D_2 receptors; 2) 33% decrease in DA binding to nuc. accumbens D_2 receptors	Lucchi et al. (1981)
	0.25% $Pb(Ac)_2$ in dams' drinking water PND 0-42	87 µg/dl (PND 42)	1) 31% increase in GABA specific binding in cerebellum; 53% increase in GMP activity; 2) 36% decrease in GABA-specific binding in striatum; 47% decrease in GMP activity	Govoni et al. (1978b, 1980)

TABLE 12-7. (continued)

Species	Exposure protocol	Peak blood lead level	Observed effect	Reference
	0.25% $Pb(Ac)_2$ in dam's drinking water PND 0-21; 0.004% or 0.25% until PND 42		1) 12 and 34% elevation of GABA binding in cerebellum for 0.004% and 0.25%, respectively; 2) 20 and 45% decreases in GABA binding in striatum for 0.004% and 0.25%, respectively	Memo et al. (1980b)
	0.5-1% $Pb(Ac)_2$ in drinking water PND 0-60		1) Increased sensitivity to seizures induced by GABA blockers; 2) increase in GABA synthesis in cortex and striatum; 3) inhibition of GABA uptake and release by synaptosomes from cerebellum and basal ganglia; 4) 70% increase in GABA-specific binding in cerebellum	Silbergeld et al. (1979, 1980a)
	0.25-1% $Pb(Ac)_2$ in drinking water PND 0-60	72-91 g/dl (PND 21)	1) 40-50% reduction of whole-brain ACh by PND 21; 2) 36% reduction by PND 30 (return to normal values by PND 60)	Modak et al. (1978)
	75 mg $Pb(Ac)_2$/kg b.w./day via gastric intubation PND 2-14	98 µg/dl (PND 15)	1) 20% decline in striatal DA levels at PND 35; 2) 35% decline in striatal DA turnover by PND 35; 3) Transient depression of DA uptake at PND 15; 4) Possible decreased DA terminal density	Jason and Kellogg (1981)
Young rat	2% $Pb(Ac)_2$ in dam's drinking water PND 0-21 then 0.002-0.008% until PND 56		1) non-dose-dependent elevations of NE in midbrain (60-90%) and DA and 5-HT in midbrain, striatum and hypothalamus (15-30%); 2) non-dose-dependent depression of NE in hypothalamus and striatum (20-30%).	Dubas et al. (1978)

PND: post-natal day
$Pb(Ac)_2$: lead acetate
$PbCl_2$: lead chloride
NE: norepinephrine
DA: dopamine
GABA: γ-aminobutyric acid
GMP: guanosine monophosphate
5-HT: serotonin

(1). Synthesis and turnover of DA and NE are depressed in the striatum, and elevated in midbrain, frontal cortex, and nucleus accumbens. This seems to be paralleled by concomitant increases in DA-specific binding in striatum and decreases in DA-specific binding in nucleus accumbens, possibly involving a specific subset (D_2) of DA receptors (Lucchi et al., 1981). These findings are probably reflective of sensitization phenomena resulting from changes in the availability of neurotransmitter at the synapse.

(2). The findings for pathways utilizing GABA show similar parallels. Increases in GABA synthesis in striatum are coupled with decreases in GABA-specific binding in that region, while the converse holds true for the cerebellum. In these cases, cyclic GMP activity mirrors the apparent changes in receptor function. This increased sensitivity of cerebellar postsynaptic receptors (probably a response to the lead-induced depression of presynaptic function) is likely the basis for the finding that lead-treated animals are more susceptible to seizures induced by GABA-blocking agents such as picrotoxin or strychnine (Silbergeld et al., 1979).

12.4.3.4.2 In vitro studies. Any alterations in the integrity of the blood-brain barrier can have serious consequences for the nervous system, especially in the developing organism. Kolber et al. (1980) examined glucose transport in isolated microvessels prepared from the brains of suckling rats given 25, 100, 200, or 1000 mg lead/kg body weight daily by intragastric gavage. On PND 25, they found that even the lowest dose blocked specific transport sites for sugars and damaged the capillary endothelium. In vitro treatment of the preparation with concentrations of lead as low as 0.1 μM produced the same effects.

Purdy et al. (1981) examined the effects in rats of varying concentrations of lead acetate on the whole-brain synthesis of tetrahydrobiopterin (BH_4), a cofactor for many important enzymes, including those regulating catecholamine synthesis. Concentrations of lead as low as 0.01 μM produced a 35 percent inhibition of BH_4 synthesis, while 100 μM inhibited the BH_4 salvage enzyme, dihydropteridine reductase, by 40 percent. This would result in a decreased conversion of phenylalanine to tyrosine and thence to DOPA (the initial steps in dopamine synthesis), as well as decreases in the conversion of trytophan to its 5-hydroxy form (the initial step in serotonin synthesis). These decrements, if occurring in vivo, could not be ameliorated by increased dietary intake of BH_4, as it does not cross the blood-brain barrier.

Lead has also been found to have an inhibitory effect on mitochondrial respiration in the cerebrum and cerebellum of immature or adult rats at concentrations greater than 50 μM (Holtzman et al., 1978b). This effect, which was equivalent in both brain regions at both ages studied, is apparently due to an inhibition of nicotinamide adenine dinucleotide (NAD)-linked dehydrogenases within the mitochondrial matrix. These same authors found that this lead-induced effect, which is an energy-dependent process, could be blocked in vitro by

addition of ruthenium red to the incubation medium (Holtzman et al., 1980b). In view of the fact that Ca^{2+} uptake and entry into the mitochondrial matrix is also blocked by ruthenium red, it is possible that both lead and Ca^{2+} share the same binding site/carrier in brain mitochondria. These findings are supported by the work of Gmerek et al. (1981) on adult rat cerebral mitochondria, with the exception that they observed respiratory inhibition at 5 μM lead acetate, which is a full order of magnitude lower than the Holtzman et al. (1978b, 1980b) studies. Gmerek and co-workers offer the possibility that this discrepancy may have been due to the inadvertent presence of EDTA in the incubation medium used by Holtzman et al.

Organolead compounds have also been demonstrated to have a deleterious effect on cellular metabolism in the nervous system. For example, Grundt and Neskovic (1980) found that concentrations of triethyl lead chloride as low as 5-7 μM caused a 40 percent decrease in the incorporation of SO_4 or serine into myelin galacto-lipids in cerebellar slices from 2-week-old rats. Similarly, Konat and coworkers (Konat and Clausen, 1978, 1980; Konat et al., 1979) observed that 3 μM triethyl lead chloride preferentially inhibited the incorporation of leucine into myelin proteins in brain stem and forebrain slices from 22-day-old rats. This apparent inhibition of myelin protein synthesis was two-fold greater than that observed for total protein synthesis (approximately 10 vs. 20 percent, respectively). In addition, acute intoxication of these animals by i.p. injection of triethyl lead chloride at 8 mg/kg produced equivalent results accompanied by a 30 percent reduction in total forebrain myelin content.

Interestingly, while a suspension of cells from the forebrain of these animals (Konat et al., 1978) exhibited a 30 percent inhibition of total protein synthesis at 20 μM triethyl lead chloride (the lowest concentration examined), a cell-free system prepared from the same tissue was not affected by triethyl lead chloride concentrations as high as 200 μM. This result, coupled with a similar, although not as severe, inhibitory effect of triethyl lead chloride on oxygen consumption in the cell suspension (20 percent inhibition at 20 μM) would tend to indicate that the inhibition of rat forebrain protein synthesis is related to an inhibition of cellular energy-generating systems.

The effects of organolead compounds on various neurotransmitter systems have been investigated in adult mouse brain homogenates. Bondy et al. (1979a,b) demonstrated that micromolar concentrations (5 μM) of tri-n-butyl lead (TBL) acetate were sufficient not only to cause a 50 percent decline in the high affinity uptake of GABA and DA in such homogenates, but also to stimulate a 25 percent increase in GABA and DA release. These effects were apparently selective for DA neurons at lower concentrations, as only DA uptake or release was affected at 0.1 μM, albeit mildly so. The effect of TBL acetate on DA uptake appears to be specific, as there is a clear dose-response relationship down to 1 μM TBL (Bondy and Agarwal, 1980) for inhibition (0-60 percent) of spiroperidol binding to rat striatal DA receptors. A concomitant

inhibition of adenyl cyclase in this dose range (50 percent) suggests that TBL may affect the entire postsynaptic binding site for DA.

12.4.3.5 Accumulation and Retention of Lead in the Brain. All too infrequently, experimental studies of the neurotoxic effects of lead exposure do not report the blood-lead levels achieved by the exposure protocols used. Even less frequently reported are the concomitant tissue levels found in brain or other tissues. From the recent information that is available, however, it is possible to draw some limited conclusions about the relationship of exposure concentrations to blood and brain lead concentrations. Table 12-8 calculates the blood lead/brain lead ratios found in recent studies where such information was available. It can be seen that, at exposure concentrations greater than 0.2 percent and for exposure periods longer than birth until weaning (21 days in rats), the ratio generally falls below unity. This suggests, that, even as blood lead levels reach a steady state and then fall due to excretion or some other mechanism, lead continues to accumulate in brain.

Further evidence bearing on this was derived from a set of studies by Goldstein et al. (1974), who reported that administration of a wide range of doses of radioactive lead nitrate to one-month-old rats resulted in parallel linear increases in both blood and brain lead levels during the ensuing 24 hours. This suggests that deposition of lead in brain occurs without threshold and that, at least initially, it is proportional to blood lead concentration. However, further studies by Goldstein et al. (1974) followed changes in blood and brain lead concentrations after cessation of lead exposure and found that, whereas blood lead levels decreased dramatically (by an order of magnitude or more) during a 7-day period, brain lead levels remained essentially constant over the one-week postexposure period. Thus, with even intermittent exposures to lead, it is not unexpected that brain concentrations would tend to remain the same or even to increase although blood lead levels may have returned to "normal" levels. Evidence confirming this comes from findings of: (1) Hammond (1971), showing that EDTA administration causing marked lead excretion in urine of young rats did not significantly lower brain lead levels in the same animals; and (2) Goldstein et al. (1974), showing that although EDTA prevented the in vitro accumulation of lead into brain mitochondria, if lead was added first then EDTA was ineffective in removing lead from the mitochondria. These results, overall, indicate that, although lead may enter the brain in rough proportion to circulating blood lead concentrations, it is then taken up by brain cells and tightly bound into certain subcellular components (such as mitochondrial membranes) and retained there for quite long after initial external exposure ceases and blood lead levels markedly decrease. This may help to account for the persistence of neurotoxic effects of various types noted above long after the cessation of external lead exposure.

TABLE 12-8. INDEX OF BLOOD LEAD AND BRAIN LEAD LEVELS FOLLOWING EXPOSURE

Species (strain)	Exposure	Time of assay	Blood lead (µg/dl)	Brain lead (µg/100g)	Blood:brain lead ratio	Reference
Suckling rat (Charles River-CD)	0.0005% $PbCl_2$ in water PND 0-21	PND 21	12	8	1.5	Bull et al. (1979)
	0.003% $PbCl_2$ in water PND 0-21	PND 21	21	11	1.9	
Suckling rat (Charles River)	0.005% $Pb(Ac)_2$ in water from conception	PND 11 PND 30	22 18	3 11	7.0 1.6	Grant et al. (1980)
	0.01% $Pb(Ac)_2$ in water from conception	PND 11 PND 30	35 48	7 22	5.0 2.2	
Suckling rat (Charles River-CD)	0.02% $PbCl_2$ in water PND 0-21	PND 21	36	25	1.4	Bull et al. (1979)
Suckling rat (Long-Evans)	0.02% $Pb(Ac)_2$ in water PND 0-21	PND 10 PND 21	21.7 25.2	6.3 13	3.4 1.9	Fox et al. (1979)
Suckling rat (Long-Evans)	0.02% $Pb(Ac)_2$ in water from PND 0-21	PND 21	29	29	1.0	Hastings et al. (1979)
Suckling rat (Holtzman-albino)	0.05% $Pb(Ac)_2$ in water PND 0-21	PND 21	12	20	0.6	Goldman et al. (1980)
	0.1% $Pb(Ac)_2$ in water PND 0-21	PND 21	20	50	0.4	
Suckling rat	0.2% $Pb(Ac)_2$ in water PND 0-21	PND 21	65	65	1.0	Hastings et al. (1979)
Suckling rat (Holtzman-albino)	0.2% $Pb(Ac)_2$ in water PND 0-21	PND 21	47	80	0.6	Goldman et al. (1980)
Suckling rat (Long-Evans)	0.2% $Pb(Ac)_2$ in water PND 0-21	PND 10 PND 21	49.6 89.4	19 82	2.6 1.1	Fox et al. (1979)
Suckling rat (Long-Evans)	0.2% $Pb(Ac)_2$ in water PND 0-21	PND 21	65.0	53	1.2	Fox et al. (1977)
Suckling rat (Long-Evans)	0.2% $Pb(Ac)_2$ in water PND 0-21	PND 21	65.1	53	1.2	Cooper et al. (1980)
Suckling mice (ICR Swiss albino)	0.25% $Pb(Ac)_2$ in water PND 0-21	PND 21	72	230	0.3	Modak et al. (1978)
Suckling rat (Wistar)	0.2% $Pb(Ac)_2$ in water PND 2-60	PND 30 PND 60	115* 35*	84 99	- -	Shigeta et al. (1979)
	0.5% $Pb(AC)_2$ in water PND 2-60	PND 30 PND 60	308* 73*	172 222	- -	

Table 12-8. (continued)

Species (strain)	Exposure	Time of assay	Blood lead (µg/dl)	Brain lead (µg/100g)	Blood:brain lead ratio	Reference
Suckling rat (Sprague-Dawley)	0.25% $Pb(Ac)_2$ in water from gestation until PND 42	PND 42	87	85	1.0	Govani et al. (1980)
	0.5% $Pb(Ac)_2$ in water PND 0-21	PND 21	70	280	0.25	
	1% $Pb(Ac)_2$ in water PND 0-21	PND 21	91	270	0.3	
Suckling rat (Sprague-Dawley)	4% $PbCO_3$ in water PND 0-27	PND 27	---	1.36	---	Wince et al. (1980)
Suckling rat (Long-Evans)	25 mg/kg $Pb(Ac)_2$ by gavage PND 2-14	PND 15	50	40	1.3	Jason and Kellogg (1981)
	75 mg/kg $Pb(Ac)_2$ by gavage PND 2-14	PND 15	98	60	1.6	
Young mice (ICR Swiss albino)	0.25% $Pb(Ac)_2$ in water PND 0-60	PND 60	91	410	0.2	Modak et al. (1978)
	0.5% $Pb(Ac)_2$ in water PND 0-60	PND 60	194	360	0.5	
	1% $Pb(Ac)_2$ in water PND 0-60	PND 60	223	810	0.3	
Adult rat (Charles River-CD)	0.0005% $Pb(Ac)_2$ in water for 21 days		9	10	0.9	Bull et al. (1979)
	0.003% $Pb(Ac)_2$ in water for 21 days		11	12	0.9	
	0.02% $Pb(Ac)_2$ in water for 21 days		29	100	0.29	
Adult rat (Wistar)	0.15% $Pb(Ac)_2$ in water for 3 months		31	12-18 (depending on region)	2.6-1.7 (depending on region)	Ewers and Erbe (1980)
	0.4% $Pb(Ac)_2$ in water for 3 months		69	16-34 (depending on region)	4.3-2.0 (depending on region)	
	1% $Pb(Ac)_2$ in water for 3 months		122	37-72 (depending on region)	3.3-1.7 (depending on region)	

PND: post-natal day

$Pb(Ac)_2$: lead acetate

$PbCl_2$: lead chloride

*Expressed as µg Pb/100g blood.

The uptake of lead into specific neural and non-neuronal elements of the brain has also been studied and provides insight into possible morphological correlates of certain lead effects discussed above and below as being observed in vivo or in vitro. For example, Stumpf et al. (1980), via autoradiographic localization of ^{210}Pb, found that ependymal cells, glial cells, and endothelial cells of brain capillaries concentrate and retain lead above background levels for several days after injections of tracer amounts of the elements. These cells are non-neural elements of brain important in the maintenance of "blood-brain barrier" functions, and their uptake and retention of lead, even with tracer doses, provides evidence of a morphological basis by which lead effects on blood-brain barrier functions may be exerted. Again, the retention of lead in these non-neuronal elements for at least several days after original exposure points towards the plausibility of lead exerting effects on blood-brain barrier functions long after external exposure ceases and blood lead levels decrease back toward normal levels. Uptake and concentration of lead in the nuclei of some cortical neurons even several days after administration of only a tracer dose of ^{210}Pb was also observed by Stumpf et al. (1980) and provide yet another plausible morphological basis by which neurotoxic effects might be exerted by lead long after external exposure terminates and blood lead levels return to apparently "normal" levels.

12.4.4 Integrative Summary of Human and Animal Studies of Neurotoxicity

An assessment of the impact of lead on human and animal neurobehavioral function raises a number of issues. Among the key points addressed here are: (1) the internal exposure levels, as indexed by blood lead levels, at which various adverse neurobehavioral effects occur; (2) the reversibility of such deleterious effects; and (3) the populations that appear to be most susceptible to neural damage. In addition, the question arises as to the utility of using animal studies to draw parallels to the human condition.

12.4.4.1 Internal Exposure Levels at Which Adverse Neurobehavioral Effects Occur. Markedly elevated blood lead levels are associated with neurotoxic effects of lead exposure (including severe, irreversible brain damage as indexed by the occurrence of acute and/or chronic encephalopathic symptoms) in both humans and and animals. For most adult humans, such damage typically does not occur until blood lead levels exceed 120 µg/dl. Evidence does exist, however, for acute encephalopathy and death occurring in some human adults at blood lead levels below 120 µg/dl. In children, the effective blood lead level for producing encephalopathy or death is lower, starting at approximately 100 µg/dl. Again, however, evidence exists for encephalopathy occurring in some children at lower blood lead levels, i.e., at 80-100 µg/dl.

It should be emphasized that, once encephalopathy occurs, death is not an improbable outcome, regardless of the quality of medical treatment available at the time of acute crisis.

In fact, certain diagnostic or treatment procedures themselves tend to exacerbate matters and push the outcome toward fatality if the nature and severity of the problem are not fully recognized or properly diagnosed. It is also crucial to note the rapidity with which acute encephalopathic symptoms can develop or death can occur in apparently asymptomatic individuals or in those apparently only mildly affected by elevated body burdens of lead. It is not unusual for rapid deterioration to occur, with convulsions or coma suddenly appearing and with progression to death within 48 hours. This strongly suggests that, even in apparently asymptomatic individuals, rather severe neural damage probably does exist at high blood lead levels even though it is not yet overtly manifested in obvious encephalopathic symptoms. This conclusion is further supported by numerous studies showing that children with high blood lead levels (over 80-100 μg/dl), but not observed to manifest acute encephalopathic symptoms, are permanently cognitively impaired, as are most children who survive acute episodes of frank lead encephalopathy.

Other evidence tends to confirm that some type of neural dysfunction exists in apparently asymptomatic children, even at much lower levels of blood lead. The body of studies on low- or moderate-level lead effects on neurobehavioral functions, as summarized in Table 12-1, presents a rather impressive array of data pointing to that conclusion. Several well-controlled studies have found effects that are clearly statistically significant, whereas others have found nonsignificant but borderline effects. Even certain studies reporting generally nonsignificant findings at times contain data confirming some statistically significant effects, which the authors attribute to various extraneous factors. It should also be noted that, given the apparent non-specific nature of some of the behavioral or neural effects probable at low levels of lead exposure, one would not expect to find striking differences in every instance. The lowest blood lead levels associated with significant neurobehavioral (e.g. cognitive) deficits both in apparently asymptomatic children and in developing rats and monkeys generally appear to be in the range of 30-50 μg/dl. Also, certain behavioral and electrophysiological effects indicative of CNS deficits have been reported at lower levels, supporting a continuous dose-response relationship between lead and neurotoxicity. Such effects, when combined with adverse social factors (such as low parental IQ, low socioeconomic status, poor nutrition, and poor quality of the caregiving environment) can place children, especially those below the age of three years, at significant risk. However, it must be acknowledged that nutritional covariates, as well as demographic social factors, have been poorly controlled in many of the pediatric neurobehavioral studies reviewed above. Socioeconomic status also is a crude measure of parenting and family structure that requires further assessment as a possible contributor to observed results of neurobehavioral studies.

Timing, type, and duration of exposure are also important factors in both animal and human studies. It is often uncertain whether observed blood lead levels represent the levels that were responsible for observed behavioral deficits. Monitoring of lead exposures in human subjects in all cases has been highly intermittent or non-existent during the period of life preceding neurobehavioral assessment. In most human studies, only one or two blood lead values are provided per subject. Tooth lead may be an important cumulative exposure index; but its modest, highly variable correlation to blood lead or FEP and to external exposure levels makes findings from various studies difficult to compare quantitatively. The complexity of the many important covariates and their interaction with dependent measures of modest validity, e.g., IQ tests, may also account for many of the discrepancies among the different studies.

The precise medical or health significance of the neuropsychological and electrophysiological effects associated with low-level lead exposure as reported in the above studies is difficult to state with confidence at this time. Observed IQ deficits and other behavioral changes, although statistically significant in some studies, tend to be relatively small as reported by the investigators, but nevertheless may still affect the intellectual development, school performance, and social development of the affected children sufficiently to be regarded as adverse. This would be especially true if such impaired intellectual development or school performance and disrupted social development were reflective of persisting, long-term effects of low-level lead exposure in early childhood. The issue of persistence of such lead effects, however, remains to be more clearly resolved. Still, some study results reviewed above suggest that significant low-level lead-induced neurobehavioral and EEG effects may, in fact, persist at least into later childhood, and a number of animal studies demonstrate long-term persistence into adulthood of neurologic dysfunctions induced by relatively moderate or low level lead exposures early in postnatal development of mammalian species.

12.4.4.2 The Question of Irreversibility. Little research on humans is available on persistence of effects. Some work suggests the possibility of reversing mild forms of peripheral neuropathy in lead workers, but little is known regarding the reversibility of lead effects on central nervous system function in humans. A recent two-year follow-up study of 28 children of battery factory workers found a persistent relation between blood lead and altered slow wave voltage of cortical slow wave potentials. Current human psychometric studies, however, will have to be supplemented by prospective longitudinal studies of the effects of lead on development in order to better elucidate persistence or reversibility of neurotoxic effects of lead exposure early in infancy or childhood.

Various animal studies provide evidence that alterations in neurobehavioral function may be long-lived, with such alterations being evident long after blood lead levels have returned

to control levels. These persistent effects have been demonstrated in monkeys as well as rats under a variety of learning performance test paradigms. Such results are also consistent with morphological, electrophysiological, and biochemical studies on animals that suggest lasting changes in synaptogenesis, dendritic development, myelin and fiber tract formation, ionic mechanisms of neurotransmission, and energy metabolism.

12.4.4.3 Early Development and Susceptibility to Neural Damage. On the question of early childhood vulnerability, the neurobehavioral data are consistent with morphological and biochemical studies of the susceptibility of the heme biosynthetic pathway to perturbation by lead. Various lines of evidence suggest that the order of susceptibility neurotoxic effects of lead is: young > adult; female > male. Animal studies also have pointed to the perinatal period of ontogeny as a particularly critical time for a variety of reasons: (1) it is a period of rapid development of the nervous system; (2) it is a period where good nutrition is particularly critical; and (3) it is a period where the caregiver environment is vital to normal development. However, the precise boundaries of a critical period for lead exposure are not yet clear and may vary depending on the species and function or endpoint that is being assessed. Nevertheless, there is general agreement that human infants and toddlers below the age of three years are at special risk because of *in utero* exposure, increased opportunity for exposure because of normal mouthing behavior of lead-containing objects, and increased rates of lead absorption due to various factors, e.g., iron and calcium deficiencies.

12.4.4.4 Utility of Animal Studies in Drawing Parallels to the Human Condition. Animal models are used to shed light on questions where it would be impractical or ethically unacceptable to use human subjects. This is particularly true in the case of exposure to environmental toxins such as lead. In the case of lead, it has been most effective and convenient to expose developing animals via their mothers' milk or by gastric gavage, at least until weaning. Very often, the exposure is continued in the water or food for some time beyond weaning. This approach does succeed in simulating at least two features commonly found in human exposure: oral intake and exposure during early development. The preweaning postnatal period in rats and mice is of particular relevance in terms of parallels with the first two years or so of human brain development.

However, important questions exist concerning the comparability of animal models to humans. Given differences between humans, rats, and monkeys in heme chemistry, metabolism, and other aspects of physiology and anatomy, it is difficult to state what constitutes an equivalent internal exposure level (much less an equivalent external exposure level). For example, is a blood lead level of 30 μg/dl in a suckling rat equivalent to 30 μg/dl in a three-year-old child? Until an answer is available to this question, i.e., until the function describing the relationship of exposure indices in different species is available, the utility of animal models for deriving dose-response functions relevant to humans will be limited.

Questions also exist regarding the comparability of neurobehavioral effects in animals with human behavior and cognitive function. One difficulty in comparing behavioral endpoints such as locomotor activity is the lack of a consistent operational definition. In addition to the lack of standardized methodologies, behavior is notoriously difficult to "equate" or compare meaningfully across species because behavioral analogies do not demonstrate behavioral homologies. Thus, it is improper to assume, without knowing more about the responsible underlying neurological structures and processes, that a rat's performance on an operant conditioning schedule or a monkey's performance on a stimulus discrimination task necessarily corresponds directly to a child's performance on a cognitive function test. Nevertheless, deficits in performance by mammalian animals on such tasks are indicative of likely altered CNS functions, which is reasonable to assume will likely parallel some type of altered CNS function in humans as well.

In terms of morphological findings, there are reports of hippocampal lesions in both lead-exposed rats and humans that are consistent with a number of independent behavioral findings suggesting an impaired ability to respond appropriately to altered contingencies for rewards. That is, subjects with hippocampal damage tend to persist in certain patterns of behavior even when changed conditions make the behavior inappropriate; the same sort of tendency seems to be common to a number of lead-induced behavioral effects. Other morphological findings in animals, such as demyelination and glial cell decline, are comparable to human neuropathologic observations only at relatively high exposure levels.

Another neurobehavioral endpoint of interest in comparing human and animal neurotoxicity of lead is electrophysiological function. Alterations of electroencephalographic patterns and cortical slow wave voltage have been reported for lead-exposed children, and various electrophysiological alterations both in vivo (e.g., in rat visual evoked response) and in vitro (e.g., in frog miniature endplate potentials) have also been noted in laboratory animals. Thus, far, however, these lines of work have not converged sufficiently to allow for much in the way of definitive conclusions regarding electrophysiological aspects of lead neurotoxicity.

Biochemical approaches to the experimental study of lead effects on the nervous system have been basically limited to laboratory animal subjects. Although their linkage to human neurobehavioral function is at this point somewhat speculative, such studies do provide insight on possible neurochemical intermediaries of lead neurotoxicity. No single neurotransmitter system has been shown to be particularly sensitive to the effects of lead exposure; lead-induced alterations have been demonstrated in various neurotransmitters, including dopamine, norepinephrine, serotonin, and gamma-aminobutyric acid. In addition, lead has been shown to have subcellular effects in the central nervous system at the level of mitochondrial function and protein synthesis. In particular, the work of McCauley, Bull, and co-workers has

indicated that delays seen in cortical synoptogenesis and metabolic maturation following prenatal lead exposure may well underly the delayed development of exploratory and locomoter function seen in other studies of the neurobehavioral effects of lead.

Given the difficulties in formulating a comparative basis for internal exposure levels among different species, the primary value of many animal studies, particularly in vitro studies, may be in the information they can provide on basic mechanisms involved in lead neurotoxicity. A number of key in vitro studies are summarized in Table 12-9. These studies show that significant, potentially deleterious effects on nervous system function occur at in situ lead concentrations of 5 μM and possibly lower. This suggests that, at least intracellularly or on a molecular level, there may exist essentially no threshold for certain neurochemical effects of lead. The relationship between blood lead levels and lead concentrations at extra- or intracellular sites of action, however, remains to be determined.

Despite the problems in generalizing from animals to humans, both the animal and the human studies show considerable internal consistency in that they both support a continuous dose-response functional relationship between lead and neurotoxic biochemical, morphological, electrophysiological, and behavioral effects.

TABLE 12-9. SUMMARY OF KEY STUDIES OF IN VITRO LEAD EXPOSURE

Preparation	Exposure concentration	Results	Reference
Adult rat brain	0.1 μM $Pb(Ac)_2$	35% inhibition of whole-brain BH_4 synthesis	Purdy et al. (1981)
Isolated microvessels from rat brain	0.1 μM $Pb(Ac)_2$	Blockade of sugar-specific transport sites in capillary endothelial cells	Kolber et al. (1980)
Adult mouse brain homogenate	0.1-5 μM tri-n-butyl lead (TBL)	1) 50% decline in high affinity uptake of DA; 2) 25% increase in release of DA	Bondy et al. (1979a,b)
Adult rat striatum	1-5 μM TBL	0-60% inhibition of spiroperidal binding to DA receptors	Bondy and Agarwal (1980)
Embryonic chick brain cell culture	3 μM $(Et_3Pb)Cl_2$	50% reduction in no. of cells exhibiting processes	Grundt et al. (1981)
Brainstem and forebrain slices from PND-22 rats	3 μM $(Et_3Pb)Cl_2$	Inhibition of leucine incorporation into myelin proteins	Konat and Clausen (1978, 1980) Konat et al. (1979)
Adult rat cerebellar homogenates	3-5 μM Pb^{++}	Inhibition of adenylate cyclase activity	Taylor et al. (1978)
Adult rat cerebellar mitochondria	5 μM $Pb(Ac)_2$	Inhibition of respiration	Gmerek et al. (1981)
Adult frog nerve/muscle preparation	5 μM Pb^{++}	Increase in frequency of MEPP's (indicative of depression of synaptic transmission)	Kolton and Yaari (1982)
Isolated, perfused bullfrog retina	5 μM Pb^{++}	Depression of rod (but not cone) receptor potentials	Fox and Sillman (1979)

PRELIMINARY DRAFT

TABLE 12-9. (continued)

Preparation	Exposure concentration	Results	Reference
Cerebellar slices from PND-14 rats	5-7 μM $(Et_3Pb)Cl_2$	Inhibition of incorporation of SO_4 and serine into myelin galactolipids	Grundt and Neskovic (1980)
In oculo culture of cerebellar tissue from PND-15 rats	5-10 μM Pb^{++}	"Disinhibition" of NE-induced inhibition of spontaneous activity in Purkinje cells	Taylor et al. (1978)
Cell suspension from forebrain of PND-22 rats	20 μM $(Et_3Pb)Cl_2$	30% inhibition of total protein synthesis	Konat et al. (1978)
Adult rat cerebral and cerebellar mitochondria	50 μM $Pb(Ac)_2$	Inhibition of respiration	Holtzman et al. (1978b, 1980b)
Adult rat caudate synaptosomes	50 μM $PbCl_2$	8-fold increase in binding of Ca^{++} to mitochondria (effectively increases Ca^{++} gradient across terminal membrane, thus increasing synatic transmission without altering firing rates)	Silbergeld and Adler (1978)
Capillary endothelial cells from rat cerecortex	100 μM $Pb(Ac)_2$	Pb preferentially sequestered in mitochondria like Ca^{++}. (Possible basis for Pb-induced disruption of transmembrane Ca^{++} transport)	Silbergeld et al. (1980b)

PND: post-natal day
$Pb(Ac)_2$: lead acetate
$PbCl_2$: lead chloride
Et_3Pb: triethyl lead
TBL: tri-n-butyl lead
DA: dopamine
NE: norepinephrine
BH_4: tetrahydrobiopterin
MEPP's: miniature endplate potentials

PRELIMINARY DRAFT

12.5 EFFECTS OF LEAD ON THE KIDNEY

12.5.1 Historical Aspects

The first description of renal disease due to lead was published by Lancereaux (1862). In a painter with lead encephalopathy and gout, Lancereaux noted tubulo-interstitial disease of the kidneys at autopsy. Distinctions between glomerular and tubulo-interstitial forms of kidney disease were not, however, clearly defined in the mid-nineteenth century. Ollivier (1863) reported observations in 37 cases of lead poisoning with renal disease and thus introduced the idea that lead nephropathy was a proteinuric disease, a confusion with primary glomerular disease that persisted for over a century. Under the leadership of Jean Martin Charcot, interstitial nephritis characterized by meager proteinuria in lead poisoning was widely publicized (Charcot, 1868; Charcot and Gombault, 1881) but not always appreciated by contemporary physicians (Danjoy, 1864; Gepper, 1882; Lorimer, 1886).

More than ninety years ago, the English toxicologist Thomas Oliver (1885, 1891) distinguished acute effects of lead on the kidney from lead-induced chronic nephropathy. Acute renal effects of lead were seen in persons dying of lead poisoning and were usually restricted to non-specific changes in the renal proximal tubular lining cells. Oliver noted that a "true interstitial nephritis" developed later, often with glomerular involvement.

In an extensive review of the earlier literature, Pejić (1928) emphasized that changes in the proximal tubules, rather than the vascular changes often referred to in earlier studies (Gull and Sutton, 1872), constitute the primary injury to the kidney in lead poisoning. Many subsequent studies have shown pathological alterations in the renal tubule with onset during the early or acute phase of lead intoxication. These include the formation of inclusion bodies in nuclei of proximal tubular cells (Blackman, 1936) and the development of functional defects as well as ultrastructural changes, particularly in renal tubular mitochondria.

12.5.2 Lead Nephropathy in Childhood

Dysfunction of the proximal tubule was first noted as glycosuria in the absence of hyperglycemia in childhood pica (McKhann, 1926). Later it was shown that the proximal tubule transport defect included aminoaciduria (Wilson et al., 1953). Subsequently, Chisolm et al. (1955) found that the full Fanconi syndrome was present: glycosuria, aminoaciduria, phosphaturia (with hypophosphatemia), and rickets. Proximal tubular transport defects appeared only when blood lead levels exceeded 80 μg/dl. Generalized aminoaciduria was seen more consistently in Chisolm's (1962, 1968) studies than were other manifestations of renal dysfunction. The condition was related to the severity of clinical toxicity, with the complete Fanconi syndrome occurring in encephalopathic children when blood lead concentrations exceeded 150 μg/dl (National Academy of Sciences, 1972). Children who were under three years of age excreted 4 to 12.8 mg of lead chelate during the first day of therapy with CaEDTA at 50 mg/kg

day. The aminoaciduria disappeared after treatment with chelating agents and clinical remission of other symptoms of lead toxicity (Chisolm, 1962). This is an important observation relative to the long-term or chronic effects of lead on the kidney.

In a group of children with slight lead-related neurological signs reported by Pueschel et al. (1972), generalized aminoaciduria was found in 8 of 43 children with blood lead levels of 40 to 120 μg/dl. It should be noted that the children reported to have aminoaciduria in this study were selected because of a blood lead level of ≧50 μg/dl or a provocative chelation test of >500 μg of lead chelate per 24 hours.

Although children are considered generally to be more susceptible than adults to the toxic effects of lead, the relatively sparse literature on childhood lead nephropathy probably reflects a greater clinical concern with the life-threatening neurologic symptoms of lead intoxication than with the transient Fanconi syndrome.

12.5.3 Lead Nephropathy in Adults

There is convincing evidence in the literature that prolonged lead exposure in humans can result in chronic lead nephropathy in adults. This evidence is reviewed below in terms of six major categories: (1) lead nephropathy following childhood lead poisoning; (2) "moonshine" lead nephropathy; (3) occupational lead nephropathy; (4) lead and gouty nephropathy; (5) lead and hypertensive nephrosclerosis; and (6) general population studies.

12.5.3.1 Lead Nephropathy Following Childhood Lead Poisoning. Reports from Queensland, Australia (Gibson et al., 1892; Nye, 1933; Henderson, 1954; Emmerson, 1963) points to a strong association between severe childhood lead poisoning, including central nervous system symptoms, and chronic nephritis in early adulthood. The Australian children sustained acute lead poisoning when confined to the enclosed, raised terraces peculiar to the houses around Brisbane. The houses were painted with white lead, which the children ingested by direct contamination of their fingers or by drinking lead-sweetened rain water as it flowed over the weathered surfaces. Two fingers brushed against the powdery paint were shown to pick up about 2 mg of lead (Murray, 1939). Henderson (1954) followed up 401 untreated children who had been diagnosed as having lead poisoning in Brisbane between 1915 and 1935. Of these 401 subjects, death certificates revealed that 165 had died under the age of 40, 108 from nephritis or hypertension. This is greatly in excess of expectation. Information was obtained from 101 of the 187 survivors, and 17 of these had hypertension and/or albuminuria.

In a more recent study, Emmerson (1963) presented a criterion for implicating lead as an etiological factor in such patients: the patients should have an excessive urinary excretion of lead following administration of CaEDTA. Leckie and Tompsett (1958) had shown that increasing the CaEDTA dosage above 2 g/day intravenously had little effect on the amount of lead chelate excreted by adults. They observed little difference in chelatable lead excretion

when 1 g was compared with 2 g (i.v.). Similarly, the magnitude of lead chelated when 1 g is given i.v. or 2 g i.m. (over 12 hr) appears to be the same (Albahary et al., 1961; Emmerson, 1963; Wedeen et al., 1975). Adult control subjects without undue lead absorption excrete less than 650 μg lead chelate during the first post-injection day if renal function is normal, or over 4 days if renal function is severely reduced. The level of reduction of glomerular filtration rate (GFR) at which the EDTA lead-mobilization test is no longer reliable has not been precisely defined but probably exceeds a reduction of 85 percent (serum creatinine concentrations in excess of about 6 mg/dl). In Emmerson's (1963) study 32 patients with chronic renal disease attributable to lead poisoning had elevated excretion of lead chelate. Intranuclear inclusions are associated with recent acute exposure but are often absent in chronic lead nephropathy or after the administration of $CaNa_2EDTA$ (Goyer and Wilson, 1975).

The Australian investigators established the validity of the EDTA lead-mobilization test for the detection of excessive past lead absorption and further demonstrated that the body lead stores were retained primarily in bone (Emmerson, 1963; Henderson, 1954; Inglis et al., 1978). Bone lead concentration averaged 94 μg/g wet weight in the young adults dying of lead nephropathy in Australia (Henderson and Inglis, 1957; Inglis et al., 1978), compared with mean values ranging from 14 to 23 μg/g wet weight in bones from non-exposed individuals (Barry, 1975; Emmerson, 1963; Gross et al., 1975; Wedeen, 1982).

Attempts to confirm the relationship between childhood lead intoxication and chronic nephropathy have not been successful in at least two studies in the United States. Tepper (1963) found no evidence of increased chronic renal disease in 139 persons with a well-documented history of childhood plumbism 20 to 35 years earlier at the Boston Children's Hospital. The study population was 165 patients (after review of 524 case records) who met any two of the following criteria: 1) a definite history of pica or use of lead nipple shields; 2) X-ray evidence of lead-induced skeletal alterations; or 3) characteristic symptoms. No uniform objective measure of lead absorption was reported in this study. In 42 of the 139 subjects clinical studies of renal function were performed and included urinalysis, endogenous creatinine clearance, urine culture, urine concentrating ability, 24-hour protein excretion, and phenolsulfonphthalein excretion. Only one patient was believed to have died of lead nephropathy; three with creatinine clearances under 90 ml/min were said to have had inadequate urine collections. Insufficient details concerning past lead absorption and patient selection were provided to permit generalized conclusions from this report.

Chisolm et al. (1976) also found no evidence of renal disease (as judged by routine urinalysis, blood urea nitrogen, serum uric acid, and creatinine clearance) in 55 adolescents known to have been treated for lead intoxication 11 to 16 years earlier. An important distinction between the Australian group and those patients in the United States studied by Chisolm et al. (1976) was that none of the latter subjects showed evidence of increased

residual body lead burden by the EDTA lead-mobilization test. This U.S. study was carried out on adolescents between 12 and 22 years of age in the late 1960s. During acute toxicity in early childhood, blood lead levels had ranged from 100 to 650 μg/dl; all received immediate chelation therapy. Follow-up chelation tests performed with 1 g EDTA i.m. (with procaine) approximately a decade later resulted in 24-hour lead-chelate excretion of less than 600 μg in 45 of 52 adolescents. The absence of renal disease in this study led Chisolm et al. to suggest that lead toxicity in the Australian children may have been of a different type, with a more protracted course than that experienced by the American children. On the other hand, chelation therapy of the American children may have removed lead stored in bone and thus prevented the development of renal failure later in life. Most children in the United States who suffer from overt lead toxicity do so early in childhood, between the ages of 1 and 4, the source often being oral ingestion of flecks of wall paint and plaster containing lead.

12.5.3.2 "Moonshine" Lead Nephropathy. In the United States, chronic lead nephropathy in adults was first noted among illicit whiskey consumers in the southeastern states. The prerevolutionary tradition of homemade whiskey ("moonshine") was modernized during the Prohibition era for large scale production. The copper condensers traditionally used in the illegal stills were replaced by truck radiators with lead-soldered parts. Illegally produced whiskey might contain up to 74 mg of lead per liter (Eskew et al., 1961). The enormous variability in moonshine lead content has recently been reiterated in a study of 12 samples from Georgia, of which five contained less than 10 μg/l but one contained 5.3 mg/l (Gerhardt et al., 1980).

Renal disease often accompanied by hypertension and gout was common among moonshiners (Eskew et al., 1961; Morgan et al., 1966; Ball and Sorenson, 1969). These patients usually sought medical care because of symptomatic lead poisoning characterized by colic, neurological disturbances, and anemia, although more subtle cases were sometimes detected by use of the i.v. EDTA lead-mobilization test (Morgan, 1968; Morgan and Burch, 1972). While acute symptomatology, including azotemia, sometimes improved during chelation therapy, residual chronic renal failure, gout, and hypertension frequently proved refractory, thus indicating underlying chronic renal disease superimposed on acute renal failure due to lead (Morgan, 1975).

12.5.3.3 Occupational Lead Nephropathy. Although rarely recognized in the United States (Brieger and Reiders, 1959; Anonymous, 1966; Greenfield and Gray, 1950; Johnstone, 1964; Kazantzis, 1970; Lane, 1949; Malcolm, 1971; Mayers, 1947), occupational lead nephropathy, often associated with gout and hypertension, was widely identified in Europe as a sequela to overt lead intoxication in the industrial setting (Albahary et al., 1961, 1965; Cramer et al., 1974; Danilović, 1958; Galle and Morel-Maroger, 1965; Lejeune et al., 1969; Lilis et al., 1967, 1968; Radosević et al., 1961; Radulescu et al., 1957; Richet et al., 1964, 1966; Tara and Francon, 1975; Vigdortchik, 1935). Some important recent studies are summarized here.

Richet et al. (1964) reported renal findings in eight lead workers, all of whom had repeated episodes of lead poisoning, including colic. Intravenous EDTA lead-mobilization tests ranged from 587 to 5930 μg lead-chelate excretion per 24 hours. Four of these men had reduced glomerular filtration rates, one had hypertension with gout, one had hypertension alone, and one had gout alone. Proteinuria exceeded 200 mg/day in only one patient. Five of seven renal biopsies were abnormal showing minor glomerular sclerosis but severe interstitial nephritis and vascular sclerosis by light microscopy. The one patient with proteinuria of 1.7 gm/day showed extensive glomerular hyalinization. Electron microscopy showed intranuclear and cytoplasmic inclusions and ballooning of mitochondria in proximal tubule cells. The presence of intranuclear inclusion bodies is helpful in establishing a relationship between renal lesions and lead toxicity, but inclusion bodies are not always present in persons with chronic lead nephropathy (Cramer et al., 1974; Wedeen et al., 1975, 1979).

Richet et al. (1966) subsequently recorded renal findings in 23 symptomatic lead workers in whom blood lead levels ranged from 30 to 87 μg/dl. Six had diastolic pressures over 90 mm Hg, three had proteinuria exceeding 200 mg/day, and five had gout. In 5 of 21 renal biopsies, glomeruli showed minor hyalinization, but two cases showed major glomerular disease (their creatinine clearances were 20 and 33 ml/min, respectively). Interstitial fibrosis and arteriolar sclerosis were seen in all but two biopsies. Intranuclear inclusion bodies were noted in 13 cases. Electron microscopy showed loss of brush borders, iron-staining intracellular vacuoles, and ballooning of mitochondria in proximal tubule epithelial cells.

Effective renal plasma flow (C_{pah}, plasma clearance of p-aminohippuric acid) by the single injection disappearance technique was measured in 14 lead-poisoned Rumanian workers before and after chelation therapy by Lilis et al. (1967). C_{pah} increased from a pre-treatment mean of 428 ml/min (significantly less than the control mean of 580 ml/min) to a mean of 485 ml/min after chelation therapy ($p < 0.02$). However, no significant increase in GFR (endogenous creatinine clearance) was found. Lilis et al. interpreted the change in effective renal plasma flow as indicating reversal of the renal vasoconstriction that accompanied acute lead toxicity. Although neither blood lead concentrations nor long-term follow-up studies of renal function were provided, it seems likely that most of these patients suffered from acute, rather than chronic, lead nephropathy.

In a subsequent set of 102 cases of occupational lead poisoning studied by Lilis et al. (1968), seven cases of clinically verified chronic nephropathy were found. In this group, endogenous creatinine clearance was less than 80 ml/min two weeks or more after the last episode of lead colic. The mean blood lead level approximated 80 μg/dl (range 42 to 141 μg/dl.) All patients excreted more than 10 mg lead chelate over 5 days during therapy consisting of 2 g $CaNa_2EDTA$ i.v. daily. Nephropathy was more common among those exposed to lead for more than 10 years than among those exposed for less than 10 years. Most of the Rumanian lead workers

had experienced lead colic, and 13 of 17 had persistent hypertension that followed the appearance of renal failure by several years. Proteinuria was absent except in two individuals who excreted 250 and 500 mg/l. Hyperuricemia was not evident in the absence of azotemia. In both studies by Lillis, reduced urea clearance preceded reduced creatinine clearance.

Cramer et al. (1974) examined renal biopsies from five lead workers exposed for 0.5 to 20 years in Sweden. Their blood lead levels ranged from 71 to 138 µg/dl, with GFR ranging from 65 to 128 ml/min, but C_{pah} exceeding 600 ml/min in all. Although plasma concentrations of valine, tyrosine, and phenylalanine were reduced, excretion of these amino acids was not significantly different from controls. A proximal tubular reabsorptive defect might, therefore, have been present without increased amino acid excretion because of low circulating levels: increased fractional excretion may have occurred without increased absolute amino acid excretion. Albuminuria and glycosuria were not present. Glomeruli were normal by electron microscopy. Intranuclear inclusions in proximal tubules were found in two patients with normal GFRs, and peritubular fibrosis was present in the remaining three patients who had had the longest occupational exposure (4 to 20 years).

Wedeen et al. (1975, 1979) reported on renal dysfunction in 140 occupationally exposed men. These investigators used the EDTA lead-mobilization test (1 g CaEDTA with 1 ml of 2 percent procaine given i.m. twice, 8 to 12 hr apart) to detect workers with excessive body lead stores. In contrast to workers with concurrent lead exposure (Alessio et al., 1979), blood lead measures have proven unsatisfactory for detection of past lead exposure (Baker et al., 1979; Havelda et al., 1980; Vitale et al., 1975). Of the 140 workers tested, 113 excreted 1000 µg or more of lead-chelate in 24 hr compared with a normal upper limit of 650 µg/day (Albahary et al., 1961; Emmerson, 1973; Wedeen et al., 1975). Glomerular filtration rates measured by ^{125}I-iothalamate clearance in 57 men with increased mobilizable lead revealed reduced renal function in 21 (GFR less than 90 ml/min per 1.73 m^2 body surface area). When workers over age 55 or with gout, hypertension, or other possible causes of renal disease were excluded, 15 remained who had previously unsuspected lead nephropathy. Their GFRs ranged between 52 and 88 ml/min per 1.73 m^2. Only three of the men with occult renal failure had ever experienced symptoms attributable to lead poisoning. Of the 15 lead nephropathy patients, one had a blood lead level over 80 µg/dl, three repeatedly had blood levels under 40 µg/dl, and eleven had blood levels between 40 and 80 µg/dl at the time of the study. Thus, blood lead levels were poorly correlated with degree of renal dysfunction. The failure of blood lead level to predict the presence of lead nephropathy probably stems from the independence of blood lead from cumulative bone lead stores (Gross, 1981; Saenger et al., 1982a,b).

Percutaneous renal biopsies from 12 of the lead workers with reduced GFRs revealed focal interstitial nephritis in six. Non-specific changes were present in proximal tubules, including loss of brush borders, deformed mitochondria, and increased lysosomal bodies. Intra-

nuclear inclusion bodies were not found in the renal biopsies from these men who had experienced long-term occupational exposure and who had had chelation tests shortly before biopsy. In experimental animals, chelation results in the rapid disapperance of lead-induced intranuclear inclusions (Goyer and Wilson, 1975). The presence of a variety of immunoglobulin deposits by fluorescent microscopy suggests (but does not prove) the possibility that some stages of lead nephropathy in adults may be mediated by immune mechanisms.

Eight patients with pre-azotemic occupational lead nephropathy were treated with 1 g CaEDTA (with procaine) i.m. three times weekly for 6 to 50 months. In four patients, GFR rose by 20 percent or more by the time the EDTA test had fallen to less than 850 µg Pb/day. The rise in GFR was paralleled by increases in effective renal plasma flow (C_{pah}) during CaEDTA treatment. These findings indicate that chronic lead nephropathy may be reversible by chelation therapy, at least during the pre-azotemic phase of the disease (Wedeen et al., 1979). However, much more information will have to be obtained on the value of long-term, low-dose chelation therapy before this regimen can be widely recommended. There is, at present, no evidence that interstitial nephritis itself is reversed by chelation therapy. It may well be that only functional derangements are corrected and that the improvement in GFR is not accompanied by disappearance of tubulo-interstitial changes in kidney. Chronic volume depletion, for example, might be caused by lead-induced depression of the renin-angiotension-aldosterone system (McAllister et al., 1971) or by direct inhibition of (Na^+, K^+)ATPase-mediated sodium transport (Nechay and Williams, 1977; Nechay and Saunders, 1978a,b,c; Raghavan et al., 1981; Secchi et al., 1973). On the other hand, volume depletion would be expected to produce prerenal azotemia, but this was not evident in these patients. The value of chelation therapy in chronic lead nephropathy once azotemia is established is unknown.

The prevalence of azotemia among lead workers has recently been confirmed in health surveys conducted at industrial sites (Baker et al., 1979; Hammond et al., 1980; Landrigan et al., 1982; Lilis et al., 1979, 1980). Interpretation of these data is, however, hampered by the weak correlation generally found between blood lead levels and chronic lead nephropathy in adults, the absence of matched prospective controls, and the lack of detailed diagnostic information on the workers found to have renal dysfunction. Moreover, blood serum urea nitrogen (BUN) is a relatively poor indicator of renal function because it is sensitive to a variety of physiological variables other than GFR, including protein anabolism, catabolism, and hydration. Several other measures of renal function are more reliable than the BUN, including in order of increasing clinical reliability: serum creatinine, endogenous creatinine clearance, and ^{125}I-iothalamate or inulin clearance. It should be noted that none of these measures of GFR can be considered reliable in the presence of any acute illness such as lead colic or encephalopathy. Elevated BUN in field surveys may, therefore, sometimes represent transient acute functional changes rather than chronic intrinsic renal disease.

The variable susceptibility of the kidneys to the nephrotoxic effects of lead suggests that environmental factors in addition to lead may participate in the expression of renal damage. Industrial workers are often exposed to a variety of toxic materials, some of which, such as cadmium (Buchet et al., 1980), are themselves nephrotoxic. In contrast to cadmium, lead does not increase urinary excretion of beta-2-microglobulins (Batuman et al., 1981; Buchet et al., 1980) or lysozyme (Wedeen et al., 1979). Multiple interactions between environmental toxins may enhance susceptibility to lead nephrotoxicity. Similarly, nephrotoxicity may be modulated by reductions in 1,25-dihydroxy vitamin D_3, increased 6-beta-hydroxycortisol production (Saenger et al., 1981, 1982a,b), or immunologic alterations (Gudbrandsson et al., 1981; Koller and Brauner, 1977; Kristensen, 1978; Kristensen and Andersen, 1978). Reductions in dietary intake of calcium, copper, or iron similarly appear to increase susceptibility to lead intoxication (Mahaffey and Michaelson, 1980).

The slowly progressive chronic lead nephropathy resulting from years of relatively low-dose lead absorption observed in adults is strikingly different from the acute lead nephropathy arising from the relatively brief but intense exposure arising from childhood pica. Typical acid-fast intranuclear inclusions are, for example, far less common in the kidneys of adults (Cramer et al., 1974; Wedeen et al., 1975). Although aminoaciduria has been found to be greater in groups of lead workers than in controls (Clarkson and Kench, 1956; Goyer et al., 1972), proximal tubular dysfunction is more difficult to demonstrate in adults with chronic lead nephropathy than in acutely exposed children (Cramer et al., 1974). It should be remembered, however, that children with the Fanconi syndrome have far more severe acute lead intoxication than is usual for workmen on the job. In contrast to the reversible Fanconi syndrome associated with childhood lead poisoning, proximal tubular reabsorptive defects in occupationally exposed adults are uncommon and subtle; clearance measurements are often required to discern impaired tubular reabsorption in chronic lead nephropathy. Hyperuricemia is frequent among lead workers (Albahary et al., 1965; Garrod, 1859; Hong et al., 1980; Landrigan et al., 1982), presumably a consequence of specific lead inhibition of uric acid excretion, increased uric acid production (Emmerson et al., 1971; Granick et al., 1978; Ludwig, 1957), and prerenal azotemia from volume depletion. The hyperuricemia in adults contrasts with the reduced serum uric acid levels usually associated with the Fanconi syndrome in childhood lead poisoning. Although aminoaciduria and glycosuria are unusual in chronic lead nephropathy, Hong et al. (1980) reported a disproportionate reduction in the maximum reabsorptive rate for glucose compared with para-aminohippuric acid (PAH) in five of six lead workers they studied. PAH transport has not been consistently altered beyond that expected in renal failure of any etiology (Hong et al., 1980; Wedeen et al., 1975). Biagini et al. (1977) have, however, reported a good negative linear correlation between the one-day EDTA lead-mobilization test

and C_{pah} in 11 patients with histologic evidence of lead-induced ultrastructural abnormalities in proximal tubules.

The differences between lead nephropathy in children and adults would not appear to be a consequence of the route of exposure, since a case of pica in an adult (geophagic lead nephropathy) studied by Wedeen et al. (1978) showed the characteristics of chronic rather than acute lead nephropathy; intranuclear inclusions were absent and the GFR was reduced out of proportion to the effective renal plasma flow.

12.5.3.4 Lead and Gouty Nephropathy. Renal disease in gout can often be attributed to well defined pathogenetic mechanisms including urinary tract stones and acute hyperuricemic nephropathy with intratubular uric acid deposition (Bluestone et al., 1977). In the absence of intra- or extra-renal urinary tract obstruction, the frequency, mechanism, and even the existence of a renal disease peculiar to gout remains in question. While some investigators have described "specific" uric acid-induced histopathologic changes in both glomeruli and tubules (Gonick et al., 1965; Sommers and Churg, 1982), rigorously defined controls with comparable degrees of renal failure were not studied simultaneously. Specific histologic changes in the kidneys in gout have not been found by others (Pardo et al., 1968; Bluestone et al., 1977). Glomerulonephritis, vaguely defined "pyelonephritis" (Heptinstall, 1974), or intra- and extra-renal obstruction may have sometimes been confused with the gouty kidney, particularly in earlier studies (Fineberg and Altschul, 1956; Gibson et al., 1980b; Mayne, 1955; McQueen, 1951; Schnitker and Richter, 1936; Talbott and Terplan, 1960; Williamson, 1920).

The histopathology of interstitial nephritis in gout appears to be non-specific and cannot usually be differentiated from that of "pyelonephritis," nephrosclerosis, or lead nephropathy on morphologic grounds alone (Barlow and Beilin, 1968; Bluestone et al., 1977; Greenbaum et al., 1961; Heptinstall, 1974; Inglis et al., 1978). Indeed, renal histologic changes in non-gouty hypertensive patients have been reported to be identical to those found in gout patients (Cannon et al., 1966). In these hypertensive patients, serum uric acid levels paralleled the BUN.

Confusion between glomerular and interstitial nephritis can in part be explained by the tendency of proteinuria to increase as renal failure progresses, regardless of the underlying etiology (Batuman et al., 1981). In the absence of overt lead intoxication it may, therefore, be difficult to recognize surreptitious lead absorption as a factor contributing to renal failure in gouty patients. Further, medullary urate deposits, formerly believed to be characteristic of gout (Brown and Mallory, 1950; Mayne, 1955; McQueen, 1951; Fineberg and Altschul, 1956; Talbott and Terplan, 1960), have more recently been reported in end-stage renal disease patients with no history of gout (Cannon et al., 1966; Inglis et al., 1978; Linnane et al., 1981; Östberg, 1968; Verger et al., 1967). Whether such crystalline deposits contribute to, or are a consequence of, renal damage cannot be determined with confidence. In

the presence of severe hyperuricemia (serum uric acid greater than 20 μg/dl), intraluminal crystal deposition may produce acute renal failure because of tubular obstruction (Emmerson, 1980) associated with grossly visible medullary streaks. In chronic renal failure without gout or massive hyperuricemia, the functional significance of such medullary deposits is unclear (Linnane et al., 1981). Moreover, medullary microtophi, presumably developing around intraluminal deposits, may extend into the renal interstitium, inducing foreign body reactions with giant cell formation. Such amorphous deposits may require alcohol fixation and deGalantha staining for identification (Verger et al., 1967). Because of the acid milieu, medullary deposits are usually uric acid, while microtophi developing in the neutral pH of the renal cortex are usually monosodium urate. Both amorphous and needle-like crystals have been demonstrated in kidneys of non-gout and hyperuricemic patients frequently in association with arteriolonephrosclerosis (Inglis et al., 1978; Cannon et al., 1966; Ostberg, 1968). Urate deposits therefore, are not only not diagnostic, but may be the result, rather than the cause, of interstitial nephritis. The problem of identifying unique characteristics of the gouty kidney has been further confounded by the coexistence of pyelonephritis, diabetes mellitis, hypertension, and the aging process itself.

Although the outlook for gout patients with renal disease was formerly considered grim (Talbott, 1949; Talbott and Terplan, 1960), more recent long-term follow-up studies suggest a benign course in the absence of renovascular or other supervening disease (Fessel, 1979; Yü and Berger, 1982; Yü, 1982). Over the past four decades the reported incidence of renal disease has varied from greater than 25 percent (Fineberg and Altschul, 1956; Henck et al., 1941; Talbott, 1949; Talbott and Terplan, 1960; Wyngaarden, 1958) to less than 2 percent, as observed by Yü (1982) in 707 patients followed from 1970 to 1980. The low incidence of renal disease in some hyperuricemic populations does not support the view that elevated serum uric acid levels of the degree ordinarily encountered in gout patients is harmful to the kidneys (Emmerson, 1980; Fessel, 1979; Ramsey, 1979; Reif et al., 1981). Similarly, the failure of the xanthine oxidase inhibitor, allopurinol, to reverse the course of renal failure in gout patients despite marked reductions in the serum uric acid (Bowie et al., 1967; Levin and Abrahams, 1966; Ogryzlo et al., 1966; Rosenfeld, 1974; Wilson et al., 1967) suggests that renal disease in gout may be due in part to factors other than uric acid. Some studies have, however, suggested a possible slowing of the rate of progression of renal failure in gout by allopurinol (Gibson et al., 1978, 1980a,b; Briney et al., 1975). While the contribution of uric acid to the renal disease of gout remains controversial, the hypothesized deleterious effect of hyperuricemia on the kidney has no bearing on other potential mechanisms of renal damage in these patients.

Although hyperuricemia is universal in patients with renal failure, gout is rare in such patients except when the renal failure is due to lead. Gout occurs in approximately half of

the patients with lead nephropathy (Emmerson, 1963, 1973; Ball and Sorenson, 1969; Richet et al., 1965). Moreover, among gout patients in Scotland without known lead exposure, blood lead levels were found to be higher than in non-gouty controls (Campbell et al., 1978). The long association of lead poisoning with gout raises the possibility that lead absorption insufficient to produce overt lead intoxication may, nevertheless, cause gout with slowly progressive renal failure. Garrod (1859), Ball and Sorenson (1969), and Emmerson et al., (1971) demonstrated that lead reduces uric acid excretion, thereby creating the internal milieu in which gout can be expected. The mechanism of hyperuricemia in lead poisoning is, however, unclear. Serum uric acid levels would be expected to rise in association with lead induced pre-renal azotemia; increased proximal tubule reabsorption of uric acid could result from reduced glomerular filtration rate due to chronic volume depletion. Increased tubular reabsorption of uric acid in lead nephropathy was suggested by the pyrazinamide suppression test (Emmerson, 1971), but interpretation of this procedure has been questioned (Holmes and Kelly, 1974). Lead inhibition of tubular secretion of uric acid, therefore, remains another possible mechanism of reduced uric acid excretion. In addition, some investigators have found increased uric acid excretion in saturnine gout patients, thereby raising the possibility that lead increases uric acid production in addition to reducing uric acid excretion (Emmerson et al., 1971; Ludwig, 1957; Granick et al., 1978).

Having specifically excluded patients with gout or hypertension from their study of occupational lead nephropathy, Wedeen and collaborators examined the possible role of lead in the etiology of the gouty kidney (Batuman et al., 1981). To test the hypothesis that surreptitious lead absorption may sometimes contribute to renal failure in gout, 44 armed service veterans with gout were examined by the EDTA lead-mobilization test. Individuals currently exposed to lead (including lead workers) were specifically excluded. Collection of urine during the EDTA lead-mobilization test was extended to three days because reduced GFR delays excretion of the lead chelate (Emmerson, 1963). Note that the EDTA test does not appear to be nephrotoxic even for patients with preexisting renal failure (Wedeen et al., 1983). Half of the gout patients had normal renal function and half had renal failure as indicated by serum creatinines over 1.5 mg/dl (mean = 3.0; standard error = 0.4 mg/dl), reflecting approximately 70 percent reduction in renal function. The groups were comparable in regard to age, duration of gout, incidence of hypertension, and history of past lead exposure. The mean (and standard error) blood lead concentration was 26 (± 3) μg/dl in the patients with reduced renal function and 24 (± 3) μg/dl in the gout patients with normal kidney function. The gout patients with renal dysfunction, however, excreted significantly more lead chelate than did those without renal dysfunction (806 ± 90 and 470 ± 52 μg Pb over 3 days, respectively).

Ten control patients with comparable renal failure excreted 424 ± 72 μg lead during the 3-day EDTA test (2 g i.m.). The non-gout control patients with renal failure had normal lead

stores (Emmerson, 1973; Wedeen et al., 1975), indicating that the excessive mobilizable lead in the gout patients with renal failure was not a consequence of reduced renal function per se. These studies suggest that excessive lead absorption may sometimes be responsible for the gouty kidney in contemporary patients, as appeared to be the case in the past (Wedeen, 1981). While the EDTA lead-mobilization test cannot prove the absence of other forms of renal disease, when other known causes are excluded by appropriate diagnostic studies, a positive EDTA test can indicate that lead may be a contributing cause of renal failure.

The source of lead exposure in these armed service veterans could not be determined with confidence. A history of transient occupational exposure and occasional moonshine consumption was common among all the veterans, but the medical histories did not correlate with either the EDTA lead-mobilization test or the presence of renal failure. The relative contributions of airborne lead, industrial sources, and illicit whiskey to the excessive body lead stores demonstrated by the EDTA lead-mobilization test could not, therefore, be determined.

12.5.3.5 Lead and Hypertensive Nephrosclerosis. Hypertension is another putative complication of excessive lead absorption that has a long and controversial history. Hypertension has often been associated with lead poisoning, frequently together with renal failure (Beevers et al., 1980; Dingwall-Fordyce and Lane, 1963; Emmerson, 1963; Legge, 1901; Lorimer, 1886; Morgan, 1976; Oliver, 1891; Richet et al., 1966; Vigdortchik, 1935). However, a number of investigators have failed to find such an association (Belknap, 1936; Brieger and Rieders, 1959; Cramer and Dahlberg, 1966; Fouts and Page, 1942; Malcolm, 1971; Mayers, 1947; Ramirez-Cervantes et al., 1978). Because of the absence of both uniform definitions of excessive lead exposure and prospective control populations, the true contribution of lead to hypertension at various levels and durations of exposure is unknown. Similarly, it is not clear whether lead-induced hypertension is mediated by renal disease, vascular effects, or mechanisms involving vasoactive hormones or sodium transport. Definitive epidemiological studies remain to be performed, but the etiologic role of lead in hypertension is likely to remain clouded as long as the etiology of "essential" hypertension is unknown.

Among non-occupationally exposed individuals, hypertension and serum uric acid levels have been found to correlate with blood lead levels (Beevers et al., 1976). Moreover, the kidneys of patients with chronic lead nephropathy may show uric acid microtophi and the vascular changes of "benign essential hypertension" even in the absence of gout and hypertension (Cramer et al., 1974; Inglis et al., 1978; Morgan, 1976; Wedeen et al., 1975). In a long-term follow-up study of 624 patients with gout, Yü and Berger (1982) reported that while hyperuricemia alone had no deleterious effect on renal function, decreased renal function was more likely to occur in gout patients with hypertension and/or ischemic heart disease than in those with uncomplicated gout.

Like gout, hypertension was specifically excluded from the study of occupational lead nephropathy by Wedeen et al. (1975, 1979) in order to isolate lead-induced renal disease. Hypertension by itself is widely accepted as a cause of renal failure. Currently, however, the renal sequelae of moderate hypertension appear to be less dramatic than in the past (Kincaid-Smith, 1982). In order to determine if unsuspected excessive body lead stores might contribute to the renal disease of hypertension, 3-day EDTA (2 g i.m.) lead mobilization tests were performed in hypertensive armed service veterans with and without renal failure (Batuman et al., 1983). A significant increase in mobilizable lead was found in hypertensive subjects with renal disease compared to those without renal disease. Control patients with renal failure again demonstrated normal mobilizable lead, thereby supporting the view that renal failure is not responsible for the excess mobilizable lead in patients with hypertension and renal failure. These findings suggest that patients who would otherwise be deemed to have essential hypertension with nephrosclerosis can be shown to have underlying lead nephropathy by the EDTA lead-mobilization test when other renal causes of hypertension are excluded.

The mechanism whereby lead induces hypertension remains unclear. Although renal disease, particularly at the end-stage, is a recognized cause of hypertension, renal arteriolar histologic changes may precede both hypertension and renal disease (Wedeen et al., 1975). Lead may therefore induce hypertension by direct or indirect effects on the vascular system.

Studies of hypertension in moonshine consumers have indicated the presence of hyporeninemic hypoaldosteronism. A blunted plasma renin response to salt depletion has been described in lead poisoned patients; this response can be restored to normal by chelation therapy (McAllister et al., 1971; Gonzalez et al., 1978; Sandstead et al., 1970a). The diminished renin-aldosterone responsiveness found in moonshine drinkers could not, however, be demonstrated in occupationally exposed men with acute lead intoxication (Campbell et al., 1979). Although the impairment of the renin-aldosterone system appears to be independent of renal failure and hypertension, hyporeninemic hypoaldosteronism due to lead might contribute to the hyperkalemia (Morgan, 1976) and the exaggerated natriuresis (Fleischer et al., 1980) of some patients with "benign essential hypertension." Since urinary kallikrein excretion is reduced in lead workers with hypertension, it has been suggested that the decrease in this vasodilator may contribute to lead-induced hypertension (Boscolo et al., 1981). The specificity of kallikrein suppression in the renal and hypertensive manifestions of excessive lead absorption cannot, however, be determined from available data, because lead workers without hypertension and essential hypertensive patients without undue lead absorption also have reduced urinary kallikrein excretion.

12.5.3.6 General Population Studies. Few studies have been performed to evaluate the possible harmful effects of lead on the kidneys in populations without suspected excessive lead absorption from occupational or moonshine exposure.

An epidemiological survey in Scotland of households with water lead concentrations in excess of WHO recommendations (100 μg/l) revealed a close correlation between water lead content and blood lead and serum urea concentrations (Campbell et al., 1977). In 970 households lead concentrations in drinking water ranged from <0.1 to >8.0 mg/l. After clinical and biochemical screening of 283 subjects from 136 of the households with water lead concentrations in excess of 100 μg/l, a subsample of 57 persons with normal blood pressure and elevated serum urea (40 μg/dl) was compared with a control group of 54 persons drawn from the study group with normal blood pressure and normal serum urea. The frequency of renal dysfunction in individuals with elevated blood lead concentratons (>41 μg/dl) was significantly greater than that of age- and sex-matched controls.

Since 62 general practitioners took part in the screening, the subsamples may have come from many different areas; however, it was not indicated if matching was done for place of residence. The authors found a significantly larger number of high blood lead concentrations among the persons with elevated serum urea and claimed that elevated water lead concentration was associated with renal insufficiency as reflected by raised serum urea concentrations. This is difficult to accept since serum urea is not the method of choice for evaluating renal function. Despite reservations concerning use of the BUN for assessing renal function (described above), these findings are consistent with the view that excessive lead absorption from household water causes renal dysfunction. However, the authors used unusual statistical methods and could not exclude the reverse causal relationship, i.e., that renal failure had caused elevated blood lead levels in their study group. A carefully matched control population of azotemic individuals from low lead households would have been helpful for this purpose. A more convincing finding in another subsample was a strong association between hyperuricemia and blood lead level. This was also interpreted as a sign of renal insufficiency, but it may have represented a direct effect of lead on uric acid production or renal excretion.

These investigators have also found a statistically significant correlation between blood lead concentration and hypertension. Tap-water lead did not, however, correlate with blood lead among the hypertensive group, thus suggesting that other environmental sources of lead may account for the presence of high blood lead concentrations among hypertensive persons in Scotland (Beevers et al., 1976, 1980).

12.5.4 Mortality Data

Cooper and Gaffey (1975) analyzed mortality data available from 1267 death certificates for 7032 lead workers who had been hired by 16 smelting or battery plants between 1900 and 1969. Standardized mortality ratios revealed an excess of observed over predicted deaths from "other hypertensive disease" and "chronic nephritis and other renal sclerosis." The authors

concluded that "high levels of lead absorption such as occurred in many of the workers in this series, can be associated with chronic renal disease." Although renal carcinomas have been observed in lead poisoned rats, no increase in cancer rates was evident in this study of lead workers (Cooper, 1976; see Section 12.7). Reports of renal carcinoma among lead workers are distinctly unusual (Baker et al., 1980).

In a more limited study of 241 Australian smelter employees who were diagnosed as lead poisoned between 1928 and 1959 by a government medical board, 140 deaths were identified between 1930 and 1977 (McMichael and Johnson, 1982). Standard proportional mortality rates of the lead-exposed workers compared with 695 non-lead-exposed employees revealed an overall three-fold excess in deaths due to chronic nephritis and a two-fold excess in deaths due to cerebral hemorrhage in the lead-exposed workers. Over the 47 years of this retrospective study the number of deaths from chronic nephritis decreased from an initial level of 36 percent to 4.6 percent among the lead-exposed workers, compared with a drop from 8.7 percent to 2.2 percent among controls. From 1965 to 1977 the age-standardized mortality rates from chronic nephritis were the same for the lead-worker and control groups, although both rates were higher than the proportional mortality rate for the general population of Australian males. The latter observation indicated that the excessive deaths from chronic nephritis among lead-poisoned workers at the smelter had declined in recent decades.

Despite substantial evidence that lead produces interstitial nephritis in adults, the impact of chronic lead nephropathy on the general population is unknown. The diagnosis of lead nephropathy is rarely made in dialysis patients in the United States. The absence of the diagnosis does not, however, provide evidence for the absence of the disease. Advanced renal failure is usually encountered only many years after excessive lead exposure. Moreover, acute intoxication may never have occurred, and neither heme enzyme abnormalities nor elevated blood lead levels may be present at the time renal failure becomes apparent. The causal relationship between lead absorption and renal disease may therefore not be evident. It is likely that such cases of lead nephropathy have previously been included among other diagnostic categories such as pyelonephritis, interstitial nephritis, gouty nephropathy, and hypertensive nephrosclerosis. Increasing proteinuria as lead nephropathy progresses may also cause confusion with primary glomerulonephritis. It should also be noted that the End Stage Renal Disease Program (Health Care Financing Administration, 1982) does not even include the diagnosis of lead nephropathy in its reporting statistics, regardless of whether the diagnosis is recognized by the attending nephrologist.

12.5.5 Experimental Animal Studies of the Pathophysiology of Lead Nephropathy

12.5.5.1 Lead Uptake by the Kidney. Lead uptake by the kidney has been studied *in vivo* and *in vitro* using renal slices. Vander et al. (1977) performed renal clearance studies in dogs

two hours after a single i.v. dose of 0.1 or 0.5 mg lead acetate containing 1-3 mCi of ^{203}Pb or 1 hour after continous i.v. infusion of 0.1-0.15 mg/kg-hour. These investigators reported that 43-44 percent of the plasma lead was ultrafiltrable, with kidney reabsorption values of 89-94 percent for the ultrafiltrable fraction. A subsequent stop-flow analysis investigation by Victery et al. (1979a), using dogs given a single i.v. dose of lead acetate at 0.2 or 10.0 mg/kg, showed both proximal and distal tubular reabsorption sites for lead. Distal reabsorption was not linked to sodium chloride or calcium transport pathways. Proximal tubule reabsorption was demonstrated in all animals tested during citrate or bicarbonate infusion. Another experiment (Victery et al., 1979b) examined the influence of acid-base status on renal accumulation and excretion of lead in dogs given 0.5-50 μg/kg hr as an infusion or in rats given access to drinking water containing 500 ppm Pb for 2-3 months. These showed that alkalosis increased lead entry into tubule cells via both luminal and basolateral membranes, with a resultant increase in both renal tissue accumulation and urinary excretion of lead. Similarly, acutely induced alkalosis increased lead excretion in rats previously given access to drinking water containing 500 ppm lead for 2-3 months. These authors also concluded that the previously reported acute exposure experiments concerning the renal handling of lead were at least qualitatively similar to results of the chronic exposure experiments and that rats were an acceptable model for investigating the effects of alkalosis on the excretion of lead following chronic exposure.

In vitro studies (Vander et al., 1979) using slices of rabbit kidney incubated with ^{203}Pb acetate at lead concentrations of 0.1 or 1.0 μM over 180-minute time intervals showed that a steady-state uptake of ^{203}Pb by slices (ratio of slice: medium uptake in the range of 10-42) was reached after 90 minutes and that lead could enter the slices as a free ion. Tissue slice uptake was reduced by a number of metabolic inhibitors, thus suggesting a possible active transport mechanism. Tin (Sn IV) was found to markedly reduce ^{203}Pb uptake into the slices but not to affect lead efflux or para-aminohippurate accumulation. This finding raises the possibility that Pb and Sn (IV) compete for a common carrier.

Subsequent studies also using rabbit kidney slices (Vander and Johnson, 1981) showed that co-transport of ^{203}Pb into the slices in the presence of organic anions such as cysteine, citrate, glutathione, histidine, or serum ultrafiltrate was relatively small compared with uptake due to ionic lead.

In summary, it is clear from the above in vivo and in vitro studies on several different animal species that renal accumulation of lead is an efficient process that occurs in both proximal and distal portions of the nephron and at both luminal and basolateral membranes. The transmembrane movement of lead appears to be mediated by an uptake process that is subject to inhibition by several metabolic inhibitors and the acid-base status of the organism.

12.5.5.2 Intracellular Binding of Lead in the Kidney. The bioavailability of lead inside renal tubule cells under low or ^{203}Pb tracer exposure conditions is mediated in part by binding to several high affinity cytosolic binding proteins (Oskarsson et al., 1982; Mistry et al., 1982) and, at higher exposure conditions, by the formation of cytoplasmic and intranuclear inclusion bodies (Goyer et al., 1970a). These inclusion bodies have been shown by both cell fractionation (Goyer et al., 1970a) and X-ray microanalysis (Fowler et al., 1980) to contain the highest intracellular concentrations of lead. Saturation analysis of the renal 63,000 dalton (63K) cytosolic binding protein has shown that it possesses an approximate dissociation constant (K_d) of 10^{-8} M (Mistry et al., 1982). These data quantify the high affinity nature of this protein for lead and explain the previously reported finding (Oskarsson et al., 1982) that this protein constitutes a major intracellular lead-binding site in the kidney cytosol. Biochemical studies on the protein components of isolated rat kidney intranuclear inclusion bodies have shown that the main component has an approximate molecular weight of 27K (Moore et al., 1973) or 32K (Shelton and Egle, 1982) and that it is rich in the dicarboxylic amino acids glutamate and aspartate (Moore et al., 1973). The isoelectric point of the main nuclear inclusion body protein has been reported to be pI = 6.3 and appeared from two-dimensional gel analysis to be unique to nuclei of lead-injected rats (Shelton and Egle, 1982). The importance of the inclusion bodies resides with the suggestion (Goyer et al., 1970a; Moore et al., 1973; Goyer and Rhyne, 1973) that, since these structures contain the highest intracellular concentrations of lead in the kidney proximal tubule and hence account for much of the total cellular lead burden, they sequester lead to some degree away from sensitive renal organelles or metabolic (e.g., heme biosynthetic) pathways until their capacity is exceeded. The same argument would apply to the high affinity cytosolic lead-binding proteins at lead exposure levels below those that cause formation of inclusion bodies. It is currently unclear whether lead-binding to these proteins is an initial step in the formation of the cytoplasmic or nuclear inclusion bodies (Oskarsson et al., 1982).

12.5.5.3 Pathological Features of Lead Nephropathy. The main morphological effects of lead in the kidney are manifested in renal proximal tubule cells and interstitial spaces between the tubules. A summary of morphological findings from some recent studies involving a number of animal species is given in Table 12-10. In all but one of these studies, formation of intranuclear inclusion bodies is a common pathognomic feature for all species examined. In addition, proximal tubule cell cytomegaly and swollen mitochondria with increased numbers of lysosomes were also observed in two of the chronic exposure studies (Fowler et al., 1980; Spit et al., 1981). Another feature reported in three of these studies (Hass et al., 1964; White, 1977; Fowler et al., 1980) was the primary localization of morphological changes in the straight (S_3) segments of the proximal tubule, thereby indicating that not all cell types of

TABLE 12-10. MORPHOLOGICAL FEATURES OF LEAD NEPHROPATHY IN VARIOUS SPECIES

Species	Pb dose regimen	Morphological findings: Nuclear inclusions	Increased mitochondrial swelling	Increased lysosomes	Interstitial fibrosis	Reference
Rabbit	0.5% Pb acetate in diet for up to 55 weeks	+	ND	ND	+	Hass et al., 1964
Rat	1% Pb in d.w. for 9 weeks	+	+	ND	+	Goyer, 1971
Dog	50 µg Pb/kg for 5 weeks*	+	ND	ND	ND	White, 1977
Monkey	0, 1.5, 6.0, 15 µg Pb/day** 6 days/week for 9 months	+	ND	ND	ND	Colle et al., 1980
Rat	0, 0.5, 5, 25, 50, 250 ppm Pb**	+	+	--	--	Fowler et al., 1980
Rabbit	0, 0.25, 0.50 µg Pb/kg*** 3 days/wk for 14 weeks	--	--	+	--	Spit et al., 1981
Ringed dove	100 µg Pb/ml**	+	+	--	--	Kendall et al., 1981

* Dosed by oral gavage

** Drinking water ad libitum

***Subcutaneous injection

ND - Not determined

PRELIMINARY DRAFT

the kidney are equally involved in the toxicity of lead to this organ. Interstitial fibrosis has also been reported in rabbits (Hass et al., 1964) given diets containing 0.5 percent lead acetate for up to 55 weeks and in rats (Goyer, 1971) given drinking water containing lead acetate for 9 weeks.

12.5.5.4 Functional Studies.

12.5.5.4.1 Renal blood flow and glomerular filtration rate. Studies by Aviv et al. (1980) concerning the impact of lead on renal function as assessed by renal blood flow (RBF) and glomerular filtration rate (GFR) have reported significant ($p < 0.01$) reductions in both of these parameters in rats at 3 and 16 weeks after termination of exposure to 1 percent lead acetate in drinking water. Statistically significant ($p < 0.05$) reduction of GFR has also been recently described (Victery et al., 1981) in dogs 2.5-4 hours after a single i.v. dose of 3.0 mg Pb/kg. In contrast, studies by others (Johnson and Kleinman, 1979; Hammond et al., 1982) were not able to demonstrate reduction in GFR or RBF using the rat as a model. The reasons behind these reported differences are presently unclear but may be related to differences in experimental design, age, or other variables.

12.5.5.4.2 Tubular function. Exposure to lead has also been reported to produce tubular dysfunction (Studnitz and Haeger-Aronsen, 1962; Goyer, 1971; Mouw et al., 1978; Suketa et al., 1979; Victery et al., 1981, 1982a,b, 1983). An early study (Studnitz and Haeger-Aronsen, 1962) reported aminoaciduria in rabbits given a single dose of lead at 125 mg/kg, with urine collected over a 15-hour period. Goyer et al. (1970b) described aminoaciduria in rats following exposure to 1 percent lead acetate in the diet for 10 weeks. Wapnir et al. (1979) confirmed a mild hyperaminoaciduria in rats injected with lead at 20 mg/kg five times a week for six weeks but found no changes in urinary excretion of phosphate or glucose.

Other studies (Mouw et al., 1978; Suketa et al., 1979; Victery et al., 1981, 1982a,b, 1983) have focused attention on increased urinary excretion of electrolytes. Mouw et al. (1978) reported increased urinary excretion of sodium, potassium, calcium, and water in dogs given a single i.v. injection of lead at 0.6 or 3.0 mg/kg over a 4-hour period despite a constant GFR, indicating decreased tubular reabsorption of these substances. Suketa et al. (1979) treated rats with a single oral dose of lead at 0, 5, 50, or 200 mg/kg and killed the animals at 0, 6, 12, or 24 hours after treatment. A dose-related increase in urinary sodium, potassium, and water was observed over time. Victery et al. (1981, 1982a,b, 1983) studied zinc excretion in dogs over a 4-hour period following an i.v. injection of lead at 0.3 or 3.0 mg/kg. These investigators reported maximal increases in zinc excretion of 140 ng/min at the 0.3 mg/kg dose and 300 ng/min at the 3.0 mg/kg dose at the end of the 4-hour period. In contrast, in studies by Mouw et al. (1978) no changes in urinary excretion of sodium or potassium were noted. Urinary protein or magnesium excretion were also unchanged.

The results of the above studies indicate that acute or chronic lead treatment is capable of producing tubular dysfunction in several species of animals, as manifested by increased urinary excretion of amino acid nitrogen and some ions such as Zn^{2+}, Ca^{2+}, Na^{+}, K^{+}, and water.

12.5.6 Experimental Studies of the Biochemical Aspects of Lead Nephrotoxicity

12.5.6.1 Membrane Marker Enzymes and Transport Functions. The biochemical effects of lead in the kidney appear to be preferentially localized in the cell membranes and mitochondrial and nuclear compartments following either acute or chronic lead exposure regimens.

Oral exposure of rats to lead acetate in the diet at concentrations of 1-2 percent for 10-40 weeks was found to produce no significant changes in renal slice water content or in accumulation of paraminohippurate (PAH) or tetraethyl-ammonium (TEA). However, tissue glucose synthesis at 40 weeks and pyruvate metabolism were both significantly ($p < 0.05$) reduced (Hirsch, 1973).

Wapnir et al. (1979) examined biochemical effects in kidneys of rats injected with lead acetate (20 mg/kg) five days per week for six weeks. They observed a significant ($p < 0.05$) reduction in renal alkaline phosphatase activity and an increase in (Mg^{2+})-ATPase, but no significant changes in (Na^{+},K^{+})-ATPase, glucose-6-phosphatase, fructose 1-6 diphosphatase, tryptophan hydroxylase, or succinic dehydrogenase. These findings indicated that preferential effects occurred only in marker enzymes localized in the brush border membrane and mitochondrial inner membrane. Suketa et al. (1979) reported marked (50-90 percent) decreases in renal (Na^{+},K^{+})-ATPase at 6-24 hours following a single oral administration of lead acetate at a dose of 200 mg/kg. A later study (Suketa et al., 1981) using this regi-men showed marked decreases in renal (Na^{+}, K^{+})-ATPase but no significant changes in (Mg^{2+})-ATPase after 24 hours, thus indicating inhibition of a cell membrane marker enzyme prior to changes in a mitochondrial marker enzyme.

12.5.6.2 Mitochondrial Respiration/Energy-Linked Transformation. Effects of lead on renal mitochondrial structure and function have been studied by a number of investigators (Goyer, 1968; Goyer and Krall, 1969a,b; Fowler et al., 1980, 1981a,b). Examination of proximal tubule cells of rats exposed to drinking water containing 0.5-1.0 percent lead acetate for 10 weeks (Goyer, 1968; Goyer and Krall, 1969a,b) or 250 ppm lead acetate for 9 months (Fowler et al., 1980) has shown swollen proximal tubule cell mitochondria *in situ*. Common biochemical findings in these studies were decreases in respiratory control ratios (RCR) and inhibition of state-3 respiration, which was most marked for NAD-linked substrates such as pyruvate/malate. Goyer and Krall (1969a,b) found these respiratory effects to be associated with a decreased capacity of mitochondria to undergo energy-linked structural transformation.

In vitro studies (Garcia-Cañero et al., 1981) using 10^{-4} M lead demonstrated decreased renal mitochondrial membrane transport of pyruvate or glutamate associated with decreased res-

piration for these two substrates. Other in vitro studies (Fowler et al., 1981a,b) have shown decreased renal mitochondrial membrane energization as measured by the fluorescent probes 1-anilino,-8 napthalenesulfonic acid (ANS) or ethidium bromide following exposure to lead acetate at concentrations of 10^{-5} to 10^{-3} M lead. High amplitude mitochondrial swelling was also observed by light scattering.

The results of the above studies indicate that lead produces mitochondrial swelling both in situ and in vitro, associated with a decrease in respiratory function that is most marked for RCR and state-3 respiration values. The structural and respiratory changes appear to be linked to lead-induced alteration of mitochondrial membrane energization.

12.5.6.3 Renal Heme Biosynthesis. There are several reports concerning the effects of lead on renal heme biosynthesis following acute or chronic exposure. Silbergeld et al. (1982) injected rats with lead at 10 μM/kg per day for three days and examined effects on several tissues including kidney. These investigators found an increase in δ-aminolevulinic acid synthetase (ALA-S) following acute injection and no change following chronic exposure (first indirectly via their dams' drinking water containing lead at 10 mg/ml until 30 days of age and then directly via this drinking water to 40-60 days of age). Renal tissue content of δ-aminolevulinic acid (ALA) was increased in both acutely and chronically exposed rats. Renal δ-aminolevulinic acid dehydrase (ALA-D) was found to be inhibited in both acute and chronic treatment groups. Gibson and Goldberg (1970) injected rabbits s.c. with lead acetate at doses of 0, 10, 30, 150, or 200 mg Pb/week for up to 24 weeks. The mitochondrial enzyme ALA-S in kidney was found to show no measurable differences from control levels. Renal ALA-D, which is found in the cytosol fraction, showed no differences from control levels when glutathione was present but was significantly reduced ($p < 0.05$) to 50 percent of control values for the pooled lead-treated groups when glutathione was absent. Mitochondrial heme synthetase (ferrochelatase) was not significantly decreased in lead-treated versus control rabbits, but this enzyme in the kidney was inhibited by 72 and 94 percent at lead-acetate concentrations of 10^{-4} and 10^{-3} M lead, respectively. Accumulation (12-15 fold) of both ALA and porphobilinogen (PBG) was also observed in kidney tissue of lead-treated rabbits relative to controls. Zawirska and Medraś (1972) injected rats with lead acetate at a dose of 3 mg Pb/day for up to 60 days and noted a similar renal tissue accumulation of uroporphyrin, coproporphyrin, and protoporphyrin. A study by Fowler et al. (1980) using rats exposed through 9 months of age to 50 or 250 ppm lead acetate in drinking water showed significant inhibition of the mitochondrial enzymes ALA-S and ferrochelatase but no change in the activity of the cytosolic enzyme ALA-D. Similar findings have been reported for ALA-D following acute i.p. injection of lead acetate at doses of 5-100 mg Pb/kg at 16 hours prior to sacrifice (Woods and Fowler, 1982). In the latter two studies, reduced glutathione was present in the assay mixture.

To summarize the above studies (also see Table 12-11), the pattern of alteration of renal heme biosynthesis by lead is somewhat different from that usually observed with this agent in other tissues (see Section 12.3). A general lack of lead-induced inhibition of renal ALA-D is one frequently reported observation in this tissue except under conditions of high-level exposure. Such a finding could result from the presence of the recently described high affinity cytosolic lead-binding proteins (Oskarsson et al., 1982; Mistry et al., 1982) in the kidney and/or the formation of lead-containing intranuclear inclusion bodies in this tissue (Goyer, 1971; Fowler et al., 1980), which would sequester most of the intracellular lead away from other organelle compartments until the capacity of these mechanisms is exceeded. Based on the observations of Gibson and Goldberg (1970), tissue or assay concentrations of glutathione may also be of importance to the effects of lead on this enzyme. The observed lack of ALA-S induction in kidney mitochondria reported in the above studies may have been caused by decreased mitochondrial protein synthesis capacity or, as previously suggested (Fowler et al., 1980), by overwhelming inhibition of this enzyme by lead, such that any inductive effects were not measurable. Further research is needed to resolve these questions.

12.5.6.4 Lead Alteration of Renal Nucleic Acid/Protein Synthesis. A number of studies have shown marked increases in renal nucleic acid or protein synthesis following acute or chronic exposure to Pb acetate. One study (Choie and Richter, 1972a) conducted on rats given a single intraperitoneal injection of lead acetate showed an increase in ^{3}H-thymidine incorporation. A subsequent study (Choie and Richter, 1972b) involved rats given intraperitoneal injections of 1-7 mg lead once per week over a 6-month period. Autoradiography of ^{3}H-thymidine incorporation into tubule cell nuclei showed a 15-fold increase in proliferative activity in the lead-treated rats relative to controls. The proliferative response involved cells both with and without intranuclear inclusions. Follow-up autoradiographic studies in rats given three intraperitoneal injections of lead acetate (0.05 mg Pb/kg) 48 hours apart showed a 40-fold increase in ^{3}H-thymidine incorporation 20 hours after the first lead dose and 6 hours after the second and third doses.

Choie and Richter (1974a) also studied mice given a single intracardiac injection of lead (5 µg Pb/g) and demonstrated a 45-fold maximal increase in DNA synthesis in proximal tubule cells as judged by ^{3}H-thymidine autoradiography 33 hours later. This increase in DNA synthesis was preceded by a general increase in both RNA and protein synthesis (Choie and Richter, 1974b). The above findings were essentially confirmed with respect to lead-induced increases in nucleic acid synthesis by Cihák and Seifertová (1976), who found a 13-fold increase in ^{3}H-thymidine incorporation into kidney nuclei of mice 4 hours after an intracardiac injection (5 µg Pb/g) of lead acetate. This finding was associated with a 34-fold increase in the mitotic index but no change in the activities of thymidine kinase or thymide monophosphate kinase. Stevenson et al. (1977) have also reported a 2-fold increase in ^{3}H-thymidine or

TABLE 12-11. EFFECTS OF LEAD EXPOSURE ON RENAL HEME BIOSYNTHESIS

Species	Pb dose regimen	ALA-S	ALA-D	FC*	Renal tissue porphyrins	Reference
Rabbit	10, 30, 150, 200 mg Pb/kg/wk (s.c.)	NC**	±↓	NC	↑ ALA, PBG (12-15 x)	Gibson and Goldberg, 1970
Rat	3 mg Pb/day (s.c.)	NM***	NM	NM	↑ uro-, copro-, proto-porphyrins	Zawirska and Medraś, 1972
Rat	10, 100, 1000, 5000 ppm Pb in d.w. for 3 wks	NM	↓	NM	↑ at 1000 and 5000 ppm ↑ ALA-urine	Buchet et al., 1976
Rat (dams)	10 ppm in d.w. during: 3 wks before mating 3 wks of pregnancy 3 wks after delivery	NM	NC	NM	NC	Hubermont et al., 1976
(newborns)		NM	NC	NM	↑	
Rat (dams)	100 ppm Pb in d.w. for 3 wks	NM	NC	NM	NC	Roels et al., 1977
(suckling)		NM	±↓	NM	↑	
Rat	0.5, 5, 25, 50, 250 ppm Pb in d.w. for 9 months	↓	NC	↓	NM	Fowler et al., 1980
Rat	5, 25, 50, 100 mg Pb/kg (i.p.) 16 hrs. prior to sacrifice	NM	NC	NM	NM	Woods and Fowler, 1982
Rat	10 μM Pb/kg/3 day (i.p.)	↑	↓	NM	↑ALA	Silbergeld et al., 1982
	10 mg Pb/ml in d.w. for 10-30 days	NC	↓	NM	↑ALA	

*FC - Ferro chelatase **NC - Not changed relative to controls ***NM - Not measured

^{14}C-orotic acid incorporation into kidney DNA or RNA of rats given a single intraperitoneal injection of lead chloride three days earlier.

The above studies clearly demonstrate that acute or chronic administration of lead by injection stimulates renal nucleic acid and protein synthesis in kidneys of rats and mice. The relationship between this proliferative response and formation of intranuclear inclusion bodies is currently unknown; nor is the basic mechanism underlying this response and the formation of renal adenomas in rats and mice following chronic lead exposure understood.

12.5.6.5 Lead Effects on the Renin-Angiotension System. A study by Mouw et al. (1978) used dogs given a single intravenous injection of lead acetate at doses of 0.6 or 3.0 mg Pb/kg and observed over a 4-hour period. Subjects showed a small but significant decrease in plasma renin activity (PRA) at 1 hour, followed by a large and significant ($p < 0.05$) increase from 2.5 to 4.0 hours. Follow-up work (Goldman et al., 1981) using dogs given a single intravenous injection of lead acetate at 3.0 mg Pb/kg showed changes in the renin-angiotensin system over a 3-hour period. The data demonstrated an increase in PRA, but increased renin secretion occurred in only three of nine animals. Hepatic extraction of renin was virtually eliminated in all animals, thus providing an explanation for the increased blood levels of renin. Despite the large observed increases in PRA, blood levels of angiotensin II (AII) did not increase after lead treatment. This suggests that lead inhibited the AII converting enzyme.

Exposure of rats to drinking water containing 0.5 mg Pb/ml for three weeks to five months (Fleischer et al., 1980) produced an elevation of PRA after six weeks of exposure in those rats on a sodium-free diet. No change in plasma renin substrate (PRS) was observed. At five months, PRA was significantly higher in the lead-treated group on a 1-percent sodium chloride diet, but the previous difference in renin levels between animals on an extremely low-sodium (1 meq) vs. 1-percent sodium diet had disappeared. The lead-treated animals had a reduced ability to decrease sodium excretion following removal of sodium from the diet.

Victery et al. (1982a) exposed rats to lead *in utero* and to drinking water solution containing 0, 100, or 500 ppm lead as lead acetate for six months. Male rats on the 100 ppm lead dose became significantly hypertensive at 3.5 months and remained in that state until termination of the experiment at six months. All female rats remained normotensive as did males at the 500-ppm dose level. PRA was found to be significantly reduced in the 100-ppm treatment males and normal in the 500-ppm treatment groups of both males and females. Dose-dependent decreases in AII/PRA ratios and renal renin content were also observed. Pulmonary AII converting enzyme was not significantly altered. It was concluded that, since the observed hypertension in the 100-ppm group of males was actually associated with reduction of PRA and AII, the renin-angiotensin system was probably not directly involved in this effect.

Webb et al. (1981) examined the vascular responsiveness of helical strips of tail arteries in rats exposed to drinking water containing 100 ppm lead for seven months. These

investigators found that the mild hypertension associated with this regimen was associated with increased vascular responsiveness to α-adrenergic agonists.

Male rats exposed to lead in utero and prior to weaning indirectly by their dams' drinking water containing 0, 5, or 25 ppm lead as lead acetate, followed by direct exposure at the same levels for five months (Victery et al., 1982b), showed no change in systolic blood pressure. Rats exposed to the 25 ppm dose showed a significant ($p < 0.05$) decrease in basal PRA. Stimulation of renin release by administration of polyethylene glycol showed a significant increase in PRA but low AII values. These yielded a significant ($p < 0.001$) decrease in the AII/PRA ratio. Basal renal renin concentrations were found to be significantly reduced in both the 5 ppm ($p < 0.05$) and 25 ppm ($p < 0.01$) dose groups relative to controls.

Victery et al. (1983) exposed rats in utero to lead by maternal administration of 0, 5, 25, 100, or 500 ppm lead as lead acetate. The animals were continued on their respective dose levels through one month of age. All exposure groups had PRA values significantly ($p < 0.05$) elevated relative to controls. Renal renin concentration was found to be similar to controls in the 5 and 25 ppm groups but significantly increased ($p < 0.05$) in the 100 and 500 ppm groups. The plasma AII/PRA ratio was similar to controls in the 100 ppm group but was significantly reduced ($p < 0.05$) in the 500 ppm group.

It appears from the above studies that lead exposure at even low dose levels is capable of producing marked changes in the renin-angiotension system and that the direction and magnitude of these changes is mediated by a number of factors, including dose level, age, and sex of the species tested, as well as dietary sodium content. Lead also appears capable of directly altering vascular responsiveness to α-adrenergic agents. The mild hypertension observed with chronic low level lead exposure appears to stem in part from this effect and not from changes in the renin-angiotensin system. (See also Section 12.9.1 for a discussion of other work on the hypertensive effects of lead.)

12.5.6.6 Lead Effects on Uric Acid Metabolism. A report by Mahaffey et al. (1981) on rats exposed concurrently to lead, cadmium, and arsenic alone or in combination found significantly ($p < 0.05$) increased serum concentrations of uric acid in the lead-only group. While the biochemical mechanism of this effect is not clear, these data support certain observations in humans concerning hyperuricemia as a result of lead exposure (see Section 12.5.3) and, also, confirm an earlier report by Goyer (1971) showing increased serum uric acid concentration in rats exposed to 1 percent lead acetate in drinking water for 84 weeks.

12.5.6.7 Lead Effects on Kidney Vitamin D Metabolism. Smith et al. (1981) fed rats vitamin D-deficient diets containing either low or normal calcium or phosphate for two weeks. The animals were subsequently given the same diets supplemented with 0.82 percent lead as lead acetate. Ingestion of lead at this dose level significantly reduced plasma levels of 1,25 dihydrocholecalciferol in cholecalciferol-treated rats and in rats fed either a low phospho-

rous or low calcium diet while it had no effect in rats fed either a high calcium or normal phosphorous diet. These data suggest decreased production of 1,25-dihydrocholecalciferol in the kidney in response to lead exposure in concert with dietary deficiencies.

12.5.7 General Summary: Comparison of Lead Effects in Kidneys of Humans and Animal Models

It seems clear from the preceding review that, in general, results of experimental animal studies have confirmed findings reported for human kidney function in individuals exposed to lead for prolonged time periods and that these studies have helped illuminate the mechanisms underlying such effects. Similar morphological changes are found in kidneys of humans and animals following chronic lead exposure, including nuclear inclusion bodies, cytomegaly, swollen mitochondria, interstitial fibrosis, and increased numbers of iron-containing lysosomes in proximal tubule cells. Physiological renal changes observed in humans have also been confirmed in animal model systems in regard to increased excretion of amino acids and elevated serum urea nitrogen and uric acid concentrations. The inhibitory effects of lead exposure on renal blood flow and glomerular filtration rate are currently less clear in experimental model systems; further research is needed to clarify the effects of lead on these functional parameters in animals. Similarly, while lead-induced perturbation of the renin-angiotensin system has been demonstrated in experimental animal models, further research is needed to clarify the exact relationships among lead exposure (particularly chronic low-level exposure), alteration of the renin-angiotensin system, and hypertension in both humans and animals.

On the biochemical level, it appears that lead exposure produces changes at a number of sites. Inhibition of membrane marker enzymes, decreased mitochondrial respiratory function/cellular energy production, inhibition of renal heme biosynthesis, and altered nucleic acid synthesis are the most marked changes thus far reported. The extent to which these mitochondrial alterations occur is probably mediated in part by the intracellular bioavailability of lead, which is determined by its binding to high affinity kidney cytosolic binding proteins and deposition within intranuclear inclusion bodies.

Recent studies in humans have indicated that the EDTA lead-mobilization test is the most reliable technique for detecting persons at risk for chronic nephropathy. Blood lead measurements are a less satisfactory indicator because they may not accurately reflect cumulative absorption some time after exposure to lead has terminated.

12.6 EFFECTS OF LEAD ON REPRODUCTION AND DEVELOPMENT

Data from human and animal studies indicate that lead may exert gametotoxic, embryotoxic, and (according to animal studies) teratogenic effects that could influence the survival and development of the fetus and newborn. It appears that prenatal viability and development may also be affected by lead indirectly, via effects on various health parameters of the expectant mother. The vulnerability of the conceptus to such effects of lead has contributed to concern that the unborn may constitute a group at risk for lead health effects. Also, certain information regarding lead effects on male reproductive functions has led to concern regarding the impact of lead on men.

12.6.1 Human Studies

12.6.1.1 Historical Evidence. Findings suggesting that lead exerts adverse effects on human reproductive functions have existed in the literature since before the turn of the century. For example, Paul (1860) observed that severely lead-poisoned pregnant women were likely to abort, while those less severely intoxicated were more likely to deliver stillborn infants. Legge (1901), in summarizing the reports of 11 English factory inspectors, found that of 212 pregnancies in 77 female lead workers, only 61 viable children were produced. Fifteen workers never became pregnant; 21 stillbirths and 90 miscarriages occurred. Of 101 children born, 40 died in the first year. Legge also noted that when lead was fed to pregnant animals, they typically aborted. He concluded that maternal exposure to lead resulted in a direct action of the element on the fetus.

Four years later, Hall and Cantab (1905) discussed the increasing use of lead in nostrums sold as abortifacients in Britain. Nine previous reports of the use of diachylon ("lead plaster") in attempts to cause miscarriage were cited and 30 further cases of known or apparent use of lead in attempts to terminate real or suspected pregnancy listed. Of 22 cases described in detail, 12 resulted in miscarriage and all 12 exhibited marked signs of plumbism, including a blue gum line (in eight cases the women were known to have attempted to induce abortion). Hall's report was soon followed by those of Cadman (1905) and Eales (1905), who described three more women who miscarried following consumption of lead-containing pills.

Oliver (1911) then published statistics on the effect of lead on pregnancy in Britain (Table 12-12). These figures showed that the miscarriage rate was elevated among women employed in industries in which they were exposed to lead. Lead compounds were said by Taussig (1936) to be known for their embryotoxic properties and their use to induce abortion.

In a more recent study by Lane (1949), women exposed to lead levels of 750 $\mu g/m^3$ were examined for effects on reproduction. Longitudinal data on 15 pregnancies indicated an increase in the number of stillbirths and abortions. No data were given on urinary lead in women, but men in this sample had urinary levels of 75 to 100 µg/liter.

TABLE 12-12. STATISTICS ON THE EFFECT OF LEAD ON PREGNANCY

Sample	Number of abortions and stillbirths per 1000 females	Number of neonatal deaths (first year) per 1000 females
Housewives	43.2	150
Female workers (mill work)	47.6	214
Females exposed to lead premaritally	86.0	157
Females exposed to lead after marriage	133.5	271

Source: Oliver (1911).

The above studies clearly demonstrate an adverse effect of lead at high levels on human reproductive functions, and include evidence of increased incidence of miscarriages and stillbirths when women are exposed to lead during pregnancy. The mechanisms underlying these effects are unknown at this time. Many factors could contribute to such results, ranging from lead effects on maternal nutrition or hormonal state before or during pregnancy to more direct gametotoxic, embryotoxic, fetotoxic, or teratogenic effects that could affect parental fertility or offspring viability during gestation. Pregnancy is a stress that may place a woman at higher risk for toxic lead exposure. Both iron deficiency and calcium deficiency increase susceptibility to lead, and women have an increased risk of both deficiencies during pregnancy and postpartum (Rom, 1976).

Such studies as those of Legge, Hall, and Oliver suffer from methodological inadequacies. They must be mentioned, however, because they provide evidence that effects of lead on reproduction occurred at times when women were exposed to high levels of lead. Nevertheless, evidence for adverse reproductive outcomes in women with obvious lead poisoning is of little help in defining the effects of lead at significantly lower exposure levels. Efforts have been made to define more precisely the points at which lead may affect reproductive functions in both the human female and male, as well as in animals, as reviewed below.

12.6.1.2 Effects of Lead Exposure on Reproduction.

12.6.1.2.1 Effects associated with exposure of women to lead. Since the time of the above reports, women have been largely, though not entirely (Khera et al., 1980), excluded from occupational exposure to lead; and lead is no longer used to induce abortion. Thus, little new information is available on reproductive effects of chronic exposure of women to lead. Various reports (Pearl and Boxt, 1980; Qazi et al., 1980; Timpo et al., 1979; Singh et al., 1978; Angle and McIntire, 1964) suggest that relatively high prenatal lead exposures do not invariably result in abortion or in major problems readily detectable in the first few years of life

These findings are based on only a few case histories, however, and are obviously not an adequate sample. The data are confounded by numerous variables, and longer follow-ups are needed.

In a sample population exposed to lead and to other toxic agents (including arsenic and sulfur dioxide) from the Rönnskär smelter, Nordström et al. (1978b) found an increased frequency of spontaneous abortions among women living closest to the smelter. In addition to the exposure to multiple environmental toxins, however, the study was confounded by failure to match exposed and control populations for socioeconomic status. A further study by the same authors (Nordström et al., 1979a) determined that female smelter workers at the Rönnskär smelter had an increased frequency of spontaneous miscarriage when the mother was employed by the smelter during pregnancy or had been so employed prior to pregnancy and still lived near the smelter. Also, women who worked in more highly polluted areas of the smelter were more likely to have aborted than were other employees. This report, however, suffers from the same deficiencies as the earlier study.

In regard to potential lead effects on ovarian function in human females, Panova (1972) reported a study of 140 women working in a printing plant for less than one year (1 to 12 months) where ambient air levels were <7 μg Pb/m^3. Using a classification of various age groups (20-25, 26-35, and 36-40 yr) and type of ovarian cycle (normal, anovular, and disturbed lutein phase), Panova claimed that statistically significant differences existed between the lead-exposed and control groups in the age range 20 to 25 years. Panova's report, however, does not show the age distribution, the level of significance, or data on the specificity of her method for classification. Zielhuis and Wibowo (1976), in a critical review of the above study, concluded that the study design and presentation of data were such that it is difficult to evaluate the author's conclusions. It should also be noted that no consideration was given to the dust levels of lead, an important factor in print shops.

Unfortunately, little else besides the above study appears to exist in regard to assessing the effects of lead on human ovarian function or other factors affecting female fertility. Studies offering firm data on maternal variables, e.g., hormonal state, that are known to affect the ability of the pregnant woman to carry the fetus full term are also lacking.

12.6.1.2.2 Effects associated with exposure of men to lead. Lead-induced effects on male reproductive functions have been reported in several instances. Among the earliest of these was the review of Stöfen (1974), who described data from the work of Neskov in the USSR involving 66 workers exposed chiefly to lead-containing gasoline (organic lead). In 58 men there was a decrease or disappearance of erection, in 41 there was early ejaculation, and in 44 there were a diminished number of spermatocytes. These results were confounded, however, by the presence of the other constituents of gasoline.

Lancranjan et al. (1975) reported lead-related interference with male reproductive functions. A group of 150 workmen who had long-term exposure to lead in varying degrees was studied. Clinical and toxicological criteria were used to categorize the men into four groups: lead-poisoned workmen (mean blood lead level = 74.5 μg/dl) and those showing moderate (52.8 μg/dl), slight (41 μg/dl), or "physiologic" (23 μg/dl) exposure to lead. Moderately increased lead absorption (52.8 μg/dl) was said to result in gonadal impairment. The effects on the testes were believed to be direct, in that tests for impaired hypothalamopituitary influence were negative. Also, semen analysis revealed asthenospermia and hypospermia in all groups except those with "physiologic" absorption levels, and increased teratospermia was seen in the two highest lead exposure groups.

An apparently exposure-related increase in erectile dysfunction was also found by Lancranjan et al. (1975). Problems with ejaculation and libido were said to be more common in the lead exposed groups, but their incidences did not seem to be dose-dependent. Control incidences of these difficulties were invariably lower than those of the lead exposed groups, however, so the lack of a clear cut dose-response relationship may have merely been due to inappropriate assignment of individuals to the high, moderate, and low exposure groups.

The Lancranjan et al. (1975) study has been criticized by Zielhuis and Wibowo (1976), who stated that the distributions of blood lead levels appeared to be skewed and that exposure groups overlapped in terms of lead intake. Thus, the means for each putative exposure group may not have been representative of the individuals within a group. It is difficult to discern, however, if the men were improperly assigned to exposure level groups, as blood lead levels may have varied considerably on a short term basis. Zielhuis and Wibowo also stated that the measured urinary ALA levels were unrealistically high for individuals with the stated blood lead levels. This suggests that if the ALA values were correct, the blood lead levels may have been underestimated. Other deficiencies include failure to use matched controls and exclusion of different proportions of individuals per exposure group for the semen analyses.

Plechaty et al. (1977) measured lead concentrations in the semen of 21 healthy men. Semen lead levels were generally less than blood lead levels, and no correlation was found between lead content of the semen and sperm counts or blood lead levels in this small sample.

Hypothalamic-pituitary-testicular relationships were investigated by Braunstein et al. (1978) in men occupationally exposed at a lead smelter. Six subjects had 2-11 years of exposure to lead and exhibited marked symptoms of lead toxicity. All had received one or more courses of EDTA chelation therapy. This group was referred to as "lead-poisoned" (LP). Four men from the same smelter had no signs of lead toxicity, but had been exposed for 1-23 years and were designated "lead-exposed" (LE). The control (C) group consisted of nine volunteers.

Mean (± standard error) blood lead levels for the LP, LE, and C groups were 38.7 (± 3), 29.0 (± 5), and 16.1 (± 1.7) μg/dl, respectively, at the time of the study. Previously, how-

ever, the LP and LE groups had exhibited values as high as 88.2 (± 4) and 80 (± 0) μg/dl, respectively. All three groups were chelated and 24-hour urinary lead excretion values were 999 (± 141), 332 (± 17), and 225 (± 31) μg for the LP, LE, and C groups, respectively. Frequency of intercourse was significantly less in both lead-exposed groups than in controls. Sperm concentrations in semen of the LP and LE men ranged from normal to severely oligospermic, and one from the LP group was unable to ejaculate. Testicular biopsies were performed on "the two most severely lead-poisoned men," one with aspermia and one with testicular pain. Both men showed increased peritubular fibrosis, decreased spermatogenesis, and Sertoli cell vacuolization. The two lead groups exhibited reduced basal serum testosterone levels, but displayed a normal increase in serum testosterone following stimulation with human chorionic gonadotrophin. A similar rise in serum follicle-stimulating hormone was seen following treatment with clomiphene citrate or gonadotrophin releasing hormone, although the LP men exhibited a lower than expected increase in luteinizing hormone (LH). The LE men also appeared to have a decreased LH response, but the difference was not significant.

The results of the Braunstein et al. (1978) study suggest that lead exposure at high levels may result in a defect in regulation of LH secretion at the hypothalamic-pituitary level, resulting in abnormal dynamics of LH secretion. They also indicate a likely direct effect on the testes, resulting in oligospermia and peritubular fibrosis. Nevertheless, the possibility remains that such effects may have been precipitated by the EDTA chelation therapy, and the numbers of men studied were quite small.

More recently, Wildt et al. (1983) compared two groups of men exposed to lead in a Swedish battery factory. The 29 high-lead group men had had blood lead levels ≥50 μg/dl at least once prior to the study, while the 30 "controls" seldom exceeded 30 μg/dl. There were two test periods eight months apart. For the first test, 15 men were in the high lead and 24 in the control groups, respectively, and 17 were in each group for the second test. Fourteen and 15 of these men from the high lead and control groups, respectively, took part in both tests. Blood lead values were obtained periodically over a six-month period. For the two high lead groups, blood lead values were 46.1 and 44.6 μg/dl, respectively (range 25-75); corresponding values for the controls were 31.1 and 21.5 μg/dl (range 8-39). The high lead men tended to exhibit decreased function of the prostate and/or seminal vesicles, as measured by seminal plasma constituents (fructose, acid phosphatase, Mg, and Zn); however, a significant difference was seen only in the case of zinc. More men in the high lead than in the control group had low semen volume values, but the numbers of individuals did not allow a reliable statistical analysis. The heads of sperm of high lead individuals were more likely to swell when exposed to a detergent solution, viz. sodium dodecyl sulfate (SDS), a test of functional maturity, but the values were still in a normal range. Conversely, the leakage of lactate dehydrogenase isoenzyme X (LDH-X) was greater in control semen samples.

The values for live and for motile sperm were lower in the control group. The data were skewed, however, by the presence of several of the same men with low values in the control groups for both sampling times. Another confounding factor was the fact that the high lead and control groups differed in a significant way: ten of the control men had present or past urogenital tract infections versus none in the high lead group, possibly explaining the incidence of control samples with lowered sperm motility and viability. The observed decrease in SDS resistance in sperm of high lead group men may have been related to their apparent abnormal prostatic function, or to an effect of lead on sperm maturation. In evaluating the above results, it must also be noted that even the "controls" had elevated blood lead levels.

12.6.1.3 Placental Transfer of Lead. The transfer of lead across the human placenta and its potential threat to the conceptus have been recognized for more than a century (Paul, 1860). Documentation of placental transfer of lead to the fetus and data on resulting fetal blood lead levels help to build the case for a potential, but as yet not clearly defined, threat of subtle embryotoxicity or other deleterious health effects.

The placental transfer of lead has been established, in part, by various studies that have disclosed measurable quantities of lead in human fetuses or newborns, as well as offspring of experimental animals. The relevant data on prenatal lead absorption have been reviewed in Chapter 10, Section 2.4 of this document, and thus work dealing only with lead levels will not be discussed further here.

12.6.1.4 Effects of Lead on the Developing Human.

12.6.1.4.1 Effects of lead exposure on fetal metabolism. Prenatal exposure of the conceptus to lead, even in the absence of overt teratogenicity, may be associated with other health effects. This is suggested by studies relating fetal and cord-blood levels to changes in fetal heme synthesis. Haas et al. (1972) examined 294 mother-infant pairs for blood lead and urinary ALA levels. The maternal blood lead mean was 16.89 μg/dl; and the fetal blood lead mean was 14.98 μg/dl, with a correlation coefficient of 0.54 ($p < 0.001$). In the infants, blood lead levels and urinary ALA were positively correlated ($r = 0.19$, $p < 0.01$), although the data were based on spot urines (which tends to limit their value). The full biological significance of the elevated ALA levels is not clear, but the positive correlation between lead in blood and urinary ALA for the group as a whole indicates that increased susceptibility of heme synthesis occurs at relatively low blood lead levels in the fetus or newborn infant.

Subsequently, Kuhnert et al. (1977) measured ALA-D activity and levels of erythrocyte lead in pregnant urban women and their newborn offspring. Cord erythrocyte lead levels ranged from 16 to 67 μg/100 ml of cells, with a mean of 32.9. Lead levels were correlated with inhibition of ALA-D activity ($r = -0.58$, $p < 0.01$), suggesting that typical urban lead exposures could affect fetal enzyme activity. Note, however, that ALA-D activity is related to blood cell age, being highest in the younger cells. Thus, results obtained with cord blood, with

its high percentage of immature cells, are not directly comparable to those obtained with adult blood. In a later study, Lauwerys et al. (1978) found no lead-related increase in erythrocyte porphyrin levels in 500 mothers or their offspring. They did, however, report negative correlations between ALA-D activity and blood lead levels in both mothers and their newborns. Maternal blood lead levels averaged 10.2 μg/dl (range 3.1-31 μg/dl). Corresponding values for the newborns were 8.4 μg/dl and 2.7-27.3. Such results indicate that ALA-D activity may be a more sensitive indicator of fetal lead toxicity than erythrocyte porphyrin or urinary ALA levels.

12.6.1.4.2 Other toxic effects of intrauterine lead exposure. Fahim et al. (1976), in a study on maternal and cord-blood lead levels, determined blood lead values in women having preterm delivery and premature membrane rupture. Such women residing in a so-called "lead belt" (mining and smelting area) had significantly higher blood lead levels than women from the same area delivering at full term. Fahim et al. (1976) also noted that among 249 pregnant women in a control group outside the lead belt area, the percentages of women having preterm deliveries and premature rupture were 3 and 0.4, respectively, whereas corresponding values for the lead area (n = 253) were 13.04 and 16.99, respectively. A confusing aspect of this study, however, is the similarity of blood lead levels in women from the nonlead and lead belt areas. In fact, no evidence was presented that women in the lead belt group had actually received a greater degree of lead exposure during pregnancy than did control individuals. Also, questions exist regarding analytical aspects of this study. Specifically, other workers (e.g., see summary table in Clark, 1977) have typically found blood lead levels in mothers and their newborn offspring to be much more similar than those of Fahim et al. (1976).

In another study, Clark (1977) detected no effects of prenatal lead exposure in newborns with regard to birth weight, hemoglobin, or hematocrit. He compared children born of 122 mothers living near a Zambian lead mine with 31 controls from another area. Maternal and infant blood lead levels for the mine area were 41.2 (± 14.4) and 37.9 (± 15.3) μg/dl, respectively. Corresponding values for control mothers and offspring were 14.7 (± 7.5) and 11.8 (± 5.6) μg/dl.

There is also some evidence that lead levels in bone samples from stillborn children are higher than would be expected (Khera et al., 1980; Bryce-Smith et al., 1977), but the data are inconclusive.

Nordström et al. (1979b) examined birth weight records for offspring of female employees of the Rönnskär smelter and found decreased birth weights related to: (1) employment of the mothers at the smelter during pregnancy, (2) distance that the mothers lived from the smelter, and (3) proximity of the mother's job to the actual smelting process. Similar results were also seen for children born to mothers merely living near the smelter (Nordström et al., 1978a). Nordström et al. (1979b) also investigated birth defects in offspring of the female

smelter workers and in populations living at various distances from the Rönnskär smelter. They concluded that the frequencies of both single and multiple malformations were increased when the mother worked at the smelter during pregnancy.

The number of smelter workers with malformed offspring was relatively small (39/1291). The incidence of children with birth defects whose mothers worked while pregnant was 5.8 percent (17 of 291). Five of the six offspring with multiple malformations were in this group, suggesting that the observed effect may have been a real one. Nevertheless, the crucial factor in evaluating all of the Rönnskär studies is the exposure of workers and the nearby population to a number of toxic substances including not only lead, but arsenic, mercury, cadmium, and sulfur dioxide as well.

Alexander and Delves (1981) found that the mean blood lead concentrations of pregnant and non-pregnant control women living in an urban area of England were approximately 4 μg/dl higher than those for similar groups living in a rural area. The mean concentrations for the urban and rural pregnant women were 15.9 and 11.9 μg/dl, respectively ($p < 0.001$), but there were no demonstrable effects of the higher maternal blood lead levels on any aspect of perinatal health. The rate for congenital abnormality was higher in the rural area, suggesting that whatever the cause, it was unlikely to be related to maternal levels of lead.

Additional studies of placental lead and stillbirths have not clarified the situation. Khera et al. (1980) measured placental and stillbirth tissue lead in occupationally exposed women in the United Kingdom. Regardless of the incidence of stillbirths, placental lead concentrations were found to increase with duration of occupational exposure, from 0.29 μg/g at <1 yr exposure to 0.48 μg/g at >6 yr exposure for a group of 26 women aged 20-29 years. Placental lead concentrations also increased with age of the mother, independently of time of occupational exposure, and ranged from 0.30 (± 0.16) μg/g for those <20 yrs old to 0.51 (± 0.44) μg/g for those ≥30 yrs old. Average placental lead concentrations for 20 occupationally exposed women whose babies were stillborn were higher [0.45 (± 0.32) μg/g] than the average level of 0.29 (± 0.09) μg/g for placentas from eight mothers who had not been occupationally exposed for at least two years. The authors noted, however, that it was not possible to say whether occupational exposure caused any of the stillbirths or whether the high lead levels were merely consequential to the fetal death. It is somewhat disconcerting that the placental lead concentrations were about three times lower than those reported earlier by this group (Wibberley et al., 1977). These differences were attributed to methodological changes and to changes in concentration during storage of placentas at -20°C (Khera et al., 1980).

The placental lead concentrations reported by Alexander (1982) are, however, similar to the earlier results of Wibberley et al. (1977), with mean values of 1.34 (± 0.15) μg/g for seven stillbirths and 1.27 (± 0.48) μg/g for seven matched healthy controls. The wide range of concentrations reported for the controls (0.34-5.56 μg/g) and the differences in concentra-

tion with site of sampling makes it difficult to draw any useful conclusions from the results presented by Alexander. Clearly these analytical discrepancies in placental lead measurements must be resolved if any interpretation of their significance is to be made.

An additional study by Roels et al. (1978b) reported placental lead values of 0.08 (± 0.05) μg/g (range = 0.01-0.40 μg/g) from a variety of locations in Belgium, but these data indicated no correlation between lead concentration and birth weight. In contrast, placental lead has been reported to be associated with decreased activity of a placental enzyme, steroid sulfatase (Karp and Robertson, 1977). A similar association was found for mercury, suggesting that either metal or both together could have affected the enzyme activity or that the authors had merely uncovered a spurious correlation.

12.6.1.4.3 Paternally mediated effects of lead. There is increasing evidence that exposure of male laboratory animals to toxic agents can result in adverse effects on their offspring, including decreased litter size, birth weight, and survival. Mutagenic effects are the most likely cause of such results, but other mechanisms have been proposed (Soyka and Joffe, 1980). In the following cases, exposure of human males to lead has been implicated as the cause of adverse effects on the conceptus.

According to Koinuma (1926) in a brief report, 24.7 percent of workmen exposed to lead in a storage battery plant had childless marriages, while the value for men not so exposed was 14.8 percent. Rates for miscarriages or stillbirths in wives of lead-exposed men and controls were 8.2 and 2.8 percent, respectively, while corresponding figures for neonatal deaths were 24.2 and 19.2 percent. These comparisons were based on 170 lead-exposed and 128 control men. These differences in fertility and prenatal mortality, while not dramatic, are suggestive of a male-mediated lead effect; however, the reliability of the methodology used in this study cannot be determined, due to the brevity of the report.

In a study of the pregnancies of 104 Japanese women before and after their husbands began lead-smelter work, miscarriages increased to 8.30 percent of pregnancies from a pre-exposure rate of 4.70 percent (Nogaki, 1957). The miscarriage rate for 75 women whose husbands were not occupationally exposed to lead was 5.80 percent. In addition, exposure to lead was related to a significant increase in the ratio of male to female offspring at birth. Lead content of paternal blood ranged from 11 to 51.7 μg/dl [mean = 25.4 (± 1.26) μg/dl], but was not correlated with reproductive outcome, except in the case of the male to female offspring ratio. The reported blood lead levels appear low, however, in view of the occupational exposure of these men, and were similar to those given for controls [mean = 22.8 (± 1.63) μg/dl]. Also, maternal age and parity appear not to have been well controlled for in the analysis of the reproductive data. Another report (Van Assen, 1958) on fatal birth defects in children conceived during a period when their fathers were lead poisoned (but neither before nor after) also hints at paternally-mediated effects of lead.

In the above study by Nordström et al. (1979b), women employed at the Rönnskär smelter were found to have higher abortion rates if their husbands were also employed at the smelter. This was true only of their third or later pregnancies, however, suggesting that the effect was related to long-term exposure of the male gametogenic stem cells. Whether this was a lead effect or that of other toxins from the smelter is not clear.

12.6.1.5 Summary of the Human Data. The literature on the effects of lead on human reproduction and development leaves little doubt that lead can, at high exposure levels, exert significant adverse health effects on reproductive functions. Most studies, however, only looked at the effects of prolonged moderate to high exposures to lead, e.g., those encountered in industrial situations, and many reports do not provide definite information on exposure levels or blood lead levels at which specific effects were observed. Also the human data were derived from studies involving relatively small numbers of individuals and therefore do not allow for discriminating statistical analysis. These reports are often additionally confounded by failure to obtain appropriate controls and, in some cases, by the presence of additional toxic agents or disease states. These and other factors obviously make interpretation of the data difficult. It appears possible that effects on sperm or on the testis may occur due to chronic exposure resulting in blood lead values of 40-50 μg/dl, based on the Lancranjan et al. (1975) and Wildt et al. (1983) studies, but additional data are greatly needed. Exposure data related to reproductive functions in the female are so lacking that even a rough estimate is impossible. Data on maternal exposure levels at which effects may be seen in human fetuses or infants are also quite meager, although the results of Haas et al. (1972), Kuhnert et al. (1977), and Lauwerys et al. (1978) suggest possible perinatal effects on heme metabolism at maternal blood levels considerably below 30 μg/dl. The human data on actual absorbed doses are even more lacking than those on blood lead values, adding to the imprecision of conclusions relating lead exposure to reproductive outcome.

12.6.2 Animal Studies

12.6.2.1 Effects of Lead on Reproduction.

12.6.2.1.1 Effects of lead on male reproductive functions. Among the first investigators to report infertility in male animals due to lead exposure were Puhac et al. (1963), who exposed rats to lead via their diet. Ability to sire offspring returned, however, 45 days after cessation of treatment. More recently, Varma et al. (1974) gave a solution of lead subacetate in drinking water to male Swiss mice for four weeks (mean total intake of lead = 1.65 g). The fertility of treated males was reduced by 50 percent. Varma and coworkers calculated the mutagenicity index (number of early fetal deaths/total implants) to be 10.4 for lead-treated mice versus 2.98 for controls ($p < 0.05$). The major differences in fecundity appeared to have been due to differing pregnancy rates, however, rather than prenatal mortality. Impairment of

male fertility by lead rather than lead-induced mutagenicity was thus likely to have been the primary toxic effect observed. Additionally, it has been suggested by Léonard et al. (1973), that effects seen following administration of lead acetate in water may be due to resulting acidity, rather than to lead. Also, Eyden et al. (1978) found no decrease in fertility of male mice fed 0.1 percent lead acetate in the diet for 64 weeks.

Several animal studies have found lead-associated damage to the testes or prostate, generally at relatively high doses. Golubovich et al. (1968) found a decrease in normal spermatogonia in the testes of rats gavaged for 20 days with lead (2 mg/kg/day). Desquamation of the germinal epithelium of the seminiferous tubules was also increased, as were degenerating spermatogonia. Hilderbrand et al. (1973) also noted testicular damage in male rats given oral lead (100 μg/day for 30 days). Egorova (cited in Stöfen, 1974) injected lead at a dose of 2 μg/kg six times over a ten-day period and reported testicular damage.

Ivanova-Chemishanska et al. (1980) investigated the effect of lead on male rats administered 0.0001 or 0.01 percent solutions of lead acetate over a four-month period. The authors reported that changes in enzymatic activity and in levels of disulfide and ATP were observed in testicular homogenates. No histopathological changes in testicular tissue were found, but the fertility index for treated males was decreased. Offspring of those males exhibited postpartum "failure to thrive" and stunted growth. Such data suggest biological effects due to chronic lead exposure of the male, but the study is difficult to evaluate due to limited information on the experimental methods, particularly the dose levels actually received.

In a more recent study of lead effects on the male reproductive tract, no histopathological changes were seen during an examination of the testes of rabbits (Willems et al., 1982). Five males per group were dosed subcutaneously with up to 0.5 mg/kg lead acetate three times weekly for 14 weeks. Blood lead levels at termination of treatment were 6.6 and 61.5 μg/dl for control and high dose rabbits, respectively.

Lead-related effects on spermatozoa have also been published. For example, Stowe et al. (1973) reported the results of a low calcium and phosphate diet containing 100 ppm lead (as acetate) fed to dogs from 6 to 18 weeks of age. This dose resulted in a number of signs of toxicity, including spermatogonia with hydropic degeneration. In the Maisin et al. (1975) study, male mice received up to 1 percent lead in the diet, and the percentage of abnormal spermatozoa increased with increasing lead exposure. Eyden et al. (1978) also fed 1 percent lead acetate in the diet to male mice. By the eighth week, abnormal sperm had increased; however, the affected mice showed weight loss and other signs of general toxicity. Thus, the spermatogenesis effect was not indicative of differential sensitivity of the gonad to lead.

Krasovskii et al. (1979) observed decreased motility, duration of motility, and osmotic stability of sperm from rats given 0.05 mg/kg lead orally for 20-30 days. Damage to gonadal

blood vessels and to Leydig cells was also seen. Rats treated for 6-12 months exhibited abnormal sperm morphology and decreased spermatogenesis. In the report of Willems et al. (1982) described above, however, no effects on sperm count or morphology were seen in rabbits.

Lead acetate effects on sperm morphology were also tested in mice given about one sixteenth to one half an LD_{50} dose by i.p. injection on five consecutive days (Bruce and Heddle, 1979; Wyrobek and Bruce, 1978; Heddle and Bruce, 1977). The two lowest doses (apparently 100 and 250 mg/kg) resulted in only a modest increase in morphologically abnormal sperm 35 days after treatment, but the 500 or 900 mg/kg doses resulted in up to 21 percent abnormal sperm.

That lead could directly affect developing sperm or their cellular precursors is made more plausible by the data of Timm and Schulz (1966), who found lead in the seminiferous tubules of rats and in their sperm. The mechanisms for lead effects on the male gonad or gamete are unknown, however, although Golubovich et al. (1968) found altered RNA levels in the testes of lead exposed rats. They suggested that testicular damage was related to diminished ribosomal activity and inhibition of protein synthesis. As noted above, Ivanova-Chemishanska et al. (1980) observed biochemical changes in testes of lead-treated mice. Nevertheless, such observations are only initial attempts to determine a mechanism for observed lead effects. A more likely mechanism for such effects on the testis may be found in the work of Donovan et al. (1980), who found that lead inhibited androgen binding by the cytosolic receptors of mouse prostate. This could provide a mechanism for the observation of Khare et al. (1978), who found that injection of lead acetate into the rat prostate resulted in decreased prostatic weight; no such changes were seen in other accessory sex glands or in the testes.

Effects on hormonal production or on hormone receptors could also explain the results of Maker et al. (1975), who observed a delay in testicular development and an increase in age of first mating in male mice of two strains whose dams were given 0.08 percent lead (C57Bl/6J) or 0.5 percent lead (Swiss-Webster albino) during pregnancy and lactation. The weanling males were fed these same doses in their diets through 60 days of age.

Another potential mechanism underlying lead effects on sperm involves its affinity for sulfhydryl groups. Mammalian sperm possess high concentrations of sulfhydryls believed to be involved in the maintenance of motility and maturation via regulation of stability in sperm heads and tails (Bedford and Calvin, 1974; Calvin and Bedford, 1971). It has also been found that blockage of membrane thiols inhibits sperm maturation (Reyes et al., 1976).

12.6.2.1.2 Effects associated with exposure of females to lead. Numerous studies have focused on lead exposure effects in females. For example, effects of lead on reproductive functions of female rats were studied by Hilderbrand et al. (1973), using animals given lead acetate orally at doses of 5 and 100 μg for 30 days. Control rats of both sexes had the same blood lead levels. Blood lead levels of treated females were higher than those of similarly treated males: 30 versus 19 μg/dl at the low dose, and 53 versus 30 μg/dl at the high dose.

The females exhibited irregular estrus cycles at both doses. When blood lead levels reached 50 μg/dl, they developed ovarian follicular cysts, with reductions in numbers of corpora lutea.

In a subsequent study (Der et al., 1974), lead acetate (100 μg lead per day) was injected s.c. for 40 days in weanling female rats. Treated rats received a low-protein (4 percent) or adequate-protein (20 percent) diet; controls were given the same diets without lead. Females on the low protein, high lead diet did not display vaginal opening during the treatment period and their ovaries decreased in weight. No estrous cycles were observed in animals from either low protein group; those of the adequate diet controls were normal, while those of the rats given adequate protein plus lead were irregular in length. Endometrial proliferation was also inhibited by lead treatment. Blood lead levels were 23 μg/dl in the two control groups, while values for the adequate and low protein lead-treated groups were 61 and 1086 μg/dl, respectively. The reports of Hilderbrand et al. (1973) and Der et al. (1974) suggest that lead chronically administered in high doses can interfere with sexual development in rats and the body burden of lead is greatly increased by protein deprivation.

Maker et al. (1975) noted a delay in age at first conception in female mice of two strains exposed to 0.08 percent (C57B1/6J) or 0.5 percent lead (Swiss-Webster) indirectly via the maternal diet (while in utero and nursing) and directly up to 60 days of age. These females were retarded in growth and tended to conceive only after reaching weights approximating those at which untreated mice normally first conceive. Litters from females that had themselves been developmentally exposed to at least 0.5 percent lead had lower survival rates and retarded development. More recently, Grant et al. (1980) reported delayed vaginal opening in rats whose mothers were given 25, 50, or 250 ppm lead (as lead acetate) in their drinking water during gestation and lactation followed by equivalent exposure of the offspring after weaning. The vaginal opening delays in the 25 ppm females occurred in the absence of any growth retardation or other developmental delays, in association with median blood lead levels of 18-29 μg/dl.

Although most animal studies have used rodents, Vermande-Van Eck and Meigs (1960) administered lead chloride i.v. to female rhesus monkeys. The monkeys were given 10 mg/ week for four weeks and 20 mg/week for the next seven months. Lead treatment resulted in cessation of menstruation, loss of color of the "sex skin" (presumably due to decreased estrogen production), and pathological changes in the ovaries. One to five months after lead treatment ceased menstrual periods resumed, the sex skin returned to a normal color, and the ovaries regained their normal appearance. Thus, there was an apparent reversal of lead effects on female reproductive functions, although there were no confirmatory tests of fertility.

The above studies indicate that pre- and/or post-natal exposure of female animals to lead can affect pubertal progression and hypothalamic-pituitary-ovarian-uterine functions. The

observations of delayed vaginal opening may reflect delayed ovarian estrogen secretion, suggesting toxicity to the ovary, hypothalamus, or pituitary. One study has demonstrated decreased levels of circulating follicle-stimulating hormone (Petrusz et al., 1979), and others discussed previously have shown lead-induced ovarian atrophy (Stowe and Goyer, 1971; Vermande-Van Eck and Meigs, 1960), again suggesting toxicity involving the hypothalamic-pituitary-ovarian-endometrial axis.

12.6.2.2 Effects of Lead on the Offspring. This section discusses developmental studies of offspring whose parents (one or both) were exposed to lead. Possible male-mediated effects as well as effects of exposure during gestation are reviewed. Results obtained for offspring of females given lead following implantation or throughout pregnancy are summarized in Tables 12-13 and 12-14.

12.6.2.2.1 Male mediated effects. A few studies have focused on male-mediated lead effects on the offspring, suggesting that paternally transmitted effects of lead may cause reductions in litter size, offspring weight, and survival rate.

Cole and Bachhuber (1914), using rabbits, were the first to report paternal effects of lead intoxication. In their study, the litters of dams sired by lead-intoxicated male rabbits were smaller than those sired by controls. Weller (1915) similarly demonstrated reduced birth weights and survival among offspring of lead-exposed male guinea pigs.

Offspring of lead-treated males from the Ivanova-Chemishanska et al. (1980) study described above were affected in a variety of ways, e.g. they exhibited "failure to thrive" and lower weights than did control progeny at one and three weeks postpartum. These results are difficult to interpret, however, without more specific information on the experimental methods and dosing procedures.

12.6.2.2.2 Results of lead exposure of both parents. Only a few studies have assessed the effects of lead exposure of both parents on reproduction. Schroeder and Mitchener (1971) found a reduction in the number of offspring of rats and mice given drinking water containing 25 ppm lead. According to the data of Schroeder et al. (1970), however, animals in the 1971 study may have been chromium deficient, and the Schroeder and Mitchener (1971) results are in marked contrast to those of an earlier study by Morris et al. (1938), who reported no significant reduction in weaning percentage among offspring of rats fed 512 ppm lead.

In another study, Stowe and Goyer (1971) assessed the relative paternal and maternal effects of lead as measured by effects on the progeny of lead-intoxicated rats. Female rats fed diets with or without 1 percent lead were mated with normal males. The pregnant rats were continued on their respective rations with or without lead throughout gestation and lactation. Offspring of these matings, the F_1 generation, were fed the rations of their dams and were mated in combinations as follows: control female to control male (CF-CM), control female to

TABLE 12-13. EFFECTS OF PRENATAL EXPOSURE TO LEAD ON THE OFFSPRING OF LABORATORY AND DOMESTIC ANIMALS: STUDIES USING ORAL OR INHALATION ROUTES OF EXPOSURE

Species	Test agent	Treatment		Effect on the offspring[a]			Reference
		Dose[b] and mode	Timing[c]	Mortality	Fetotoxicity	Malformation	
Rat	Lead acetate	512 ppm in diet	all	-	-	?	Morris et al. (1938)
		10,000 ppm in diet	all	+	+	?	Stowe and Goyer (1971)
	Lead acetate	45.2 mg/kg/day, po	6-16	-	-	-	Kennedy et al. (1975)
		45.5 mg/kg/day, po	6-8	+	+	-	
		31.9-47.8 mg/kg/day, po	all	-	-	-	Miller et al. (1982)
		63.7 mg/kg/day, po	all	-	+	-	
		150 mg/kg/day, po	6-18	+	-	-	Wardell et al. (1982)
		255-478 mg/kg/day in water	all,LAC	?	+[d]	?	Murray et al. (1978)
		31.9-319 ppm in water	all	±[e]	+	-	Dilts and Ahokas (1979, 1980)
		0.32-159 ppm in water	all	-	+	-	Kimmel et al. (1980)
	Tetraethyl lead	1.6-3.2 mg/kg/day, po	9-11 or 12-14	±	+	-	McClain and Becker (1972)
		0.064 mg/kg/day, po	6-16	-	-	-	Kennedy et al. (1975)
		0.64 mg/kg/day, po	6-16	+	+	-	
		6.4 mg/kg/day, po	6-8	+	+	-	
	Tetramethyl lead	10-28.7 mg/kg/day, po	9-11 or 12-14	±	+	-	McClain and Becker (1972)
	Trimethyl lead chloride	3.6-7.2 mg/kg/day, po	9-11 or 12-14	-	+	-	
	Lead nitrate	1 ppm in water	all	-	-	?	Hubermont et al. (1976)
		10 ppm in water	all	-	+[f,g]	?	
	Lead (aerosol)	1 or 3 mg/m^3, inhaled	1-21	?	-[f]	?	Prigge and Greve (1977)
		10 mg/m^3, inhaled	1-21	?	+[h]	?	
Mouse	Lead acetate	3,185 ppm in diet	1-7	+	±	N/A	Jacquet (1977)
		780-1,593 ppm in diet	1-16,17, or 18	?	+[f,i]	?	Jacquet et al. (1977b)
		3,185 ppm in diet	1-16,17, or 18	?	+[f,i,j]	?	
		1,593-6,370 ppm in diet	1-15,16, or 17	?	+[k]	?	Gerber and Maes (1978)
		1,595-3,185 ppm in diet	7-16,17, or 18	?	+[l]	?	Gerber et al. (1978)
		45.3 mg/kg/day, po	6-16	-	-	-	Kennedy et al. (1975)
		455 mg/kg/day, po	6-8	+	+	-	

PRELIMINARY DRAFT

TABLE 12-13. (continued)

Species	Test agent	Treatment: Dose[b] and mode	Treatment: Timing[c]	Effect on the offspring[a]: Mortality	Effect on the offspring[a]: Fetotoxicity	Effect on the offspring[a]: Malformation	Reference
Mouse		0.1-1.0 g/l in water	all	-	?	?	Léonard et al. (1973)
		637-3,185 ppm in diet	1-18	+	?	?	Maisin et al. (1975)
		1,593 ppm in diet	1-16,17, or 18	+	-	-	Jacquet et al. (1975)
		3,185 ppm in diet	1-16,17, or 18	+	+	-	
		1,250 ppm in diet	all	-	+	-	
		3,185 ppm in diet	1-16,17, or 18	+	+	-	
		1,250 ppm in diet	all	-	+	-	
		2,500-5,000 ppm in diet	all	+	+	-	Jacquet (1976)
		1,250 ppm in diet	all	-	+	-	
	Tetraethyl lead	0.06 mg/kg/day, po	6-16	-	-	-	Kennedy et al. (1975)
		0.64 mg/kg/day, po	6-16	+	+	-	
		6.4 mg/kg/day, po	6-8	+	+	-	
Sheep	Lead powder	0.5-16 mg/kg/day, in diet[l]	all	+	?	-	Sharma and Buck (1976)

[a]+ = present; - = effect not seen; ± = ambiguous effect; ? = effect not examined or insufficient data.

[b] As elemental lead.

[c]Specific gestation days when exposed; LAC = also during lactation.

[d]Decreased numbers of dendritic spines and malformed spines at day 30 postpartum.

[e]Litter size values for high dose group suggestive of an effect.

[f]ALAD activity was decreased.

[g]Free tissue porphyrins increased in kidneys.

[h]Hematocrit was decreased.

[i]Fetal porphyrins were increased, except in the low dose fetuses assayed on gestation day 18.

[j]Decreased heme and fetal weight.

[k]Incorporation of Fe into heme decreased, and growth was retarded.

[l]Decreased placental blood flow.

PRELIMINARY DRAFT

TABLE 12-14. EFFECTS OF PRENATAL LEAD EXPOSURE ON OFFSPRING OF LABORATORY ANIMALS: RESULTS OF STUDIES EMPLOYING ADMINISTRATION OF LEAD BY INJECTION

Species	Test agent	Treatment		Effect on the offspring[a]			Reference
		Dose[b] and mode	Timing[c]	Mortality	Fetotoxicity	Malformation	
Rat	Lead acetate	15.9 mg/kg, ip	9	+	+	+	Zegarska et al. (1974)
	Lead nitrate	31.3 mg/kg, iv	8	-	+	+	McClain and Becker (1975)
		31.3 mg/kg, iv	9 or 16	+[d]	+	+	
		31.3 mg/kg, iv	10-14, 15,17	+	+	-	
		3.13 mg/kg, iv	9 or 15	-	-	-	Hackett et al. (1978a,b)
		15.6 mg/kg, iv	9	+	+	+	
		15.6 mg/kg, iv	15	+	?	?	
		unknown, iv	8 or 9	+	?	+	Coro Antich and Amoedo Mon (1980)
		31.3 mg/kg, iv	17	-	+	-	Minsker et al. (1982)
		15.6 mg/kg, iv	17	+	+	-	
		5 mg/kg, iv	9 or 15	-	-	-	Hackett et al. (1982)
		25 mg/kg, iv	9 or 15	+	+	+,-[e]	
	Lead chloride	7.5 mg/kg,[f]	9	±	-	-	McLellan et al. (1974)
		75 mg/kg,[f]	9	+	+	-	
	Trimethyl lead chloride	20.2 mg/kg, iv	12	-	+	-	
		23.8 mg/kg, iv	9,10,13, or 15	+[g]	+	-	
Mouse	Lead acetate	9.56-22.3 mg/kg, ip	8	-	+	+	Jacquet and Gerber (1979)
		9.56 mg/kg, ip	9	+	-	+	
		22.3 mg/kg, ip	9	+	+	+	
		22.3 mg/kg, ip	10 or 12	-	-	-	
	Lead chloride	29.8 mg/kg, iv	3 or 4	+	?	?	Wide and Nilsson (1977)
		29.8 mg/kg, iv	6	+	N/A	N/A	

PRELIMINARY DRAFT

TABLE 12-14. (continued)

Species	Test agent	Treatment: Dose[b] and mode	Treatment: Timing[c]	Effect on the offspring[a]: Mortality	Effect on the offspring[a]: Fetotoxicity	Effect on the offspring[a]: Malformation	Reference
Hamster	Lead acetate	31.9 mg/kg, iv	8	+	?	+	Ferm (1969)
	Lead acetate or chloride	31.9 or 37.3 mg/kg, iv	8	?	?	+	Ferm and Carpenter (1967)
	Lead nitrate	31.3 mg/kg, iv	7, 8, or 9	?	?	+	Ferm and Carpenter (1967)
		15.6-31.3 mg/kg, iv	8 or 9	+	?	+	Ferm and Ferm (1971)
		31.3 mg/kg, iv	8	+	+	+	Carpenter and Ferm (1977)
		31.3 mg/kg, iv	8	+	+[h]	+	Gale (1978)

[a] + = effect present; - = effect not seen; ± = ambiguous effect; ? = effect not examined or insufficient data.

[b] As elemental lead.

[c] Specific gestation days when exposed.

[d] With the exception of day 17.

[e] No fetuses survived to be examined for malformation.

[f] No dosage route specified.

[g] Only after day 10 treatment.

[h] Delayed ossification (fetal weights not given).

[i] Dosage was varied daily to maintain a blood lead level of ≅ 40 μg/dl (range = 30 to 70 μg/dl).

PRELIMINARY DRAFT

lead-intoxicated male (CF-PbM), lead-intoxicated female to control male (PbF-CM), and lead-intoxicated female to lead-intoxicated male (PbF-PbM). The results are shown in Table 12-15.

The paternal effects of lead included reductions of 15 percent in the number of pups per litter, 12 percent in mean pup birth weight, and 18 percent in pup survival rate. The maternal effects of lead included reductions of 26 percent in litter size, 19 percent in pup birth weight, and 41 percent in pup survival. The combined male and female effects of lead toxicity resulted in reductions of 35 percent in the number of pups per litter, 29 percent in pup birth weight, and 67 percent in pup survival to weaning. Stowe and Goyer classified the effects of lead upon reproduction as gametotoxic, intrauterine, and extrauterine. The gametotoxic effects of lead seemed to be irreversible and had additive male and female components. Intrauterine effects were presumed to be due to lead uptake by the conceptus, plus gametotoxic effects. The extrauterine effects were due to the passage of lead from the dam to the nursing pups, adding to the gametotoxic and intrauterine effects.

Léonard et al. (1973), however, found no effect on the reproductive performance of groups of 20 pairs of mice given lead in their drinking water over a nine-month period. Lead doses ranged from 0.1 to 1.0 g/l. A total amount of 31 g/kg was ingested at the high dose, equivalent to ingestion of 2.2 kg lead by a 70 kg man over the same time period.

12.6.2.2.3 Lead effects on implantation and early development. Numerous studies have been performed to elucidate mechanisms by which lead causes prenatal death. They suggest two mechanisms of action for lead, one on implantation and the other (mainly at higher doses) on fetal development. The latter is discussed primarily in Section 12.6.2.2.4.5.

Maisin et al. (1975) exposed female mice to dietary lead for 18 days after mating; both the number of pregnancies and surviving embryos decreased. Similarly, exposure of female mice to lead via their diet (0.125-1.00 percent) from mating to 16-18 days afterward (Jacquet, 1976; Jacquet et al., 1975) resulted in decreased pregnancy incidence and number of corpora lutea; increased number of embryos dying after implantation at the highest dosages; decreased body weights of surviving fetuses; and treated dam fatalities at the high dose.

Jacquet and co-workers also described effects of maternal dietary lead exposure on preimplantation mouse embryos (Jacquet, 1976; Jacquet et al., 1976). They found lead in the diet to be associated with retardation of cleavage in embryos, failure of trophoblastic giant cells to differentiate, and absence of an uterine decidual reaction. Maisin et al. (1978) also found delayed cleavage in embryos of mice fed lead acetate prior to mating and up to 7 days afterwards.

Giavini et al. (1980) further confirmed the ability of lead to affect the preimplantation embryo in studies of rats transplacentally exposed to lead nitrate, and Wide and Nilsson (1977, 1979) reported that inorganic lead had similar effects on mice. Jacquet (1978) was able to force implantation in that species by use of high doses of progesterone, while Wide

TABLE 12-15. REPRODUCTIVE PERFORMANCE OF F_1 LEAD-INTOXICATED RATS

Parameter	Type of mating			
	CF-CM	CF-PbM	PbF-CM	PbF-PbM
Litters observed	22	24	36	16
Pups per litter	11.90 ± 0.40[a]	10.10 ± 0.50	8.78 ± 0.30[b]	7.75 ± 0.50[c]
Pup birth weight, g	6.74 ± 0.15	5.92 ± 0.13[c]	5.44 ± 0.13[c,d]	4.80 ± 0.19[c,d,e]
Weaned rats per litter	9.84 ± 0.50	7.04 ± 0.77[c]	5.41 ± 0.74[c,d]	2.72 ± 0.70[c,d,e]
Survival rate, %	89.80 ± 3.20	73.70 ± 7.90	52.60 ± 7.20	30.00 ± 8.20[c,d,f]
Litter birth weight / Dam breeding weight, %	28.04 ± 1.30	22.30 ± 0.90[c]	19.35 ± 1.00[c]	15.38 ± 1.10[c,d,f]
Litter birth weight / Dam whelping weight, %	19.09 ± 0.80	15.97 ± 0.58[c]	14.28 ± 0.66[c]	11.58 ± 0.78[c,d,f]
Gestational gain / Pups per litter, g	11.54 ± 0.60	11.20 ± 0.74	11.17 ± 0.54	12.34 ± 1.24
Nonfetal gestational gain per fetus, g	3.93 ± 0.38	4.83 ± 0.47	4.15 ± 0.42	3.96 ± 0.46

[a]Mean ± S.E.M.

[b]Significantly ($p < 0.05$) less than mean for CF-CM.

[c]Significantly ($p < 0.01$) less than mean for CF-CM.

[d]Significantly ($p < 0.01$) less than mean for CF-PbM.

[e]Significantly ($p < 0.01$) less than mean for PbF-CM.

[f]Significantly ($p < 0.05$) less than mean for PbF-CM.

Source: Stowe and Goyer (1971).

PRELIMINARY DRAFT

(1980) determined that administration of estradiol-17β and progesterone could reverse the effects of lead on implantation. Wide suggested that the lead-induced implantation blockage was mediated by a decrease in endometrial responsiveness to both sex steroids. Jacquet (1976) and Jacquet et al. (1977b) had attributed lead-induced prevention of implantation in the mouse to a lack of endogenous progesterone alone, stating that estrogen levels were unaffected. Later, however, Jacquet et al. (1977a) stated that estrogen levels also decreased, a finding not supported by Wide and Wide (1980). The latter authors did find a lead-induced increase in uterine estradiol receptors, but no change in binding affinities.

In order to examine lead effects early in gestation, Wide and Nilsson (1977) examined embryos from untreated mice and from mothers given 1 mg lead chloride on days 3, 4, or 6 of pregnancy. Embryonic mortality was greater in lead-treated litters; in the day-6 group some abnormal embryos were observed by day 8. In a later experiment, Wide (1978) removed blastocysts from lead-treated mice. She found that they attached and grew normally during three days of in vitro culture. Other blastocysts from untreated mothers were cultured in media containing lead, and a dose-dependent decrease in the number of normally developing embryos was seen.

A study employing domestic sheep was reported by Sharma and Buck (1976), who fed lead powder to pregnant ewes throughout gestation. Levels in the diet were varied from 0.5 to 16 mg/kg/day in an effort to keep blood lead levels near 40 μg/dl (actual levels ranged from 30 to 70 μg/dl). Such treatment resulted in a greatly decreased lambing percentage but no gross malformations. However, the number of subjects was small.

12.6.2.2.4 Teratogenicity and prenatal toxicity of lead in animals.

12.6.2.2.4.1 High dose effects on the conceptus. Teratogenic effects refer to physical defects (malformations) in the developing offspring. Prenatal toxicity (embryotoxicity, fetotoxicity) includes premature birth, prenatal death, stunting, histopathological effects, and transient biochemical or physiological changes. Behavioral teratogenicity, consisting of behavioral alterations or functional (e.g., motor, sensory) deficits resulting from in utero exposure, is dealt with in Section 12.4 of this chapter.

Teratogenicity of lead, at high exposure levels, has been demonstrated in rodents and birds, with some results suggesting a species-related specificity of certain gross teratogenic effects. Ferm and Carpenter (1967), as well as Ferm and Ferm (1971), reported increased embryonic resorption and malformation rates when various lead salts were administered i.v. to pregnant hamsters. Teratogenic effects were largely restricted to the tail region, including malformations of sacral and caudal vertebrae resulting in absent or stunted tails. Gale (1978) found the same effects plus hydrocephalus, among six strains of hamsters and noted differences in susceptibility, suggesting a genetic component in lead-induced teratogenicity.

Zegarska et al. (1974) performed a study with rats injected with lead acetate at midgestation. They reported embryonic mortality and malformations. McClain and Becker (1975) subsequently administered lead nitrate i.v. to rats on one of days 8-17 of gestation, producing malformations and embryo- and feto-toxicity. Hackett et al. (1978, 1982a,b) also gave lead i.v. to rats and found malformations and high incidences of prenatal mortality. Minsker et al. (1982) gave lead i.v. to dams on day 17 of gestation and observed decreased birth weights, as well as decreased weight and survival by postpartum day 7.

In another study, Miller et al. (1982) used oral doses of lead acetate up to 100 mg/kg given daily to rats before breeding and throughout pregnancy and found fetal stunting at the high dose, but no other effects. Maternal blood lead values ranged from 80 to 92 μg/dl prior to mating and from 53 to 92 μg/dl during pregnancy. Pretreatment and control blood lead levels averaged 6 to 10 μg/dl. Also, Wardell et al. (1982) gavaged rats daily with lead doses of up to 150 mg/kg from gestation day 6 through day 18 and observed decreased prenatal survival at the high dose, but no malformations.

Ferm (1969) reported that teratogenic effects of i.v. lead in hamsters are potentiated in the presence of cadmium, leading to severe caudal dysplasia. This finding was duplicated by Hilbelink (1980). In addition to caudal malformations, lead appears to influence the morphology of the developing brain. For example, Murray et al. (1978) described a significant decrease in number of dendritic spines and a variety of morphological abnormalities of such spines in parietal cortex of 30-day-old rat pups exposed to lead during gestation and nursing, during the postweaning period only, or during both periods. Morphometric analysis of rats transplacentally exposed to lead indicated that cellular organelles were altered as a function of dose and stage of development at exposure (Klein et al., 1978). These results indicate that morphologically apparent effects of lead on the brain could be produced by exposure during pregnancy alone, a question not addressed by Murray et al. (1978).

A variety of studies relating neurobehavioral effects to prenatal lead exposure have also been published. These studies are discussed in Section 12.4.3 of this chapter.

12.6.2.2.4.2 Low dose effects on the conceptus. There is a paucity of information regarding the teratogenicity and developmental toxicity of prolonged low-level lead exposure. Kimmel et al. (1980) exposed female rats chronically to lead acetate via drinking water (0.5, 5, 50, and 250 μg/g) from weaning through mating, gestation, and lactation. They observed a decrease in fetal body length of female offspring at the high dose, and the female offspring from the 50 and 250 μg/g groups weighed less at weaning and showed delays in physical development. Maternal toxicity was evident in the rats given 25 μg/g or higher doses, corresponding to blood lead levels of 20 μg/dl or higher. Reiter et al. (1975) observed delays in the development of the nervous system in offspring exposed to 50 μg/g lead throughout gestation

and lactation. Whether these delays in development resulted from a direct effect of lead on the nervous system of the pups or reflect secondary changes (resulting from malnutrition, hormonal imbalance, etc.) is not clear. Whatever the mechanisms involved, these studies suggest that low-level, chronic exposure to lead may induce postnatal developmental delays.

12.6.2.2.4.3 Prenatal effects of organolead compounds. In an initial study of the effects of organolead compounds in animals, McClain and Becker (1972) treated rats orally with 7.5-30 mg/kg tetraethyl lead, 40-160 mg/kg tetramethyl lead, or 15-38 mg/kg trimethyl lead chloride, given in three divided doses on gestation days 9-11 or 12-14. The last compound was also given i.v. at doses of 20 to 40 mg/kg on one of days 8-15 of pregnancy. The highest dose of each agent resulted in maternal death, while lower doses caused maternal toxicity. At all dose levels, fetuses from dams given multiple treatment weighed less than controls. Single treatments at the highest doses tended to have similar effects. In some cases delayed ossification was observed. In addition, direct intra-amniotic injection of trimethyl lead chloride at levels up to 100 μg per fetus caused increasing fetal mortality.

Kennedy et al. (1975) administered tetraethyl lead by gavage to mice and rats during the period of organogenesis at dose levels up to 10 mg/kg. Maternal toxicity, prenatal mortality, and developmental retardation were noted at the highest doses in both species, although maternal treatment was discontinued after only three days due to excessive toxicity. In a subsequent study involving alkyl lead, Odenbro and Kihlström (1977) treated female mice orally with triethyl lead at doses of up to 3.0 mg/kg/day on days 3 to 5 following mating. The highest treatment levels resulted in decreased pregnancy rates, while at 1.5 mg/kg, lower implantation rates were seen. In order to elucidate the mechanism of implantation failure in organolead-intoxicated mice, Odenbro et al. (1982) measured plasma sex steroid levels in mice five days after mating. Levels of both estradiol and progesterone, but not estrone, were decreased following intraperitoneal triethyl lead chloride on days three and four of gestation. Such results suggest a hormonal mechanism for blockage of implantation, a finding also suggested for inorganic lead (Wide, 1980; Jacquet et al., 1977a).

12.6.2.2.4.4 Effects of lead on fetal physiology and metabolism. Biochemical indicators of developmental toxicity have been the subject of a number of investigations, as possible indicators of subtle prenatal effects. Hubermont et al. (1976) exposed female rats to lead in drinking water before mating, during pregnancy, and after delivery. In the highest exposure group (10 ppm), maternal and offspring blood lead values were elevated and approached 68 and 42 μg/dl, respectively. Inhibition of ALA-D and elevation of free tissue porphyrins were also noted in the newborns. Maternal diets containing up to 0.5 percent lead were associated with increased fetal porphyrins and decreased ALA-D activity by Jacquet et al. (1977a). Fetuses in the high dose group had decreased weights, but no data were presented on maternal weight gain or food consumption (which could have influenced fetal weight).

In the only inhalation exposure study (Prigge and Greve, 1977), rats were exposed throughout gestation to an aerosol containing 1, 3, or 10 mg Pb/m^3 or to a combination of 3 mg Pb/m^3 and 500 ppm carbon monoxide CO. Both maternal and fetal ALA-D activities were strongly inhibited by lead exposure in a dose-related manner. In the presence of lead plus CO, however, fetal (but not maternal) ALA-D activity was higher than in the group given lead alone, possibly due to the increase in total ALA-D seen in the CO-plus-lead treated fetuses. Fetal body weight and hematocrit were decreased in the high-dose lead group, while maternal values were unchanged, thus suggesting that the fetuses were more sensitive to lead effects than were the mothers. Granahan and Huber (1978) also reported decreased hematocrit, as well as reduced hemoglobin levels, in fetal rats from lead intoxicated dams (1000 ppm in the diet throughout gestation).

Gerber and Maes (1978) fed pregnant mice diets containing up to one percent lead from day 7 to 18 of pregnancy and determined levels of heme synthesis. Incorporation of iron into fetal heme was inhibited, but glycine incorporation into heme and protein was unaffected. Gerber et al. (1978) also found that dietary lead given late in gestation resulted in diminished placental blood flow but did not decrease uptake of a non-metabolizable amino acid, alpha-amino isobutyrate. The authors could not decide whether lead-induced fetal growth retardation was due to placental insufficiency or to the previously described reduction in heme synthesis (Gerber and Maes, 1978). They did not mention the possibility that the treated mothers may have reduced their food consumption, resulting in a reduced nutrient supply to the fetus, regardless of fetal ability to absorb nutrients.

More recently, Wardell et al. (1982) exposed rat fetuses <u>in utero</u> to lead by gavaging their pregnant mothers with 150 mg/kg lead from gestation days 6 to 18. On day 19, fetal limb cartilage was tested for ability to synthesize protein, DNA, and proteoglycans, but no adverse effects were seen.

12.6.2.2.4.5 <u>Possible mechanisms of lead-induced teratogenesis</u>. The reasons for the localization of many of the gross teratogenic effects of lead are unknown at this time. Ferm and Ferm (1971) have suggested that the observed specificity could be explained by an interference with specific enzymatic events. Lead alters mitochondrial function and enhances or inhibits enzymes (Vallee and Ulmer, 1972); any or all such effects could interfere with normal development. Similarly, inhibition of ALA has been suggested as a mechanism of teratogenesis by Cole and Cole (1976).

In an attempt to study the mechanics of lead induction of sacral-tail region malformations, Carpenter and Ferm (1977) examined hamster embryos treated at mid-gestation during the critical stage for response to teratogens in this species. The initial effects were edema of the tail region of embryos 30 hours after maternal exposure, followed by blisters and hema-

tomas. These events disrupted normal caudal development, presumably by mechanical displacement. The end results seen in surviving fetuses were missing, stunted, or malformed tails and anomalies of the lower spinal cord and adjacent vertebrae.

12.6.2.2.4.6 Maternal factors in lead-induced teratogenesis and fetotoxicity. Nutritional factors may also have a bearing on the prenatal toxicity of lead. Jacquet and Gerber (1979) reported increased mortality and defects in fetuses of mice given i.p. injections of lead while consuming a calcium deficient diet during gestation. In several treatment groups, lead-treated calcium deficient mothers had low blood calcium levels, while controls on the same diet had normal values. It is not certain how meaningful these data are, however, as there was no clear dose-response relationship within diet groups. In fact, fetal weights were said to be significantly higher in two of the lead-treated groups (on the normal diet) than in the untreated controls. Another problem with the study was that litter numbers were small.

Another study on interactions of lead with other elements was done by Dilts and Ahokas (1979), who exposed rats to lead in their drinking water throughout gestation. Controls were pair-fed or fed *ad libitum*. Lead treatment was said to result in decreased fetal weight, and dietary zinc supplementation was claimed to be associated with a protective effect against fetal stunting. The data presented do not allow differentiation of effects due to maternal stress (e.g., decreased food consumption) from direct effects on the fetus. Litter numbers were small, and some of the data were confusing (e.g., a lead-treated and a pair-fed group with very similar litter sizes and total litter weights, but rather dissimilar average fetal weights; live litter weight divided by live litter size does not give the authors' values for average fetal weight). Also, no data were given on maternal or fetal lead or zinc levels. In a further report on apparently the same animals as above, Dilts and Ahokas (1980) found that lead inhibited cell division and decreased protein contents of the fetal placentas, eviscerated carcasses, and livers. Such lead-related effects were not influenced by maternal zinc supplementation.

12.6.2.3 Effects of Lead on Avian Species. The effects of lead on the reproduction and development of various avian species have been studied by a number of investigators, primarily out of interest in the effects of lead shot ingested by wildlife or out of interest in an avian embryo model for the experimental analysis of ontogenetic processes. The relevance of such studies to the health effects of lead on humans is not clear. Consequently, these studies are not discussed further here.

12.6.3 Summary

The most clear-cut data described in this section on reproduction and development are derived from studies employing high lead doses in laboratory animals. There is still a need for

more critical research to evaluate the possible subtle toxic effects of lead on the fetus, using biochemical, ultrastructural, or behavioral endpoints. An exhaustive evaluation of lead-associated changes in offspring will require consideration of possible additional effects due to paternal lead burden. Neonatal lead intake via consumption of milk from lead-exposed mothers may also be a factor at times. Also, it must be recognized that lead effects on reproduction may be exacerbated by other environmental factors (e.g., dietary influences, maternal hyperthermia, hypoxia, and co-exposure to other toxins).

There are currently no reliable data pointing to adverse effects in human offspring following paternal exposure to lead, and the early studies of high dose exposure in pregnant women indicate toxic--but not teratogenic--effects on the conceptus. Effects on reproductive performance in women are not well documented, but industrial exposure of men to lead at levels resulting in blood lead values of 40-50 μg/dl appear to have resulted in altered testicular function. Unfortunately, the human data regarding lead effects during development currently do not lend themselves to accurate estimation of no-effect levels.

The paucity of human exposure data forces an examination of the animal studies for indications of threshold levels for effects of lead on the conceptus. It must be noted that the animal data are almost entirely derived from rodents. Based on these rodent data, it seems likely that fetotoxic effects have occurred in animals at chronic exposures to 600-1000 ppm lead in the diet. Subtle effects appear to have been observed at 10 ppm in the drinking water, while effects of inhaled lead have been seen at levels of 10 mg/m^3. With acute exposure by gavage or by injection, the values are 10-16 mg/kg and 16-30 mg/kg, respectively. Since humans are most likely to be exposed to lead in their diet, air, or water, the data from other routes of exposure are of less value in estimating harmful exposures. Indeed, it seems likely that teratogenic effects occur only when the maternal dose is given by injection.

Although human and animal responses may be dissimilar, the animal evidence does document a variety of effects of lead exposure on reproduction and development. Measured or apparent changes in production of or response to reproductive hormones, toxic effects on the gonads, and toxic or teratogenic effects on the conceptus have all been reported. The animal data also suggest subtle effects on such parameters as metabolism and cell structure that should be monitored in human populations. Well-designed human epidemiological studies involving large numbers of subjects are still needed. Such data could clarify the relationship of exposure levels and durations to blood lead values associated with significant effects and are needed for estimation of no-effect levels.

12.7 GENOTOXIC AND CARCINOGENIC EFFECTS OF LEAD

12.7.1 Introduction

Potential carcinogenic, genotoxic (referring to alteration in structure or metabolism of DNA), and mutagenic roles of lead are considered here. Epidemiological studies of occupationally exposed populations are considered first. Such studies investigate possible associations of lead with induction of human neoplasia. Epidemiological studies are important because they assess the incidence of disease in humans under actual ambient exposure conditions. However, such studies have many limitations that make it difficult to assess the carcinogenic activity of any specific agent. These include general problems in accurately determining the amount and nature of exposure to a particular chemical agent; in the absence of adequate exposure data it is difficult to determine whether each individual in a population was equally exposed to the agent in question. It is also often difficult to assess other factors, such as exposure to carcinogens in the diet, and to control for confounding variables that may have contributed to the incidence of any neoplasms. These factors tend to obscure the effect of lead alone. Also, in an occupational setting a worker is often exposed to various chemical compounds, making it more difficult to assess epidemiologically the injurious effect resulting specifically from exposure to one, such as lead.

A second approach considered here examines the ability of specific lead compounds to induce tumors in experimental animals. The advantage of these studies over epidemiological investigations is that a specific lead compound, its mode of administration, and level of exposure can be well defined and controlled. Additionally, many experimental procedures can be performed on animals that for ethical reasons cannot be performed on humans, thereby allowing a better understanding of the course of chemically induced injury. For example, animals may be sacrificed and necropsies performed at any desired time during the study. Factors such as diet and exposure to other environmental conditions can be well controlled, and genetic variability can be minimized by use of well established and characterized animal lines. One problem with animal studies is the difficulty of extrapolating such data to humans; however, this drawback is perhaps more important in assessing the toxicity of organic chemicals than in assessing inorganic agents. The injury induced by many organic agents is highly dependent upon reactive intermediates formed in vivo by the action of enzymatic systems (e.g., microsomal enzymes) upon the parent compound. Both qualitative and quantitative differences between the metabolic capabilities of humans and experimental animals have been documented (Neal, 1980). With inorganic compounds of lead, however, the element of interest undergoes little alteration in vivo and, therefore, the ultimate toxic agent is less likely to differ between experimental animals and humans (Costa, 1980). The carcinogenic action of most

organic chemicals is dependent upon activation of a parent pro-carcinogen, whereas most metallic carcinogens undergo little alteration in vivo to produce their oncogenic effects (Costa, 1980).

A third approach discussed below is in vitro studies. Animal carcinogen bioassays are presently the preferred means for assessing carcinogenic activity but they are extremely expensive and time consuming. As a result, much effort has been directed toward developing suitable in vitro tests to complement in vivo animal studies in evaluating potential oncogenicity of chemicals. The cell transformation assay has as its endpoint neoplastic transformation of mammalian cells and is among the most suitable in vitro systems because it examines cellular events closely related to carcinogenesis (Heck and Costa, 1982a). A general problem with this assay system, which is less troublesome with reference to metal compounds, is that it employs fibroblastic cells in culture, which lack many in vivo metabolic systems. Since lead is not extensively metabolized in vivo, addition of liver microsomal extracts (which has been attempted in this and similar systems) is not necessary to generate ultimate carcinogen(s) from this metal (see above). However, if other indirect factors are involved with lead carcinogenesis in vivo, then these might be absent in such culture systems (e.g., specific lead-binding proteins that direct lead interactions in vivo with oncogenically relevant sites). There are also technical problems related to the culturing of primary cells and difficulties with the final microscopic evaluation of morphological transformations, which are prone to some subjectivity. However, if the assay is performed properly it can be very reliable and reproducible. Modifications of this assay system (i.e., exposure of pregnant hamsters to a test chemical followed by culturing and examination of embryonic cells for transplacentally induced transformation) are available for evaluation of in vivo metabolic influences, provided that the test agent is transported to the fetus. Additionally, cryopreservation of primary cultures isolated from the same litter of embryos can control for variation in cell populations exposed to test chemicals and give more reproducible responses in replicate experiments (Pienta, 1980). A potential advantage of the cell transformation assay system is the possibility that cultured human cells can be transformed in vitro. Despite numerous attempts, however, no reproducible human-cell transformation system has yet been sucessfully established which has been evaluated with a number of different chemicals of defined carcinogenic activity.

Numerous processes have been closely linked with oncogenic development, and specific assay systems that utilize events linked mechanistically with cancer as an endpoint have been developed to probe whether a chemical agent can affect any of these events. These systems include assays for mutations, chromosomal aberrations, development of micronuclei, enhancement of sister chromatid exchange, effects on DNA structure, and effects on DNA and RNA polymerase. These assay systems have been used to examine the genotoxicity of lead and facilitate the

assessment of possible lead carcinogenicity. Chromosomal aberration studies are useful because human lymphocytes cultured from individuals after exposure to lead allow evaluation of genotoxic activity that occurred under the influence of an in vivo metabolic system. Such studies are discussed below in relationship to genotoxic effects of lead. However, a neoplastic change does not necessarily result, and evaluations of some less conspicuous types of chromosomal aberrations are somewhat subjective since microscopy is exclusively utilized in the final analyses. The sensitivity of detection of chromosomal changes also tends to be less than other measurable DNA effects, e.g., the induction of DNA repair. However, it is reasonable to assume that if an agent produces chromosomal aberrations it may have potential carcinogenic activity. Many carcinogens are also mutagenic and this fact, combined with the low cost and ease with which bacterial mutation assays can be performed, has resulted in wide use of these systems in determining potential carcinogenicity of chemicals. Mutation assays can also be performed with eukaryotic cells and several studies are discussed below that examined the mutagenic role of lead in these systems. However, in bacterial systems such as the Ames test, metal compounds with known human carcinogenic activity are generally negative and, therefore, this system is not useful for determining the potential oncogenicity of lead. Similarly, even in eukaryotic systems, metals with known human cancer-causing activity do not produce consistent mutagenic responses. Reasons for this lack of mutagenic effect remain unclear, and it appears that mutagenicity studies of lead cannot be weighed heavily in assessing its genotoxicity.

Other test systems that probe for effects of chemical agents on DNA structure may be useful in assessing the genotoxic potential of lead. Sister chromatid exchange represents the normal movement of DNA in the genome and enhancement of this process by potentially carcinogenic agents is a sensitive indicator of genotoxicity (Sandberg, 1982). However, these studies usually involve tissue cultures; consequently, in vivo interactions related to such effects have not been addressed with this system. Numerous recently developed techniques can be used to assess DNA damage induced by chemical carcinogens. One of the most sensitive is alkaline elution (Kohn et al., 1981), which may be used to study DNA lesions produced in vivo or in cell culture. This technique can measure DNA strand breaks or crosslinks in DNA, as well as repair of these lesions, but lead compounds have not been studied with this technique. Assessment of the induction of DNA repair represents one of the most sensitive techniques for probing genotoxic effects. The reason for this is that the other procedures measure DNA lesions that have persisted either because they were not recognized by repair enzymes or because their number was sufficiently great to saturate DNA repair systems. Measurement of DNA repair activation is still possible even if the DNA lesion has been repaired, but effects of lead compounds on DNA repair have not been studied. There are a few isolated experiments within publications that examined the ability of lead compounds to induce DNA damage, but this

line of investigation requires further work. There are some well-conducted studies of the effect of lead along with other water soluble metals on isolated DNA and RNA polymerases, which suggest mutagenic mechanisms occurring in intact cells. The ability of lead to affect the transcription of DNA and RNA merits concern in regard to its potential oncogenic and mutagenic properties.

12.7.2 Carcinogenesis Studies with Lead and its Compounds

12.7.2.1 Human Epidemiological Studies. Epidemiological studies of industrial workers, where the potential for lead exposure is usually greater than for a "normal population," have been conducted to evaluate the role of lead in the induction of human neoplasia (Cooper, 1976, 1981; Cooper and Gaffey, 1975; Chruściel, 1975; Dingwall-Fordyce and Lane, 1963; Lane, 1964; McMichael and Johnson, 1982; Neal et al., 1941; Nelson et al., 1982). In general, these studies made no attempt to consider types of lead compounds to which workers were exposed or to determine probable routes of exposure. Some information on specific lead compounds encountered in the various occupational settings, along with probable exposure routes, would have made the studies more interpretable and useful. As noted in Chapter 3, with the exception of lead nitrate and lead acetate, many inorganic lead salts are relatively water insoluble. If exposure occurred by ingestion, the ability of water-insoluble lead salts (e.g., lead oxide and lead sulfide) to dissolve in the gastrointestinal tract may contribute to understanding of their ultimate systemic effects in comparison to their local actions in the gastrointestinal tract. Factors such as particle size are also important in the dissolution of any water insoluble compounds in the gastrointestinal system (Mahaffey, 1983). When considering other routes of exposure (e.g., inhalation), the water solubility of the lead compound in question, as well as the particle size, are extremely important, both in terms of systemic absorption and contained injury in the immediate locus of the retained particle (see Chapter 10). A hypothetical example is the inhalation of an aerosol of lead oxide versus a water soluble lead salt such as lead acetate. Lead oxide particles having a diameter of <5 μm would tend to deposit in the lung and remain in contact with cells there until they dissolved, while soluble lead salts would dissipate systemically at a much more rapid rate. Therefore, in the case of inhaled particulate compounds, localized exposure to lead might produce injury primarily in respiratory tissue, whereas with soluble salts systemic (i.e., CNS, kidney, and erythropoietic) effects might predominate.

The studies of Cooper and Gaffey (1975) and Cooper (1976, 1981) examined the incidence of cancer in a large population of industrial workers exposed to lead. Two groups of individuals were identified as the lead-exposed population under consideration: smelter workers from six lead production facilities and battery plant workers (Cooper and Gaffey, 1975). The authors reported (see Table 12-16) that total mortality from cancer was higher in lead smelter workers

than in a control population in two ways: (1) the difference between observed and expected values for the types of malignancies reported; and (2) the standardized mortality ratio, which indicates a greater than "normal" response if it is in excess of 100 percent. These studies report not only an excess of all forms of cancer in smelter workers but also a greater level of cancer in the respiratory and digestive systems in both battery plant and smelter workers. The incidence of urinary system cancer was also elevated in the smelter workers (but not in the battery plant workers), although the number of individuals who died from this neoplasm was very small. As the table indicates, death from neoplasm at other sites was also elevated compared with a normal population, but these results were not discussed in the report. Kang et al. (1980) examined the Cooper and Gaffey (1975) report and noted an error in the statistical equation used to assess the significance of excess cancer mortality. Table 12-17, from Kang et al., 1980, shows results based on a corrected form of the statistical equation used by Cooper and Gaffy; it also employed another statistical test claimed to be more appropriate. Statistical significance was observed in every category listed with the exception of battery plant workers, whose deaths from all forms of neoplasia were not different from a control population.

TABLE 12-16. EXPECTED AND OBSERVED DEATHS FOR MALIGNANT NEOPLASMS JAN. 1, 1947 - DEC. 31, 1979 FOR LEAD SMELTER AND BATTERY PLANT WORKERS

Causes of Death (ICD† Code)	Obs	Exp	Smelters SMR*	Obs	Battery plant Exp	SMR+
All malignant neoplasms (140-205)	69	54.95	133	186	180.34	111
Buccal cavity & pharynx (140-248)	0	1.89	--	6	6.02	107
Digestive organs peritoneum (150-159)	25	17.63	150	70	61.48	123
Respiratory system (160-164)	22	15.76	148	61	49.51	132
Genital organs (170-179)	4	4.15	101	8	18.57	46
Urinary organs (180-181)	5	2.95	179	5	10.33	52
Leukemia (204)	2	2.40	88	6	7.30	88
Lymphosarcoma lymphatic and hematopoietic (200-203, 205)	3	3.46	92	7	9.74	77
Other sites	8	6.71	126	23	17.39	142

†International Classification of Diseases.

*Correction of +5.55% applied for 18 missing death certificates.

+Correction of +7.52% applied for 71 missing death certificates.

Source: Cooper and Gaffey (1975).

TABLE 12-17. EXPECTED AND OBSERVED DEATHS RESULTING FROM SPECIFIED MALIGNANT NEOPLASMS FOR LEAD SMELTER AND BATTERY PLANT WORKERS AND LEVELS OF SIGNIFICANCE BY TYPE OF STATISTICAL ANALYSIS ACCORDING TO ONE-TAILED TESTS

Causes of death (ICD† code)	Number of deaths		SMR*	Probability		
	Observed	Expected		Poisson**	This analysis***	Cooper and Gaffey****
Lead smelter workers:						
All malignant neoplasms (140-205)	69	54.95	133	<0.02	<0.01	<0.02
Cancer of the digestive organs peritoneum (250-159)	25	17.63	150	<0.03	<0.02	<0.05
Cancer of the respiratory system (160-164)	22	15.76	148	<0.05	<0.03	>0.05
Battery plant workers:						
All malignant neoplasms (140-205)	186	180.34	111	>0.05	>0.05	>0.05
Cancer of the digestive organs, peritoneum (150-159)	70	61.48	123	<0.05	<0.04	>0.05
Cancer of the respiratory system (160-164)	61	49.51	132	<0.03	<0.02	<0.03

†International Classification of Diseases.

*SMR values were corrected by Cooper and Gaffey for missing death certificates under the assumption that distribution of causes of death was the same in missing certificates as in those that were obtained.

**Observed deaths were recalculated as follows: adjusted observed deaths = (given SMR/100) x expected deaths.

***Given $z = (SMR - 100)\sqrt{expected}/100$.

****Given $z = (SMR - 100)/\sqrt{100 \times SMR/expected}$.

Source: Kang et al. (1980).

Cooper and Gaffey (1975) did not discuss types of lead compounds that these workers may have been exposed to in smelting operations, but workers thus employed likely ingested or inhaled oxides and sulfides of lead. Since these and other lead compounds produced in the industrial setting are not readily soluble in water it could be that the cancers arising in respiratory or gastrointestinal systems were caused by exposure to water-insoluble lead compounds. Although the Cooper and Gaffey (1975) study had a large sample (7032), only 2275 of the workers (32.4 percent) were employed when plants monitored urinary lead. Urinary lead values were available for only 9.7 percent of the 1356 deceased employees on whom the cancer

mortality data were based. Only 23 (2 percent) of the 1356 decedents had blood lead levels measured. Cooper and Gaffey (1975) did report some average urinary and blood lead levels, where 10 or more urine or at least three blood samples were taken (viz., battery plant workers: urine lead = 129 μg/l, blood lead = 67 μg/dl; smelter workers: urine lead = 73 μg/l, blood lead = 79.7 μg/dl). Cooper (1976) noted that these workers were potentially exposed to other materials, including arsenic, cadmium, and sulfur dioxide, although no data on such exposures were reported. In these and other epidemiological studies in which selection of subjects for monitoring exposure to an agent such as lead is left to company discretion, it is possible that individual subjects are selected primarily on the basis of obvious signs of lead exposure, while other individuals who show no symptoms of lead poisoning would not be monitored (Cooper and Gaffey, 1975). It is also not clear from these studies when the lead levels were measured, although the timing of measurement would make little difference since no attempt was made to match an individual's lead exposure to any disease process.

In a follow-up study of the same population of workers, Cooper (1981) concluded that lead had no significant role in the induction of neoplasia. However, he did report standardized mortality ratios (SMRs) of 149 percent and 125 percent for all types of malignant neoplasms in lead battery plant workers with < 10 and > 10 years of employment, respectively. SMR is a percentage value that is based upon comparison of an exposed population relative to a control population. If the value exceeds 100 percent, the incidence of death is greater than normal but not necessarily statistically significant. In battery workers employed for 10 years or more there was an unusually high incidence of cancer listed as "other site" tumors (SMR = 229 percent; expected = 4.85, observed = 16). Respiratory cancers were elevated in the battery plant workers employed for less than 10 years (SMR = 172 percent). Similarly, in workers involved with lead production facilities for more than 10 years the SMR was 151 percent. Again, in the absence of good lead exposure documentation, it is difficult to assess the contribution of lead to the observed results. Cooper (1981) suggested that the excess of respiratory cancers could have been due to a lack of correction for smoking histories.

A recent study (McMichael and Johnson, 1982) examined the historical incidence of cancers in a population of smelter workers diagnosed as having lead poisoning. The incidence of cancer in a relatively small group of 241 workers was compared with 695 deceased employees from the same company. The control group had been employed during approximately the same period and was asserted to be free from lead exposure, although there were no data to indicate lead levels in either the control or the experimental group. Based upon diagnoses of lead poisoning made in the 1920s and 1930s for a majority of the deaths, the authors concluded that there was a considerably lower incidence of cancer in lead-poisoned workers. However, there is no indication of how lead poisoning was diagnosed. It is difficult to draw any conclusions from this study with regard to the role of lead in human neoplasia.

Evaluation of the ability of lead to induce human neoplasia must await further epidemiological studies in which other factors that may contribute to the observed effects are well controlled for and the disease process is assessed in individuals with well documented exposure histories. Little can now be reliably concluded from available epidemiological studies.

12.7.2.2 Induction of Tumors in Experimental Animals. As discussed in the preceding sections it is difficult to obtain conclusive evidence of the carcinogenic potential of an agent using only epidemiological studies. Experiments testing the ability of lead to cause cancer in experimental animals are an essential aspect of understanding its oncogenicity in humans. However, a proper lifetime animal feeding study to assess the carcinogenic potential of lead following National Cancer Institute guidelines (Sontag et al., 1976) has not been conducted. The cost of such studies exceed $1 million and consequently are limited only to those agents in which sufficient evidence based upon *in vitro* or epidemiological studies warrants such an undertaking. The literature on lead carcinogenesis contains many smaller studies where only one or two doses were employed and where toxicological monitoring of experimental animals exposed to lead was generally absent. Some of these studies are summarized in Table 12-18. Most mainly serve to illustrate that numerous different laboratories have induced renal tumors in rats by feeding them diets containing 0.1 percent or 1.0 percent lead acetate. In some cases, other lead formulations were tested, but the dosage selection was not based upon lethal dose values. In most cases, only one dose level was used. Another problem with many of these studies was that the actual concentrations of lead administered and internal body burdens achieved were not measured. Some of these studies are discussed very briefly; others are omitted entirely because they contribute little to our understanding of lead carcinogenesis.

Administration of 1.0 percent lead acetate (10,000 ppm) resulted in kidney damage and a high incidence of mortality in most of the studies in Table 12-18. However, kidney tumors were also evident at lower dosages (e.g., 0.1 percent lead acetate in the diet), which produced less mortality among the test animals. As discussed in Section 12.5, renal damage is one of the primary toxic effects of lead. At 0.1 percent lead acetate (1000 ppm) in the diet, the concentration of lead measured in the kidney was 30 μg/g while 1 percent lead acetate resulted in 300 μg/g of lead in the kidneys of necropsied animals (Azar et al., 1973). In most of the studies with rats fed 0.1 or 1.0 percent lead in the diet, the incidence of kidney tumors increased between the lower and higher dosage, suggesting a relationship between the deposition of lead in the kidney and the carcinogenic response. Renal tumors were also induced in mice at the 0.1 percent oral dosage of lead subacetate but not in hamsters that were similarly exposed to this agent (Table 12-18).

Other lead compounds have also been tested in experimental animals, but in these studies only one or two dosages (generally quite high) were employed, making it difficult to assess

TABLE 12-18. EXAMPLES OF STUDIES ON THE INCIDENCE OF TUMORS IN EXPERIMENTAL ANIMALS EXPOSED TO LEAD COMPOUNDS

Species	Pb compound	Dose and mode	Incidence (and type) of neoplasms	Reference
Rat	Pb phosphate	120-680 mg (total dose s.c.)	19/29 (renal tumors)	Zollinger (1953)
Rat	Pb acetate	1% (in diet)	15/16 (kidney tumors) 14/16 (renal carcinomas)	Boyland et al. (1962)
Rat	Pb subacetate	0.1% and 1.0% (in diet)	11/32 (renal tumors) 13/24 (renal tumors)	Van Esch et al. (1962)
Mouse	Pb naphthenate	20% in benzene (dermal 1-2 times weekly)	5/59 (renal neoplasms) (no control with benzene)	Baldwin et al. (1964)
Rat	Pb phosphate	1.3 g (total dosage s.c.)	29/80 (renal tumors)	Balo et al. (1965)
Rat	Pb subacetate	0.5 - 1% (in diet)	14/24 (renal tumors)	Hass et al. (1967)
Rat	Pb subacetate	1% (in diet)	31/40 (renal tumors)	Mao and Molnar (1967)
Mouse	Tetraethyl lead in tricaprylin	0.6 mg (s.c.) 4 doses between birth and 21 days	5/41 (lymphomas) in females, 1/26 in males, and 1/39 in controls	Epstein and Mantel (1968)
Rat	Pb acetate	3 mg/day for 2 months; 4 mg/day for 16 months (p.o.)	72/126 (renal tumors) 23/94 males (testicular [Leydig cell] tumors)	Zawirska and Medraś (1968)
Hamster	Pb subacetate	1.0% (in 0.5% diet)	No significant incidence of renal neoplasms	Van Esch and Kroes (1969)
Mouse	Pb subacetate	0.1% and 1.0% (in diet)	7/25 (renal carcinomas) at 0.1% Substantial death at 1.0%	Van Esch and Kroes (1969)
Rat	Pb nitrate	25 g/l in drinking water	No significant incidence of tumors	Schroeder et al. (1970)
Rat	Pb acetate	3 mg/day (p.o.)	89/94 (renal, pituitary, cerebral gliomas, adrenal, thyroid, prostatic, mammary tumors)	Zawirska and Medraś, 1972

TABLE 12-18. (continued)

Species	Pb Compound	Dose and mode	Incidence (and type) of neoplasms	Reference
Rat	Pb acetate	0, 10, 50, 100, 1000, 2000 ppm (in diet) for 2 yr	No tumors 0-100 ppm; 5/50 (renal tumors) at 500 ppm; 10/20 at 1000 ppm; 16/20 males, 7/20 females at 2000 ppm	Azar et al. (1973)
Hamster	Pb oxide	10 intratracheal administrations (1 mg)	0/30 without benzopyrene, 12/30 with benzopyrene (lung cancers)	Kobayashi and Okamoto (1974)
Rat	Pb powder	10 mg orally 2 times each month	5/47 (1 lymphoma, 4 leukemias)	Furst et al. (1976)
		10 mg/monthly for 9 months; then 3 monthly injections of 5 mg	1/50 (fibrosarcoma)	

the potential carcinogenic activity of lead compounds at relatively nontoxic concentrations. It is also difficult to assess the true toxicity caused by these agents, since properly designed toxicity studies were generally not performed in parallel with these cancer studies.

As shown in Table 12-18, lead nitrate produced no tumors in rats when given at very low concentrations, but lead phosphate administered subcutaneously at relatively high levels induced a high incidence of renal tumors in two studies. Lead powder administered orally resulted in lymphomas and leukemia; when given intramuscularly only one fibrosarcoma was produced in 50 animals. Lead naphthenate applied as a 20 percent solution in benzene two times each week for 12 months resulted in the development of four adenomas and one renal carcinoma in a group of 50 mice (Baldwin et al., 1964). However, in this study control mice were not painted with benzene. Tetraethyl lead at 0.6 mg given in four divided doses between birth and 21 days to female mice resulted in 5/36 surviving animals developing lymphomas while 1/26 males treated similarly and 1/39 controls developed lymphomas (Epstein and Mantel, 1968).

Lead subacetate has also been tested in the mouse lung adenoma bioassay (Stoner et al., 1976). This assay measures the incidence of nodules forming in the lung of strain A/Strong mice following parenteral administration of various test agents. Nodule formation in the lung does not actually represent the induction of lung cancer but merely serves as a general measure of carcinogenic potency independent of lung tissue (Stoner et al., 1976). Lead subacetate was administered to mice at 150, 75, and 30 mg (total dose), which represented the maximum

tolerated dose (MTD), 1/2 MTD, and 1/5 MTD, respectively, over a 30-week period using 15 separate i.p. injections (Stoner et al., 1976). Survivals at the three doses were 15/20 (MTD), 12/20 (1/2 MTD), and 17/20 (1/5 MTD), respectively, with 11/15, 5/12, and 6/17 survivors having lung nodules. Only at the highest doses was the incidence of nodules greater than in the untreated 1 or 2 highest groups. However, these authors concluded that on a molar-dose basis lead subacetate was the most potent of all the metallic compounds examined. Injection of 0.13 mmol/kg lead subacetate was required to produce one lung tumor per mouse, indicating that this compound was about three times more potent than urethane (at 0.5 mmol/kg) and approximately 10 times more potent than nickelous acetate (at 1.15 mmol/kg). The mouse lung adenoma bioassay has been one of the most utilized systems for examining carcinogenic activity in experimental animals and is well recognized as a highly accurate test system for assessing potential carcinogenic hazard (Stoner et al., 1976). Lead oxide combined with benzopyrene administered intratracheally resulted in 11 adenomas and 1 adenocarcinoma in a group of 15 hamsters, while no lung neoplasias were observed in groups receiving benzopyrene or lead oxide alone (Kobayashi and Okamoto, 1974).

Administration of lead acetate to rats has been reported to produce other types of tumors, e.g., testicular, adrenal, thyroid, pituitary, prostate, lung (Zawirska and Medras, 1968), and cerebral gliomas (Oyasu et al., 1970). However, in other animal species, such as dogs (Azar et al., 1973; Fouts and Page, 1942) and hamsters (Van Esch and Kroes, 1969), lead acetate induced either no tumors or only kidney tumors (Table 12-18).

The above studies seem to implicate some lead compounds as carcinogens in experimental animals but were not designed to address the question of lead carcinogenesis in a definitive manner. In contrast, a study by Azar et al. (1973) examined the oncogenic potential of lead acetate at a number of doses and in addition monitored a number of toxicological parameters in the experimental animals. Azar et al. (1973) gave 0, 10, 50, 100, 1000 and 2000 ppm dose levels of lead (as lead acetate) to rats during a two-year feeding study. Fifty rats of each sex were utilized at doses of 10 to 500 ppm, while 100 animals of each sex were used as controls. After the study was under way for a few months, a second 2-year feeding study was initiated using 20 animals of each sex in groups given doses of 0, 1000, or 2000 ppm. The study also included four male and four female beagle dogs at each dosage of lead ranging from 0 to 500 ppm in a 2-year feeding study. During this study, the clinical appearance and behavior of the animals were observed, and food consumption, growth, and mortality were recorded. Blood, urine, fecal, and tissue lead analyses were done periodically using atomic absorption spectrophotometry. A complete blood analysis was done periodically, including blood count, hemoglobin, hematocrit, stippled cell count, prothrombin time, alkaline phosphatase, urea nitrogen, glutamic-pyruvate transaminase, and albumin-to-globulin ratio. The activity of the enzyme alpha-aminolevulinic acid dehydrase (ALA-D) in the blood and the excretion of its sub-

strate, delta-aminolevulinic acid (δ-ALA) in the urine were also determined. A thorough necropsy, including both gross and histologic examination, was performed on all animals. Reproduction was also assessed (see Section 12.6).

Table 12-19 depicts the mortality and incidence of kidney tumors reported by Azar et al. (1973). At 500 ppm (0.05 percent) and above, male rats developed a significant number of renal tumors. Female rats did not develop tumors except when fed 2000 ppm lead acetate. Two out of four male dogs fed 500 ppm developed a slight degree of cytomegaly in the proximal convoluted tubule but did not develop any kidney tumors. The number of stippled red blood cells increased at 10 ppm in the rats but not until 500 ppm in the dogs. ALA-D was decreased at 50 ppm in the rats but not until 100 ppm in the dogs. Hemoglobin and hematocrit, however, were not depressed in the rats until they received a dose of 1000 ppm lead. These results illustrate that the induction of kidney tumors coincides with moderate to severe toxicological doses of lead acetate, for it was at 500-1000 ppm lead in the diet that a significant increase in mortality occurred (see Table 12-19). At 1000 and 2000 ppm lead, 21-day-old weanling rats showed no tumors but did show histological changes in the kidney comparable to those seen in adults receiving 500 ppm or more lead in their diet. Also of interest from the Azar et al. (1973) study is the direct correlation obtained in dogs between blood lead level and kidney lead concentrations. A dietary lead level of 500 ppm produced a blood lead concentration of 80 μg/dl, which corresponds to a level at which humans often show clinical signs of lead poisoning (see Section 12.4.1). The kidney lead concentration corresponding to this blood lead level was 2.5 μg/g (wet weight), while at 50 μg/dl in blood the kidney lead levels were 1.5 μg/g. Presumably blood and kidney lead were determined at about the same time, although this was not clear from the report. At this level of lead, kidney tumors were induced in the rats but not the dogs. However, it is apparent from the above differences in hematological parameters that dogs tolerate higher levels of lead than rats. As shown in Figure 12-5, the induction of renal tumors by lead acetate was linearly proportional to the dietary levels of lead fed to male rats. It may be concluded, therefore, that chronic lead exposure of rats producing blood lead levels comparable to those at which clinical signs of toxicity would be evident in humans results in a significant elevation in the incidence of kidney tumors.

Animal carcinogenesis studies conducted with lead and its compounds are numerous; however, with the exception of the study by Azar et al., (1973) they do not provide much useful information. Most of the studies shown in Table 12-18 were conducted with only one lead compound in one animal species, the rat. In cases where other lead compounds were tested or where other animal species were used, only a single high dosage level was administered, and parameters of toxicity such as those monitored in the Azar et al. (1973) study were not measured. Although it is clear from these studies as a whole that lead is a carcinogen in experimental

TABLE 12-19. MORTALITY AND KIDNEY TUMORS IN RATS FED LEAD ACETATE FOR TWO YEARS

Nominal (actual)[a] concentration in ppm of Pb in diet	No. of rats of each sex	% Mortality[b]		% Kidney tumors	
		Male	Female	Male	Female
0 (5)	100	37	34	0	0
10 (18)	50	36	30	0	0
50 (62)	50	36	28	0	0
100 (141)	50	36	28	0	0
500 (548)	50	52	36	10	0
0 (3)	20	50	35	0	0
1000 (1130)	20	50	50	50	0
2000 (2102)	20	80	35	80	35

[a]Measured concentration of lead in diet.
[b]Includes rats that either died or were sacrificed in extremis.

Source: Azar et al. (1973).

animals, until more investigations such as that of Azar et al. (1973) are conducted it is difficult to determine the relative carcinogenic potency of lead. There remains a need to test thoroughly the carcinogenic activity of lead compounds in experimental animals. These tests should include several modes of administration, many dosages spanning non-toxic as well as toxic levels, and several different lead compounds or at least a comparison of a relatively water-soluble form such as lead acetate with a less soluble form such as lead oxide.

12.7.2.3 Cell Transformation. Although cell transformation is an in vitro experimental system, its end point is a neoplastic change. There are two types of cell transformation assays: (1) those employing continuous cell lines, and (2) those employing cell cultures prepared from embryonic tissue. Use of continuous cell lines has the advantage of ease in preparation of the cell cultures, but these cells generally have some properties of a cancer cell. The absence of a few characteristics of a cancer cell in these continuous cell lines allows for an assay of cell transforming activity. End points include morphological transformation (ordered cell growth to disordered cell growth), ability to form colonies in soft agar-containing medium (a property characteristic of cancer cells), and ability of cells to form tumors when inoculated into experimental animals. Assays that utilize freshly isolated embryonic cells are generally preferred to those that use cell lines, because embryonic cells have not yet acquired any of the characteristics of a transformed cell. The cell transformation assay system has been utilized to examine the potential carcinogenic activity of a number of chemical agents, and the results seem to agree generally with the results of carcinogenesis

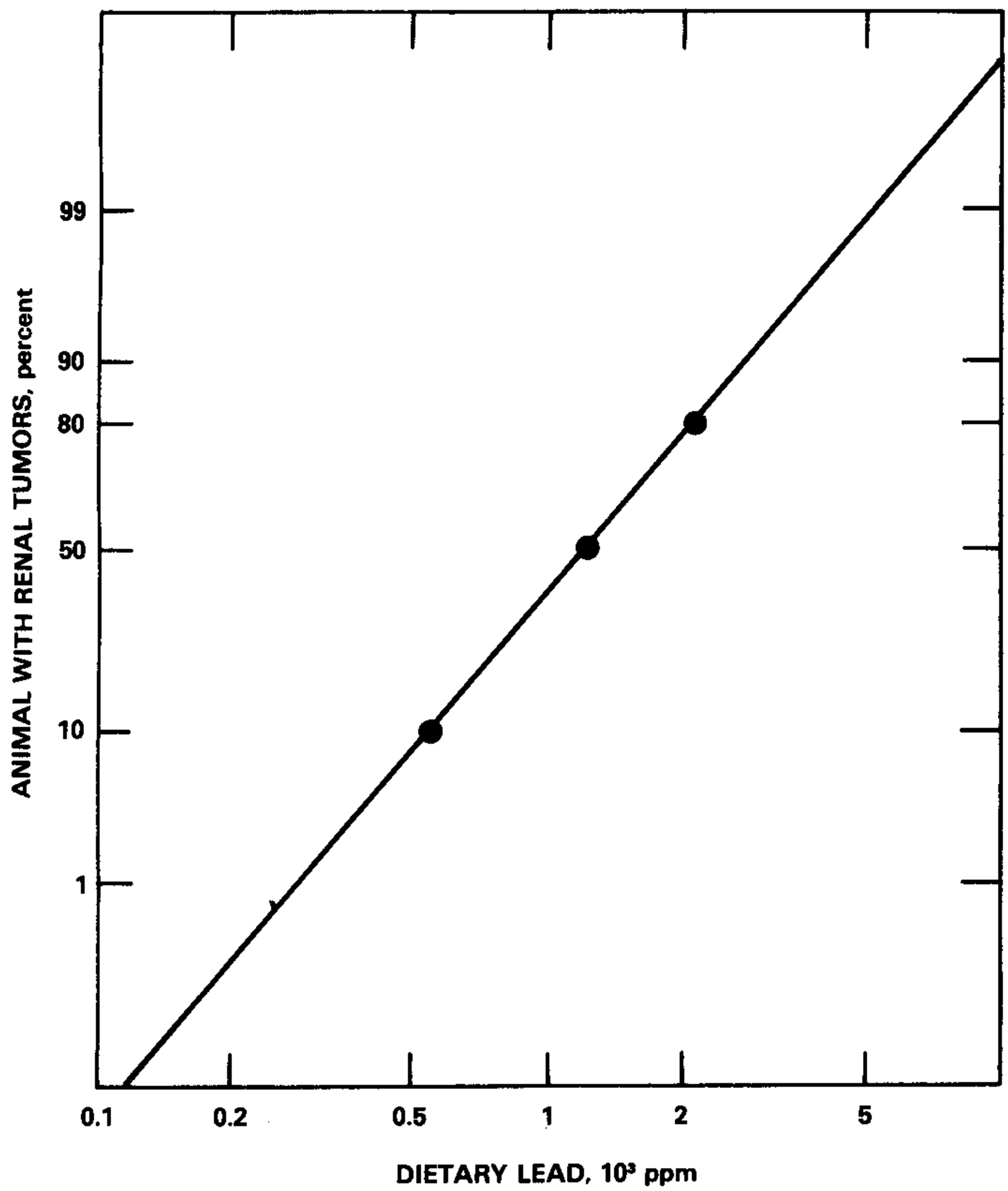

Figure 12-5. Probit plot of incidence of renal tumors in male rats.

Source: U.S. Environmental Protection Agency (1980) based on Azar et al. (1973).

tests using experimental animals. Cell transformation assays can be made quantitative by assessing the percentage of surviving colonies exhibiting morphological transformation. Verification of a neoplastic change can be accomplished by cloning these cells and testing their ability to form tumors in animals.

Lead acetate has been shown to induce morphological transformation in Syrian hamster embryo cells following a continuous exposure to 1 or 2.5 μg/ml of lead in culture medium for nine days (Dipaolo et al., 1978). The incidence of transformation increased from 0 percent in untreated cells to 2.0 and 6.0 percent of the surviving cells, respectively, following treatment with lead acetate. Morphologically transformed cells were capable of forming fibrosarcomas when cloned and administered to "nude" mice and Syrian hamsters, while no tumor growth resulted from similar inoculation with untreated cells (Dipaolo et al., 1978). In the same study lead acetate was shown to enhance the incidence of simian adenovirus (SA-7) induction of Syrian hamster embryo cell transformation. Lead acetate also caused significant enhancement (~2-3 fold) at 100 and 200 μg/ml following three hours of exposure. In another study (Casto et al., 1979), lead oxide also enhanced SA-7 transformation of Syrian hamster embryo cells almost 4 fold at 50 μM following three hours of exposure (Casto et al., 1979). The significance of enhanced virally induced carcinogenesis in relationship to the carcinogenic potential of an agent is not well understood.

Morphological transformation induced by lead acetate was correlated with the ability of the transformed cells to form tumors in appropriate hosts (see above), indicating that a truly neoplastic change occurred in tissue culture. The induction of neoplastic transformation by lead acetate suggests that this agent is potentially carcinogenic at the cellular level. However, with in vitro systems such as the cell transformation assay it is essential to compare the effects of other, similar types of carcinogenic agents in order to evaluate the response and to determine the reliability of the assay. The incidence of transformation obtained with lead acetate was greater than the incidence following similar exposure to $NiCl_2$, but less than that produced by $CaCrO_4$ (Heck and Costa, 1982a). Both nickel and chromium have been implicated in the etiology of human cancer (Costa, 1980). These results thus suggest that lead acetate has effects similar to those caused by other metal carcinogens. In particular, the ability of lead acetate to induce neoplastic transformation in cells in a concentration-dependent manner is highly suggestive of potential carcinogenic activity. It should also be noted that lead acetate induced these transformations at concentrations that decreased cell survival by only 27 percent (Heck and Costa, 1982a). Further studies from other laboratories utilizing the cell transformation assay and other lead compounds are needed.

12.7.3 Genotoxicity of Lead

Since cancer is known to be a disease of altered gene expression, numerous studies have evaluated changes in DNA consequent to exposure to suspected carcinogenic agents. The general response associated with such alterations in regulation of DNA function has been called genotoxicity. Various assay systems developed to examine specific changes in DNA structure and function caused by carcinogenic agents include assays that evaluate chromosomal aberrations, sister chromatid exchange, mutagenicity, and functional and structural features of DNA metabolism. Lead effects on these parameters are discussed below.

12.7.3.1 Chromosomal Aberrations. Two approaches have been used in the analysis of effects of lead on chromosomal structure. The first approach involves culturing lymphocytes either from humans exposed to lead or from experimental animals given a certain dosage of lead. The second approach involves exposing cultured lymphocytes directly to lead. For present purposes, emphasis will not be placed on the type of chromosomal aberration induced, since most of the available studies do not appear to associate any specific type of chromosomal aberration with lead exposure. It should be noted, however, that moderate aberrations include gaps and fragments, whereas severe aberrations include dicentric rings, translocations, and exchanges. Little is known of the significance of chromosomal aberrations in relationship to cancer, except that in a number of instances genetic diseases associated with chromosomal aberrations often enhance the probability of neoplastic disease. However, implicit in a morphologically distinct change in genetic structure is the possibility of an alteration in gene expression that represents a salient feature of neoplastic disease.

Contradictory reports exist regarding lead effects in inducing chromosomal aberrations (Tables 12-20 and 12-21). These studies have been grouped in two separate tables based upon their conclusions. Those studies reporting a positive effect of lead on chromosomal aberrations are indexed in Table 12-20, whereas studies reporting no association between lead exposure and chromosomal aberrations are indexed in Table 12-21. Unfortunately, these studies are difficult to evaluate fully because of many unknown variables (e.g., absence of sufficient evidence of lead intoxication, no dose-response relationship, and absence of information regarding lymphocyte culture time). To illustrate, in a number of the studies where lead exposure correlated with an increased incidence of chromosomal aberrations (Table 12-20), lymphocytes were cultured for 72 hours. Most cytogenetic studies have been conducted with a maximum culture time of 48 hours to avoid high background levels of chromosomal aberrations due to multiple cell divisions during culture. Therefore, it is possible that the positive effects of lead on chromosomal aberrations may have been due to the longer culture period. Nonetheless, it is evident that in the negative studies the blood lead concentration was generally lower than in the studies reporting a positive effect of lead on chromosomal aberrations, although in many of the latter instances blood lead levels indicated severe exposure.

TABLE 12-20. CYTOGENETIC INVESTIGATIONS OF CELLS FROM INDIVIDUALS EXPOSED TO LEAD: POSITIVE STUDIES

Number of exposed subjects	Number of controls	Cell culture time (hrs.)	Blood (μg/dl) or urine (μg/l) level	Exposed subjects	Type of damage	Remarks	References
8	14	?	62.-89. (blood)	Workers in a lead oxide factory	Chromatid and chromosome	Increase in chromosomal damage correlated with increased δ-ALA excretion	Schwanitz et al. (1970)
10	10	72	60.-100. (blood)	Workers in a chemical factory	Chromatid gaps, breaks	No correlation with blood lead levels	Gath & Thiess (1972)
14	5	48	155-720 (urine)	Workers in a zinc plant, exposed to fumes & dust of cadmium, zinc & lead	Gaps, fragments, exchanges, dicentrics, rings	Thought to be caused by lead, not cadmium or zinc	Deknudt et al. (1973)
105	-	72	11.6-97.4 mean, 37.7 (blood)	Blast-furnace workers, metal grinders, scrap metal processers	"Structural abnormalities," gaps, breaks, hyperploidy	No correlation with δ-ALA excretion or blood lead levels	Schwanitz et al. (1975)
11 (before and after exposure)	-	68-70	34.-64. (blood)	Workers in a lead-acid battery plant and a lead foundry	Gaps, breaks, fragments	No correlation with ALA-D activity in red cells	Forni et al. (1976)
44	15	72	30.-75. (blood)	Individuals in a lead oxide factory	Chromatid and chromosome aberrations	Positive correlation with length of exposure	Garza-Chapa et al. (1977)
23	20	48	44.-95. (not given)	Lead-acid battery melters, tin workers	Dicentrics, rings, fragments	Factors other than lead exposure may be required for severe aberrations	Deknudt et al. (1977b)
20	20	46-48	53.-100. (blood)	Ceramic, lead & battery workers	Breaks, fragments	Positive correlation with blood lead levels	Sarto et al. (1978)
26 (4 low, 16 medium, 6 high exposure)	not given	72	22.5-65. (blood)	Smelter workers	Gaps, chromatid and chromosome aberrations	Positive correlation with blood lead levels	Nordenson et al. (1978)
12	18	48-72	24-49 (blood)	Electrical storage battery workers	Chromatid and chromosome aberrations		Forni et al. (1980)

Source: International Agency for Research on Cancer (1980), with modifications.

PRELIMINARY DRAFT

TABLE 12-21. CYTOGENETIC INVESTIGATIONS OF CELLS FROM INDIVIDUALS EXPOSED TO LEAD: NEGATIVE STUDIES

Number of exposed subjects	Number of controls	Cell culture time (hrs.)	Blood lead level (μg/dl)	Exposed subjects	References
29	20	46-48	Not given, stated to be 20-30% higher than controls	Policemen "permanently in contact with high levels of automotive exhaust"	Bauchinger et al. (1972)
32	20	46-48	Range not given; highest level was 590 mg/l [sic]	Workers in lead manufacturing industry; 3 had acute lead intoxication	Schmid et al. (1972)
35	35	45-48	Control, <4.; exposed, 4. - >12.	Shipyard workers employed as "burners" cutting metal structures on ships	O'Riordan and Evans (1974)
24	15	48	19.3 (lead) 0.4 (cadmium)	Mixed exposure to zinc, lead, and cadmium in a zinc-smelting plant; significant increase in chromatid breaks and exchanges. Authors suggest that cadmium was the major cause of this damage	Bauchinger et al. (1976)
9	9	72	40.0 ± 5.0, 7 weeks	Volunteers ingested capsules containing lead acetate	Bulsma & De France (1976)
30	20	48	Control, 11.8-13.2; exposed, 29-33	Children living near a lead smelter	Bauchinger et al. (1977)

Source: International Agency for Research on Cancer (1980).

PRELIMINARY DRAFT

In some of these positive studies there was a correlation in the incidence of gaps, fragments, chromatid exchanges, and other chromosomal aberrations with blood lead levels (Sarto et al., 1978; Nordenson et al., 1978). However, as indicated in Table 12-20, in other studies there were no direct correlations between indices of lead exposure (i.e., δ-ALA excretion) and numbers of chromosomal aberrations. Nutritional factors such as Ca^{2+} levels *in vivo* or *in vitro* are also important since it is possible that the effects of lead on cells may be antagonized by Ca^{2+} (Mahaffey, 1983). As is usually the case in studies of human populations exposed to lead, exposure to other metals (zinc, cadmium, and copper) that may produce chromosomal aberrations was prevalent. None of the studies attempted to determine the specific lead compound that the individuals were exposed to.

In a more recent study by Forni et al. (1980), 18 healthy females occupationally exposed to lead were evaluated for chromosomal aberrations in their lymphocytes cultured for 48 or 72 hours. There were more aberrations at the 72-hour culture time compared with the 48-hour culture period in both control and lead-exposed groups, but this difference was not statistically significant. However, statistically significant differences from the 72-hour controls were noted in the 72-hour culture obtained from the lead exposed group. These results demonstrate that the extended 72-hour culture time results in increased chromosomal aberrations in the control lymphocytes and that the longer culture time was apparently necessary to detect the effects of lead on chromosomal structure. However, the blood lead levels in the exposed females ranged from 24 to 59 μg/dl, while control females had blood lead levels ranging from 22 to 37 μg/dl. Thus, there was a marginal effect of lead on chromosomal aberration, but the two groups may not have been sufficiently different in their lead exposure to show clear differences in frequency of chromosomal aberrations.

Some studies have also been conducted on the direct effect of soluble lead salts on cultured human lymphocytes. In a study by Beek and Obe (1974), longer (72-hr) culture time was used and lead acetate was found to induce chromosomal aberrations at 100 μM. Lead acetate had no effect on chromatid aberrations induced with X-rays or alkylating agents (Beek and Obe, 1975). In another study (Deknudt and Deminatti, 1978), lead acetate at 1 and 0.1 mM caused minimal chromosomal aberrations. Both cadmium chloride ($CdCl_2$) and zinc chloride ($ZnCl_2$) were more potent than lead acetate in causing these changes; however, both $CdCl_2$ and $ZnCl_2$ also displayed greater toxicity than lead acetate.

Chromosomal aberrations have been demonstrated in lymphocytes from cynomolgus monkeys treated chronically with lead acetate (6 mg/day, 6 days/week for 16 months), particularly when they were kept on a low calcium diet (Deknudt et al., 1977a). These aberrations accompanying a low Ca^{2+} diet were characterized by the authors as severe (chromatid exchanges, dispiralization, translocations, rings, and polycentric chromosomes). Similar results were observed in mice (Deknudt and Gerber, 1979). The effect of low calcium on chromosomal aberrations induced

by lead is most likely due to interaction of Ca^{2+} and Pb^{2+} at the level of the chromosome (Mahaffey, 1983). Léonard and his coworkers found no effect of lead on the incidence of chromosomal aberrations in accidentally intoxicated cattle (Léonard et al., 1974) or in mice given 1 gram of lead per liter of drinking water for 9 months (Léonard et al., 1973). However, Muro and Goyer (1969) found gaps and chromatid aberrations in bone marrow cells cultured for four days after isolation from mice that had been maintained on 1 percent dietary lead acetate for two weeks. Chromosomal loss has been reported (Ahlbert et al., 1972) in Drosophila exposed to triethyl lead (4 mg/l), but inorganic lead had no effect (Ramel, 1973). Lead acetate has also been shown to induce chromosomal aberrations in cultured cells other than lymphocytes, viz. Chinese hamster ovary cells (Bauchinger and Schmid, 1972).

These studies demonstrate that under certain conditions lead compounds are capable of inducing chromosomal aberrations in vivo and in tissue cultures. The ability of lead to induce these chromosomal changes appears to be concentration-dependent and highly influenced by calcium levels. In lymphocytes isolated from patients or experimental animals, relatively long (72-hr) culture conditions are required for the abnormalities to be expressed.

Sister chromatid exchange represents the normal movement of DNA in the genome. The sister chromatid exchange assay offers a very sensitive probe for the effects of genotoxic compounds on DNA rearrangement, as a number of chemicals with carcinogenic activity are capable of increasing these exchanges (Sandberg, 1982). The effect of lead on such movement has been examined in cultured lymphocytes (Beek and Obe, 1975), with no increase in exchanges observed at lead acetate concentrations of 0.01 mM. However, one study with lead at one dose in one system is not sufficient to rule out whether lead increases the incidence of these exchanges.

The ability of agents such as lead to cause abnormal rearrangements in the structure of DNA, as revealed by the appearance of chromosomal aberrations, and sister chromatid exchanges has become an important focus in carcinogenesis research. Current theories suggest that cancer may result from an abnormal expression of oncogenes (genes that code for protein products associated with virally induced cancers). Numerous oncogenes are found in normal human DNA, but the genes are regulated such that they are not expressed in an carcinogenic fashion. Rearrangement of these DNA sequences within the genome can lead to oncogenic expression. Evidence has been presented suggesting that chromosomal aberrations such as translocations are associated with certain forms of cancer and with the movement of oncogenes in regions of the DNA favoring their expression in cancer cells (Shen-Ong et al., 1982). By inducing aberrations in chromosomal structure, lead may enhance the probability of an oncogenic event.

12.7.3.2 Lead Effects on Bacterial and Mammalian Mutagenesis Systems. Bacterial and mammalian mutagenesis test systems examine the ability of chemical agents to induce changes in DNA sequences of a specific gene product that is monitored by selection procedures. They measure the potential of a chemical agent to produce a change in DNA, but this change is not likely to

be the same alteration in gene expression that occurs during oncogenesis. However, if an agent affects the expression of a particular gene product that is being monitored, then it could possibly affect other sequences which may result in cancer. Since many carcinogens are also mutagens, it is useful to employ such systems to evaluate genotoxic effects of lead.

Use of bacterial systems for assaying metal genotoxicity must await further development of bacterial strains that are appropriately responsive to known mutagenic metals (Rosenkranz and Poirier, 1979; Simmon, 1979; Simmon et al., 1979; Nishioka, 1975; Nestmann et al., 1979). Mammalian cell mutagenic systems that screen for specific alterations in a defined gene mutation have not been useful in detecting mutagenic activity with known carcinogenic metals (Heck and Costa, 1982b). In plants, however, chromosomal aberrations in root tips (Mukherji and Maitra, 1976) and other mutagenic activity, such as chlorophyll mutations (Reddy and Vaidyanath, 1978), have been demonstrated with lead.

12.7.3.3 Lead Effects on Parameters of DNA Structure and Function. There are a number of very sensitive techniques for examining the effect of metals on DNA structure and function in intact cells. Although these techniques have not been extensively utilized with respect to metal compounds, future research will probably be devoted to this area. Considerable work has been done to understand the effects of metals on enzymes involved in DNA transcription.

Sirover and Loeb (1976) examined effects of lead and other metal compounds upon the fidelity of transcription of DNA by a viral DNA polymerase. High concentrations of metal ions (in some cases in the millimolar range) were required to decrease the fidelity of transcription, but there was a good correlation between metal ions that are carcinogenic or mutagenic and their activity in decreasing the fidelity of transcription. This assay system measures the ability of a metal ion to incorporate incorrect (non-homologous) bases using a defined polynucleotide template. In an intact cell, this would cause the induction of a mutation if the insertion of an incorrect base is phenotypically expressed. Since the interaction of metal ions with cellular macromolecules is relatively unstable, misincorporation of a base during semi-conservative DNA replication or during DNA repair synthesis following breakage of DNA with a metal could alter the base sequence of DNA in an intact cell. Lead at 4 mM was among the metals listed as mutagenic or carcinogenic that caused a decrease in the fidelity of transcription (Sirover and Loeb, 1976). Other metals active in decreasing fidelity included: Ag^{+}, Be^{2+}, Cd^{2+}, Co^{2+}, Cr^{2+}, Cr^{3+}, Cu^{2+}, Mn^{2+}, and Ni^{2+}. No change in fidelity was produced by Al^{3+}, Ba^{2+}, Ca^{2+}, Fe^{3+}, K^{+}, Rb^{+}, Mg^{2+}, Mg^{+}, Se^{2+}, Sr^{2+}, and Zn^{2+}. Metals that decreased fidelity are metals also implicated as carcinogenic or mutagenic (Sirover and Loeb, 1976).

In a similar study, Hoffman and Niyogi (1977) demonstrated that lead chloride was the most potent of 10 metals tested in inhibiting RNA synthesis (i.e., $Pb^{2+} > Cd^{2+} > Co^{2+} > Mn^{2+} > Li^{+} > Na^{+} > K^{+}$) for both types of templates tested, i.e., calf thymus DNA and T_4 phage DNA.

These results were explained in terms of the binding of these metal ions more to the bases than to the phosphate groups of the DNA (i.e., $Pb^{2+} > Cd^{2+} > Zn^{2+} > Mn^{2+} > Mg^{2+} > Li^{+} = Na^{+} = K^{+}$). Additionally, metal compounds such as lead chloride with carcinogenic or mutagenic activity were found to stimulate mRNA chain initiation at 0.1 mM concentrations.

These well-conducted mechanistic studies provide evidence that lead can affect a molecular process associated with normal regulation of gene expression. Although far removed from the intact cell situation, these effects suggest that lead may be genotoxic.

12.7.4 Summary and Conclusions

It is evident from studies reviewed above that, at relatively high concentrations, lead displays some carcinogenic activity in experimental animals (e.g. the rat). An agent may act as a carcinogen in two distinct ways: (1) as an initiator or (2) as a promoter (Weisburger and Williams, 1980). By definition, an initiator must be able to interact with DNA to produce a genetic alteration, whereas a promoter acts in a way that allows the expression of an altered genetic change responsible for cancer. Since lead is capable of transforming cells directly in culture and affecting DNA-to-DNA and DNA-to-RNA transcription, it may have some initiating activity. Its ability to induce chromosomal aberrations is also indicative of initiating activity. There are no studies that implicate or support a promotional activity of lead; however, its similarity to Ca^{2+} suggests that it may alter regulation of this cation in processes (e.g., cell growth) related to promotion. Intranuclear lead inclusion bodies in the kidney may pertain to lead's carcinogenic effects, since both the formation of these bodies and the induction of tumors occur at relatively high doses of lead. The interaction of lead with key non-histone chromosomal proteins in the nucleus to form the inclusion bodies or the presence of inclusion bodies in the nucleus may alter genetic function, thus leading to cell transformation. Obviously, elucidating the mechanism of lead carcinogenesis requires further research efforts and only theories can be formulated regarding its oncogenic action at present.

It is hard to draw clear conlusions concerning what role lead may play in the induction of human neoplasia. Epidemiological studies of lead-exposed workers provide no definitive findings. However, statistically significant elevations in respiratory tract and digestive system cancer in workers exposed to lead and other agents warrant concern. Also, since lead acetate can produce renal tumors in some experimental animals, it may be prudent to assume that at least that lead compound may be carcinogenic in humans. However, this statement is qualified by noting that lead has been observed to increase tumorogenesis rates in animals only at relatively high concentrations, and therefore does not appear to be an extremely potent carcinogen. *In vitro* studies further support the genotoxic and carcinogenic role of lead, but also indicate that lead is not extremely potent in these systems either.

12.8 EFFECTS OF LEAD ON THE IMMUNE SYSTEM

12.8.1 Development and Organization of the Immune System

Component cells of the immune system arise from a pool of pluripotent stem cells in the yolk sack and liver of the developing fetus and in the bone marrow and spleen of the adult. Stem cell differentiation and maturation follows one of several lines to produce lymphocytes, macrophages, and polymorphonuclear leukocytes. These cells have important roles in immunological function and host defense.

The predominant lymphocyte class develops in the thymus, which is derived from the third and fourth pharyngeal pouches at 9 weeks of gestation in man (day 9 in mice). In the thymus microenvironment they acquire characteristics of thymus-derived lymphocytes (T-cells), then migrate to peripheral thymic-dependent areas of the spleen and lymph nodes. T-cells are easily distinguished from other lymphocytes by genetically defined cell surface markers that allow them to be further subdivided into immunoregulatory amplifier cells (helper T-cells) and suppressor T-cells that regulate immune responses. T-cells also participate directly as cytolytic effector cells against virally infected host cells, malignant cells, and foreign tissues as well as in delayed-type hypersensitivity (DTH) reactions where they elaborate lymphokines that modulate the inflammatory response. T-cells are long-lived lymphocytes and are not readily replaced. Thus, any loss or injury to T-cells may be detrimental to the host and result in increased susceptibility to viral, fungal, bacterial, or parasitic diseases. Individuals with acquired immune deficiency syndrome (AIDS) are examples of individuals with T-cell dysfunction. There is ample evidence that depletion by environmental agents of thymocytes or stem cell progenitors during lymphoid organogenesis can produce permanent immunosuppression.

The second major lymphocyte class differentiates from a lymphoid stem-cell in a yet undefined site in man, which would correspond functionally to the Bursa of Fabricius in avian species. In man, B-lymphocyte maturation and differentiation probably occur embryologically in gut-associated lymphoid tissue (GALT) and fetal liver, as well as adult spleen and bone marrow. This is followed by the peripheral population of thymic-independent areas of spleen and lymph nodes. Bone marrow-derived lymphocytes (B-cells), which mature independently of the thymus, possess specific immunoglobulin receptors on their surfaces. The presence of cell surface immunoglobulin (sIg) at high density is the major characteristic separating B-cells from T-cells. Following interaction with antigens and subsequent activation, B-lymphocytes proliferate and differentiate into antibody-producing plasma cells. In contrast to the long-lived T-cell, B-cells are rapidly replaced by newly differentiating stem cells. Therefore, lesions in the B-cell compartment may be less serious than those in the T-cell compartment since they are more easily reversed. Insult to B-cells at the stem cell or terminal maturation stage can result in suppression of specific immunoglobulin and enhanced susceptibility to infectious agents whose pathogenesis is limited by antibodies.

Pluripotent stem cells also give rise to lymphocytes whose lineages are still unclear. Some possess natural cytolytic activity for tumor cells (natural killer cell activity), while others, devoid of T- and B-cell surface markers (null cells), participate in antibody-dependent cell-mediated cytotoxicity (ADCC). The pluripotent stem cell pool also contains precursors of monocyte-macrophages and polymorphonuclear leukocytes (PMN). The macrophage has a major role in presentation and processing of certain antigens, in cytolysis of tumor target cells, and in phagocytosis and lysis of persistent intracellular infectious agents. Also, it actively phagocytizes and kills invading organisms. Defects in differentiation or function of PMNs or macrophages predispose the host to infections by bacteria and other agents.

This introduction should make it evident that the effects of an element such as lead on the immune system may be expressed in complex or subtle ways. In some cases, lead might produce a lesion of the immune system not resulting in markedly adverse health effects, especially if the lesion did not occur at an early stem cell stage or during a critical point in lymphoid organogenesis. On the other hand, some lead-induced immune system effects might adversely affect health through increasing susceptibility to infectious agents or neoplastically transformed cells if, for example, they were to impair cytocidal or bactericidal function.

12.8.2 Host Resistance

One way of ascertaining if a chemical affects the immune response of an animal is to challenge an exposed animal with a pathogen such as an infectious agent or oncogen. This provides a general approach to determine if the chemical interferes with host immune defense mechanisms. Host defense is a composite of innate immunity, part of which is phagocyte activities, and acquired immunity, which includes B- and T-lymphocyte and enhanced phagocyte reactivities. Analysis of host resistance constitutes a holistic approach. However, dependent on the choice of the pathogen, host resistance can be evaluated somewhat more selectively. Assessment of host resistance to extracellular microbes such as *Staphylococci*, *Salmonella typhimurium*, *Escherichia coli*, or *Streptococcus pneumoniae* and to intracellular organisms such as *Listeria monocytogenes* or *Candida albicans* primarily measures intact humoral immunity and cell-mediated immunity, respectively. Immune defense to extracellular organisms requires T-lymphocyte, B-lymphocyte, and macrophage interactions for the production of specific antibodies to activate the complement cascade and to aid phagocytosis. Antibodies can also directly neutralize some bacteria and viruses. Resistance to intracellular organisms requires T-lymphocyte and macrophage interactions for T-lymphocyte production of lymphokines, which further enhance immune mechanisms including macrophage bactericidal activities. An additional T-lymphocyte subset, the cytolytic T-cell, is involved in resistance to tumors; immune defenses against tumors are also aided by NK- and K-lymphocytes and macrophages.

12.8.2.1 Infectivity Models. Numerous studies designed to assess the influence of lead on host resistance to infectious agents consistently have shown that lead impairs host resistance, regardless of whether the defense mechanisms are predominantly dependent on humoral- or cell-mediated immunity (Table 12-22).

TABLE 12-22. EFFECT OF LEAD ON HOST RESISTANCE TO INFECTIOUS AGENTS

Species	Infectious agent	Lead dose	Lead exposure	Mortality[a]	Reference
Mouse	*S. typhimurium*	200 ppm	i.p.; 30 days	54% (13%)	Hemphill et al. (1971)
Rat	*E. coli*	2 mg/100 g	i.v.; 1 day	96% (0%)	Cook et al. (1975)
Rat	*S. epidermidis*	2 mg/100 g	i.v.; 1 day	80% (0%)	Cook et al. (1975)
Mouse	*L. monocytogenes*	80 ppm	orally; 4 wk	100% (0%)	Lawrence (1981a)
Mouse	EMC virus	2000 ppm	orally; 2 wk	100% (19%)	Gainer (1977b)
Mouse	EMC virus	13 ppm	orally; 10 wk	80% (50%)	Exon et al. (1979)
Mouse	Langat virus	50 mg/kg	orally; 2 wk	68% (0%)	Thind and Kahn (1978)

[a]The percent mortality is reported for the lowest dose of lead in the study that significantly altered host resistance. The percent mortality in parentheses is that of the non-lead-treated, infected control group.

Mice (Swiss Webster) injected i.p. for 30 days with 100 or 250 μg (per 0.5 ml) of lead nitrate and inoculated with *Salmonella typhimurium* had higher mortality (54 and 100 percent, respectively) than non-lead-injected mice (13 percent) (Hemphill et al., 1971). These concentrations of lead, by themselves, did not produce any apparent toxicity. Similar results were observed in rats acutely exposed to lead (one i.v. dose of 2 mg/100 g) and challenged with *Escherichia coli* (Cook et al., 1975). In these two studies, lead could have interfered with the clearance of endotoxin from the *S. typhimurium* or *E. coli*, and the animals may have died from endotoxin shock and not septicemia due to the lack of bacteriostatic or bactericidal activities. However, the study by Cook et al. (1975) also included a non-endotoxin-producing gram-positive bacterium, *Staphylococcus epidermidis*, and lead still impaired host resistance. In another study, lead effects on host resistance to the intracellular parasite *Listeria monocytogenes* were monitored (Lawrence, 1981a). CBA/J mice orally exposed to 16, 80, 400, and 2000 ppm lead for four weeks were assayed for viable *Listeria* after 48 and 72 hours, and for mortality after 10 days. Only 2000 ppm lead caused significant inhibition of early bactericidal activity (48-72 hr), but 80-2000 ppm lead produced 100 percent mortality, compared with 0 percent mortality in the 0-16 ppm lead groups. Other reports have suggested that host

resistance is impaired by lead exposure of rodents. Salaki et al. (1975) indicated that lead lowered resistance of mice to Staphylococcus aureus, Listeria, and Candida; and observed higher incidence of inflammation of the salivary glands in lead-exposed rats (Grant et al., 1980) may be due, in part, to lead-induced increased susceptibility to infections.

Inhalation of lead has also been reported to lower host resistance to bacteria. Schlipköter and Frieler (1979) exposed NMRI mice to an aerosol of 13-14 μg/m^3 lead chloride and clearance of Serratia marcesens in the lungs was reduced significantly. Microparticles of lead in lungs of mice were also shown to lower resistance to Pasteurella multocida, in that 6 μg of lead increased the percentage of mortality by 27 percent (Bouley et al., 1977).

Lead has also been shown to increase host susceptibility to viral infections. CD-1 mice, administered 2,000 and 10,000 ppm lead in drinking water for two weeks and subsequently inoculated with encephalomyocarditis (EMC) virus, had a significant increase in mortality (100 percent at 2,000 ppm; 65 percent at 10,000 ppm) compared with control EMC virus-infected mice (13 percent) (Gainer, 1977b). In another study (Exon et al., 1979), Swiss Webster mice were exposed to 13, 130, 1300, or 2600 ppm lead for 10 weeks in their drinking water and were infected with EMC virus. Although as low as 13 ppm lead caused a significant increase in mortality (80 percent) in comparison with the non-lead-treated EMC virus-infected mice (50 percent), there were no dose-response effects, in that 2600 ppm lead resulted in only 64 percent mortality. The lack of a dose-response relationship in the two studies with EMC virus (Gainer, 1977b; Exon et al., 1979) suggests that the higher doses of lead may directly inhibit EMC infectivity as well as host defense mechanisms. Additional studies have confirmed that lead inhibits host resistance to viruses. Mice treated orally with lead nitrate (10-50 mg/kg/ day) for two weeks had suppressed antibody titers to Langat virus (Type B arbovirus) and increased titers of the virus itself (Thind and Singh, 1977), and the lead-inoculated, infected mice had higher mortalities (25 percent at 10 mg/kg; 68 percent at 50 mg/kg) than the non-lead-infected mice (0 percent) (Thind and Khan, 1978).

The effects of lead on bacterial and viral infections in humans have never been studied adequately; there is only suggestive evidence that human host resistance may be lowered by lead. Children with persistently high blood lead levels who were infected with Shigella enteritis had prolonged diarrhea (Sachs, 1978). In addition, lead workers with blood lead levels of 22-89 μg/dl have been reported to have more colds and influenza infections per year (Ewers et al., 1982). This study also indicated that secretory IgA levels were suppressed significantly in lead workers with a median blood lead level of 55 μg/dl. Secretory IgA is a major factor in immune defense against respiratory as well as gastrointestinal infections.

Hicks (1972) points out that there is need for systematic epidemiological studies on the effects of elevated lead levels on the incidence of infectious diseases in humans. The current paucity of information precludes formulation of any clear dose-response relationship for

humans. Epidemiological investigations may help to determine if lead alters the immune system of man and consequently increases susceptibility to infectious agents and neoplasia.

12.8.2.2 Tumor Models and Neoplasia. The carcinogenicity of lead has been studied both as a direct toxic effect of lead (see Section 12.7) and as a means of better understanding the effects of lead on the body's defense mechanisms. Studies by Gainer (1973, 1974) demonstrated that exposure of CD-1 mice to lead acetate potentiated the oncogenicity of a challenge with Rauscher leukemia virus (RLV), resulting in enhanced splenomegaly and higher virus titers in the spleen presumably through an immunosuppressive mechanism. Recent studies by Kerkvliet and Baecher-Steppan (1982) revealed that chronic exposure of C57BL/6 mice to lead acetate in drinking water at 130-1300 ppm enhanced the growth of primary tumors induced by Moloney sarcoma virus (MSV). Regression of MSV-induced tumors was not prevented by lead exposure, and lead-treated animals resisted late sarcoma development following primary tumor resistance. Depressed resistance to transplantable MSV tumors was associated with a reduced number of macrophages, which also exhibited reduced phagocytic activity.

In addition to enhancing the transplantability of tumors or the oncogenicity of leukemia viruses, lead has also been shown to facilitate the development of chemically induced tumors. Kobayashi and Okamoto (1974) found that intratracheal dosing of benzo(a)pyrene (BaP) combined with lead oxide resulted in an increased frequency of lung adenomas and adenocarcinomas over mice exposed to BaP alone. Similarly, exposure to lead acetate enhanced the formation of N(4'-fluoro-4-biphenyl) acetamide-induced renal carcinomas from 70 to 100 percent and reduced the latency to tumor appearance (Hinton et al., 1980). Recently, Koller et al. (1983) found that exposure to lead for 18 months increased the frequency of spontaneous tumors, predominantly renal carcinomas, in rats. Similarly, Schrauzer et al. (1981) found that adding lead at 5 ppm to drinking water of C3H/St mice infected with Bittner milk factor diminished the uptake of selenium and reduced its anticarcinogenic effects, causing mammary tumors to appear at the same high incidence as in selenium-unsupplemented controls. Lead likewise significantly accelerated tumor growth and shortened survival in this model.

The above studies on host susceptibility to various pathogens, including infectious agents and tumors, indicate that lead could be detrimental to health by methods other than direct toxicity. In order to understand the mechanisms by which lead suppresses host resistance maintained by phagocytes, humoral immunity, and/or cell-mediated immunity, the immune system must be dissected into its functional components and the effects of lead on each, separately and combined, must be examined in order that the mechanism(s) of the immunomodulatory potential of lead can be understood.

12.8.3 Humoral Immunity

12.8.3.1 Antibody Titers. A low antibody titer in animals exposed to lead could explain the increased susceptibility of animals to extracellular bacteria and some viruses (see Table

12-23), as well as to endotoxins (Selye et al., 1966; Filkins, 1970; Cook et al., 1974; Schumer and Erve, 1973; Rippe and Berry, 1973; Truscott, 1970). Specific antibodies can directly neutralize pathogens, activate complement components to induce lysis, or directly or indirectly enhance phagocytosis via Fc receptors or C3 receptors, respectively. Studies in animals and humans have assayed the effects of lead on serum immunoglobulin levels, specific antibody levels, and complement levels. Analysis of serum immunoglobulin levels is not a good measure of specific immune reactivity, but it would provide evidence for an effect on B-lymphocyte development.

TABLE 12-23. EFFECT ON LEAD ON ANTIBODY TITERS

Species	Antigen	Lead dose and exposure	Effect	Reference
Rabbit	Pseudorabies virus	2500 ppm; 10 wk	Decrease	Koller (1973)
Rat	S. typhimurium	5000-20000 ppm; 3 wk	Decrease	Stanković and Jugo (1976)
Rat	Bovine serum albumin	10-1000 ppm; 10 wk	Decrease	Koller et al. (1983)
Mouse	Sheep red blood cells	0.5-10 ppm[a]; 3 wk	Decrease	Blakley et al. (1980)

[a]Lead was administered as tetraethyl lead; other studies used inorganic forms.

Lead had little effect on the serum immunoglobulin levels in rabbits (Fonzi et al., 1967a), children with blood lead levels of 40 µg/dl (Reigart and Garber, 1976), or lead workers with 22-89 µg/dl (Ewers et al., 1982). On the other hand, most studies have shown that lead significantly impairs antibody production. Acute oral lead exposure (50,000 ppm/kg) produced a decreased titer of anti-typhus antibodies in rabbits immunized with Typhus vaccine (Fonzi et al., 1967b). In New Zealand white rabbits challenged with pseudorabies virus, lead (oral exposure to 2500 ppm for 70 days) caused a 9-fold decrease in antibody titer to the virus (Koller, 1973). However, lead has not always been shown to reduce titers to virus. Vengris and Mare (1974) did not observe depressed antibody titers to Newcastle disease virus in lead-treated chickens, but their lead treatment was only for 35 days prior to infection. Lead-poisoned children also had normal anti-toxoid titers after booster immunizations with tetanus toxoid (Reigart and Garber, 1976). In another study, Wistar rat dams were exposed to 5,000, 10,000, or 20,000 ppm lead for 20 days following parturition (Stanković and Jugo, 1976). The progeny were weaned at 21 days of age and given standard laboratory chow for an

additional month. At that time, they were injected with Salmonella typhimurium, and serum antibody titers were assessed. Each dosage of lead resulted in significantly reduced antibody titers. More recently, rats (Sprague-Dawley) given 10 ppm lead acetate orally for 10 weeks had a significant suppression in antibody titers when challenged with bovine serum albumin (BSA) and compared with BSA-immunized non-lead-exposed rats (Koller et al., 1983). Development of a highly sensitive, quantitative, enzyme-linked immunosorbent assay (ELISA) contributed to detecting the immunosuppressive activity of lead at this dosage.

Tetraethyl lead also has been responsible for reduced antibody titers in Swiss-cross mice (Blakley et al., 1980). The mice were exposed orally to 0.5, 1.0, and 2.0 ppm tetraethyl lead for 3 weeks. A significant reduction in hemagglutination titers to sheep red blood cells (SRBC) occurred at all levels of exposure.

12.8.3.2 Enumeration of Antibody Producing Cells (Plaque-Forming Cells). From the above results, it appears that lead inhibits antibody production. To evaluate this possible effect at the cellular level, the influence of lead on the number of antibody producing cells after primary or secondary immunization can be assessed. In primary humoral immune responses (mostly direct), IgM plaque-forming cells (PFC) are measured, whereas in secondary or anamnestic responses (mostly indirect), IgG PFC are counted. The primary immune response represents an individual's first contact with a particular antigen. The secondary immune response represents re-exposure to the same antigen weeks, months, or even years after the primary antibody response has subsided. The secondary immune response is attributed to persistence, after initial contact with the antigen, of a substantial number of antigen-sensitive memory cells. Impairment of the memory response, therefore, results in serious impairment of humoral immunity in the host.

Table 12-24 summarizes the effects of lead on IgM or IgG PFC development. Mice exposed orally to tetraethyl lead (0.5, 1, or 2 ppm) for three weeks produced a significant reduction in the development of IgM and IgG PFC (Blakley et al., 1980). Mice (Swiss Webster) exposed orally to 13, 137, or 1375 ppm inorganic lead for eight weeks had reduced numbers of IgM PFC in each lead-exposed group (Koller and Kovacic, 1974). Even the lowest lead group (13 ppm) had a decrease. The secondary response (IgG PFC, induced by a second exposure to antigen SRBC seven days after the primary immunization) was inhibited to a greater extent than the primary response. This study indicated that chronic exposure to lead produced a significant decrease in the development of IgM PFC and IgG PFC. When Swiss Webster mice were exposed to 13, 130, and 1300 ppm lead for 10 weeks and hyperimmunized by SRBC injections at week 1, 2, and 9, the memory response as assessed by the enumeration of IgG PFC was significantly inhibited at 1300 ppm (Koller and Roan, 1980a). This suggests that the temporal relationships between lead exposure and antigenic challenge may be critical. Other studies support this interpretation.

TABLE 12-24. EFFECT OF LEAD ON THE DEVELOPMENT OF ANTIBODY-PRODUCING CELLS (PFC)

Species	Antigen[a]	Lead dose and exposure	Effect[b]	Reference
Mouse	SRBC (in vivo)	13-1370 ppm; 8 wk	IgM PFC (D) IgG PFC (D)	Koller and Kovacic (1974)
Mouse	SRBC (in vivo)	0.5-2 ppm tetraethyl lead; 3 wk	IgM PFC (D) IgG PFC (D)	Blakley et al. (1980)
Mouse	SRBC (in vivo)	13-1370 ppm; 10 wk	IgG PFC (D)	Koller and Roan (1980a)
Mouse	SRBC (in vivo)	4 mg (i.p. or orally)	IgM PFC (I) IgG PFC (D)	Koller et al. (1976)
Mouse	SRBC (in vivo) SRBC (in vitro + 2-ME)	16-2000 ppm; 1-10 wk 16-80 ppm; 4 wk 2000 ppm; 4 wk	IgM PFC (N) IgM PFC (I) IgM PFC (D)	Lawrence (1981a)
Rat	SRBC (in vivo)	25-50 ppm; pre/postnatal	IgM PFC (D)	Luster et al. (1978)
Mouse	SRBC (in vitro) SRBC (in vitro + 2-ME)	50-1000 ppm; 3 wk 50-1000 ppm; 3 wk	IgM PFC (D) IgM PFC (N or I)	Blakley and Archer (1981)
Mouse	SRBC (in vitro + 2-ME)	2-20 ppm (in vitro)	IgM PFC (I)	Lawrence (1981b,c)

[a]The antigenic challenge with sheep red blood cells (SRBC) was in vivo or in vitro after in vivo exposure to lead unless otherwise stated. The in vitro assays were performed in the presence or absence of 2-mercaptoethanol (2-ME).

[b]The letters in parentheses are defined as follows: D = decreased response; N = unaltered response; I = increased response.

Female Sprague-Dawley rats with pre- and post-natal exposure to lead (25 or 50 ppm) had a significant reduction in IgM PFC (Luster et al., 1978). In contrast, CBA/J mice exposed orally to 16-2000 ppm lead for 1-10 weeks did not have altered IgM PFC responses to SRBC (Lawrence, 1981a). Furthermore, when Swiss Webster mice were exposed to an acute lead dose (4 mg lead orally or i.p.), the number of IgG PFC was suppressed, but the number of IgM PFC was enhanced (Koller et al., 1976).

The influence of lead on the development of PFC in mice was assessed further by in vivo exposure to lead, removal of spleen cells, and in vitro analysis of PFC development. Initially it appeared that low doses of lead (16 and 80 ppm) enhanced development, and only a high

dose (2000 ppm) inhibited the development of IgM PFC (Lawrence, 1981a). However, a later study by Blakley and Archer (1981) indicated that 50-1000 ppm lead consistently inhibited IgM PFC. Through the analysis of mixed cultures of lead-exposed lymphocytes (nonadherent cells) and unexposed macrophages (adherent cells), and vice versa, as well as of in vitro responses to antigens that do not require macrophage help (i.e., lipopolysaccharide, LPS), their data indicated that the effects of lead may be at the level of the macrophage. This was substantiated by the fact that 2-mercaptoethanol (2-ME, a compound that can substitute for at least one macrophage activity) was able to reverse the inhibition by lead. This may explain why in vivo lead exposure (16 and 80 ppm) appeared to enhance the in vitro IgM PFC responses in the study by Lawrence (1981a), because 2-ME was present in the in vitro assay system. Furthermore, in vitro exposure to lead (2 or 20 ppm) in spleen cell cultures with 2-ME enhanced the development of IgM PFC (Lawrence, 1981b,c).

These experiments indicate that lead modulates the development of antibody-producing cells as well as serum antibody titers, which supports the notion that lead can suppress humoral immunity. However, it should be noted that the dose and route of exposure of both lead and antigen may influence the modulatory effects of lead. The adverse effects of lead on humoral immunity may be due more to lead's interference with macrophage antigen processing and/or antigen presentation to lymphocytes than to direct effects on B-lymphocytes. These mechanisms require further investigation.

12.8.4 Cell-Mediated Immunity

12.8.4.1 Delayed-Type Hypersensitivity. T-lymphocytes (T-helper and T-suppressor cells) are regulators of humoral and cell-mediated immunity as well as effectors of two aspects of cell-mediated immunity. T-cells responsive to delayed-type hypersensitivity (DTH) produce lymphokines that induce mononuclear infiltrates and activate macrophages, which are aspects of chronic inflammatory responses. In addition, another subset of T-cells, cytolytic T-cells, cause direct lysis of target cells (tumors or antigenically modified autologous cells) when in contact with the target. To date, the effects of lead on cytolytic T-cell reactivity have not been measured, but the influence of lead on inducer T-cells has been studied (Table 12-25). Groups of mice injected i.p. daily for 30 days with 13.7 to 137 ppm lead were subsequently sensitized i.v. with SRBC. The DTH reaction was suppressed in these animals in a dose-related fashion (Müller et al., 1977). The secondary DTH response was inhibited in a similar fashion. In another study (Faith et al., 1979), the effects of chronic low level pre- and post-natal lead exposure on cellular immune functions in Sprague-Dawley rats was assessed. Female rats were exposed to 25 or 50 ppm lead acetate continuously for seven weeks before breeding and through gestation and lactation. The progeny were weaned at three weeks of age and continued on the respective lead exposure regimen of their mothers for an additional 14 to 24 days.

TABLE 12-25. EFFECT OF LEAD ON CELL-MEDIATED IMMUNITY

Species	Lead dose and exposure	Parameter*	Effect	Reference
Mouse	13.7-137 ppm; 4 wk	DTH	Decrease	Müller et al. (1977)
Rat	25-50 ppm; 8 wk	DTH	Decrease	Faith et al. (1979)
Mouse	13-1300 ppm; 10 wk	MLC	None	Koller and Roan (1980b)
Mouse	16-2000 ppm; 4 wk	MLC	Decrease	Lawrence (1981a)

*DTH =delayed-type hypersensitivity; MLC = mixed lymphocyte culture.

Thymic weights and DTH responses were significantly decreased by both lead dosages. These results indicate that chronic low levels of lead suppress cell-mediated immune function.

The in vitro correlate of the analysis of DTH responsive T-cells in vivo is the analysis of mixed lymphocyte culture (MLC) responsive T-cells. When two populations of allogeneic lymphoid cells are cultured together, cellular interactions provoke blast cell transformation and proliferation of a portion of the cultured cells (Cerottini and Brunner, 1974; Bach et al., 1976). The response can be made one-way by irradiating one of the two allogeneic preparations, in which case the irradiated cells are the stimulators (allogeneic B-cells and macrophages) and the responders (T-cells) are assayed for their proliferation. The mixed lymphocyte reaction is an in vitro assay of cell-mediated immunity analogous to in vivo host versus graft reactions.

Mice (DBA/2J) fed 13, 130, or 1300 ppm lead for 10 weeks were evaluated for responsiveness in mixed lymphocyte cultures. The 130-ppm lead dose tended to stimulate the lymphocyte reaction, although no change was observed at the other dose levels (Koller and Roan, 1980b). In another study (Lawrence, 1981a), mice (CBA/J) were fed 16, 80, 400, or 2000 ppm lead for four weeks. The 16 and 80 ppm doses slightly stimulated, while the 2000 ppm dose suppressed, the mixed lymphocyte reaction. It is important to note that in these in vitro MLC assays, 2-ME was present in the culture medium, and the 2-ME may have reversed the in vivo effects of lead, as was observed for the in vitro PFC responses (Blakley and Archer, 1981).

The data on the effects of lead on humoral and cell-mediated immunity indicate that in vivo lead usually is immunosuppressive, but additional studies are necessary to fully understand the temporal and dose relationship of lead's immunomodulatory effects. The in vitro analysis of immune cells exposed to lead in vivo suggest that the major cell type modified is the macrophage; the suppressive effects of lead may be readily reversed by thiol reagents possibly acting as chelators.

12.8.4.3 Interferon. Interferons (IF) are a family of low molecular weight proteins which exhibit antiviral activity in sensitive cells through processes requiring new cellular RNA and protein synthesis (Stewart, 1979). It has been speculated that the enhanced susceptibility of lead-treated mice to infectious virus challenge might be due to a decreased capacity of these animals to produce viral or immune interferons or to respond to them. Studies by Gainer (1974, 1977a) appeared to resolve this question and indicated that exposure of CD-1 mice to lead does not inhibit the antiviral action of viral IF *in vivo* or *in vitro*. In the later of the two studies, lead exposure inhibited the protective effects of the IF inducers Newcastle disease virus and poly I:poly C against encephalomyocarditis virus (EMC)-induced mortality. These data suggest that, although lead did not directly interfere with the antiviral activity of interferon, it might suppress viral IF production *in vivo*. Recently, Blakley et al. (1982) re-examined this issue and found that female BDF_1 mice exposed to lead acetate in drinking water at concentrations ranging from 50 to 1000 μg/ml for three weeks produced amounts of IF similar to controls given a viral IF inducer, Tilorone. Similarly, the *in vitro* induction of immune IF by the T-cell mitogens phytohemagglutinin, concanavalin A, and staphylococcal enterotoxin in lymphocytes from lead-exposed mice were unaltered compared with controls (Blakley et al., 1982). Thus, lead exposure does not appear to significantly alter the lymphocyte's ability to produce immune interferon. Therefore, it must be assumed that increased viral susceptibility associated with chronic lead exposure in rodents is by mechanisms other than interference with production of or response to interferon.

12.8.5 Lymphocyte Activation by Mitogens

Mitogens are lectins that induce activation, blast-cell transformation, and proliferation in resting lymphocytes. Certain lectins bind specifically to (1) T-cells (i.e., phytohemagglutinin [PHA] and concanavalin A [Con A]), (2) B-cells (i.e., lipopolysaccharide [LPS] of gram-negative bacteria) or (3) both (i.e., pokeweed mitogen [PWM]). The blastogenic response produced can be used to assess changes in cell division of T- and B-lymphocytes. The biological significance of the following studies is difficult to interpret since exposure to lead was either *in vivo* or *in vitro* at different doses and for different exposure periods.

12.8.5.1 In Vivo Exposure. Splenic lymphocytes from Swiss Webster mice exposed orally to 2000 ppm lead for 30 days had significantly depressed proliferative responses to PHA (Table 12-26) which were not observed after 15 days of exposure (Gaworski and Sharma, 1978). Suppression was likewise observed with PWM, a T- and B-cell mitogen. These observations with T-cell mitogens were confirmed in Sprague-Dawley rats exposed orally to 25 or 50 ppm lead pre- and postnatally for seven weeks (Faith et al., 1979). Splenic T-cell responses to Con A and PHA were significantly diminished. A similar depression of Con A and PHA responses occurred

TABLE 12-26. EFFECT OF LEAD EXPOSURE ON MITOGEN ACTIVATION OF LYMPHOCYTES

Species	Lead dose	Mitogen	Effect	Reference
Mice	In vivo, 250 and 2000 ppm, 30 days	PHA (T-Cell)	Significantly depressed at 2000 ppm on day 30 only[1]	Gaworski and Sharma (1978)
		PWM (T and B-Cell)	Significantly depressed at 2000 ppm on both days 15 and 30[1]	
Mice	In vivo, 13, 130 and 1300 ppm, 10 weeks	Con A (T-Cell)	No effect	Koller et al. (1979)
		LPS (B-Cell)	No effect	
Rats	In vivo, pre/postnatal 25 and 50 ppm, 7 weeks	Con A	Significantly depressed at 25 and 50 ppm	Faith et al. (1979)
		PHA	Significantly depressed at 50 ppm only	
Mice	In vivo, .08 - 10 mM, 4 weeks	Con A, PHA	No effect	Lawrence (1981c)
		LPS	Depressed at 2 and 10 mM	
Mice	In vivo, 1300 ppm, 8 weeks	Con A, PHA	Significantly depressed	Neilan et al. (1980)
		LPS	No effect	
Mice	In vivo, 50, 200 and 1000 ppm 3 weeks	Con A, PHA, SEA	Increased to all[2]	Blakley and Archer (1982)
		LPS	No effect	
Mice	In vitro, 10^{-4} - 10^{-6} for full culture period	Con A, PHA	Slightly increased at highest dose at day 2, no effect at day 3.5	Lawrence (1981a,b)
		LPS	Increased up to 245%[1]	
Mice	In vitro, 0.1, 0.5, 1.0 mM for full culture period	PHA	Increased at all doses by up to 453%[3]	Gaworski and Sharma (1978)
		PWM	Increased by approximately 250% at 0.1 and 0.5 mM only	
Mice	In vitro, 10^{-3} - 10^{-7} M	LPS	Increased by up to 312%	Shenker et al. (1977) Gallagher et al. (1979)

1. Difficult to interpret since data were reported only as % of control response rather than CPM of ^{3}H-TdR incorporation.
2. Untreated control values unusually low for T-cell response. Lead treated had much higher response with highest dose showing cytotoxicity.
3. Noted white precipitate thought to be lead carbonate in cultures.

PRELIMINARY DRAFT

in lymphocytes from C57B1/6 mice exposed to 1300 ppm lead for 8 weeks (Neilan et al., 1980). Lead impaired blastogenic transformation of lymphocytes by both T-cell mitogens, although the B-cell proliferative response to LPS was not impaired.

In contrast to reports that lead exposure suppressed the blastogenic response of T-cells to mitogens, several laboratories have reported that lead exposure does not suppress T-cell proliferative responses (Koller et al., 1979; Lawrence, 1981c; Blakley and Archer, 1982). These differences are not easily reconciled since analysis of the lead dose employed and exposure period (Table 12-25) provides little insight into the observed differences in T-cell responses. In one case, a dose of 2000 ppm for 30 days produced a clear depression while a lesser dose of 1300 ppm produced no effect at 10 weeks in another laboratory. These data are confusing and may reflect technical differences in performing the T-cell blastogenesis assay in different laboratories, a lack of careful attention to lectin response kinetics, or the influence of suppressor macrophages. Thus, no firm conclusion can be drawn regarding the ability of in vivo exposure to lead to impair the proliferative capacity of T-cells.

The blastogenic response of B-cells to LPS was unaffected in four different in vivo studies at lead exposure levels from 25 to 1300 ppm (Koller et al., 1979; Faith et al., 1979; Neilan et al., 1980; Blakley and Archer, 1982). Lawrence (1981c), however, reported that the LPS response was suppressed after 4 weeks exposure at 2 and 10 mM lead. The weight of the data suggests that the proliferative response of B-cells to LPS is probably not severely impaired by lead exposure.

12.8.5.2 In Vitro Exposure. The biological relevance of immunological studies in which lead was added in vitro to normal rodent splenocytes in the presence of a mitogen (Table 12-26) is questionable since differences probably reflect either a direct toxic or stimulatory effect by the metal. These models may, however, provide useful information regarding metabolic and functional responses in lymphocytes using lead as a probe.

In one study, lymphocytes were cultured in the presence of lead (10^{-4}, 10^{-5}, and 10^{-6} M). A slight but significant increase in lymphocyte transformation occurred on day 2 at the highest lead dosage when stimulated with Con A or PHA (Lawrence, 1981b). In a follow-up study where the kinetics of the lectin response were examined (Lawrence, 1981a), lead (10^{-4}, 10^{-5}, and 10^{-6} M) significantly suppressed the Con A- and PHA-induced proliferative responses of lymphocytes on day 2, but not on days 3 to 5. In yet another in vitro exposure study, lymphocytes cultured in the presence of 0.1, 0.5, or 1.0 mM lead had a significantly enhanced response to PHA (Gaworski and Sharma, 1978). It should be kept in mind when considering these in vitro exposure observations that lead has been demonstrated to be directly mitogenic to lymphocytes (Shenker et al., 1977). The data discussed here suggest that lead may also be slightly co-mitogenic with T-cell mitogens. Direct exposure of lymphocytes in culture to lead can also result in decreased lymphocyte viability (Gallagher et al., 1979). In vitro studies

on the effect of lead on the B-cell blastogenic response to LPS indicated that lead is potently co-mitogenic with LPS and enhanced the proliferative response of B-cells by 245 percent (Lawrence 1981b,c) to 312 percent (Shenker et al., 1977; Gallagher et al., 1979).

12.8.6 Macrophage Function

The monocyte/macrophage is involved with phagocytosis, bactericidal activity, processing of complex antigens for initiation of antibody production, interferon production, endotoxin detoxification, and immunoregulation. Since some of these functions are altered in lead-treated rodents (Table 12-27), the monocyte/macrophage or comparable phagocytic cell in the liver has been suggested as a possible cellular target for lead (Trejo et al., 1972; Cook et al., 1974; Müller et al., 1977; Luster et al., 1978; Blakley and Archer, 1981).

Several laboratories have shown that a single i.v. injection of lead impaired the phagocytic ability of the reticuloendothelial system (RES) (Trejo et al., 1972; Cook et al., 1974; Filkins and Buchanan, 1973). Trejo et al. (1972) found that an i.v. injection of 5 mg lead impaired vascular clearance of colloidal carbon that resulted from an impaired phagocytic ability of liver Kupffer cells. Similarly, others have confirmed that lead injected i.v. depressed intravascular clearance of colloidal carbon (Filkins and Buchanan, 1973) as well as a radiolabeled lipid emulsion (Cook et al., 1974). Opposite effects on RES function have been seen when lead was given orally (Koller and Roan, 1977). Similarly, Schlick and Friedberg (1981) noted that a 10-day exposure to 10-1000 μg lead enhanced RES clearance and endotoxin hypersensitivity.

Lead has likewise been demonstrated to suppress macrophage-dependent immune responses (Blakley and Archer, 1981). Exposure of BDF_1 mice to lead (50 ppm) for three weeks in drinking water suppressed *in vitro* antibody PFC responses to the macrophage-dependent antigens, sheep red blood cells or dinitrophenyl-Ficoll, but not to the macrophage-independent antigen *E. coli* lipopolysaccharide. The macrophage substitute 2-mercaptoethanol and macrophages from non-exposed mice restored lead-suppressed response. Castranova et al. (1980) found that cultured rat alveolar macrophages exposed to lead had depressed oxidative metabolism.

The effects of heavy metals on endotoxin hypersensitivity were first observed by Selye et al. (1966), who described a 100,000-fold increase in bacterial endotoxin sensitivity in rats given lead acetate. The increased sensitivity to endotoxin was postulated to be due to a blockade of the RES. Filkins (1970) subsequently demonstrated that endotoxin detoxification is primarily a hepatic macrophage-mediated event that is profoundly impaired by lead exposure (Trejo and Di Luzio, 1971; Filkins and Buchanan, 1973). The several types of data described above suggest that macrophage dysfunction may be contributing to impairment of immune function, endotoxin detoxification, and host resistance following lead exposure.

TABLE 12-27. EFFECT OF LEAD ON MACROPHAGE AND RETICULOENDOTHELIAL SYSTEM FUNCTION

Species	Lead dose and exposure	Parameter	Effect	Reference
Rat	2.25 μmol i.v., single injection	Vascular clearance; lipid emulsion endotoxin sensitivity	Depressed Increased	Cook et al. (1974); Trejo et al. (1972)
Rat	5 mg i.v., single injection	Vascular clearance; colloidal carbon endotoxin sensitivity	Depressed Increased	Trejo et al. (1972); Filkins and Buchanan (1973)
Mouse	13, 130, 1300 ppm oral, 10-12 weeks	Phagocytosis	Depressed	Kervliet and Braecher-Steppan (1982)
Guinea Pig	10^{-3}-10^{-6} M	Macrophage migration	Depressed	Kiremidjian-Schumacher et al. (1981)
Rat	10^{-3}-10^{-6} M	Macrophage oxygen metabolism	Depressed	Castranova et al. (1980)
Mouse	50-1000 ppm oral, 3 weeks	Macrophage dependent antigens PFC response	Depressed	Blakley and Archer (1981)
Mouse	10-1000 μg, 10 days	Vascular clearance	Enhanced at 10 days; no effect at >30 days.	Schlick and Friedberg (1981)
		Endotoxin sensitivity	Increased	

PRELIMINARY DRAFT

12.8.7 Mechanisms of Lead Immunomodulation

The mechanism of toxic action of lead on cells is complex (see Section 12.2). Since lead has a high affinity for sulfhydryl groups, a likely subcellular alteration accounting for the immunomodulatory effects of lead on immune cells is its association with cellular thiols. Numerous studies have indicated that surface and intracellular thiols are involved in lymphocyte activation, growth, and differentiation. Furthermore, the study by Blakley and Archer (1981) suggests that lead may inhibit the macrophage's presentation of stimulatory products to the lymphocytes. This process may rely on cellular thiols since the inhibitory effects of lead can be overcome by an exogenous thiol reagent. Goyer and Rhyne (1973) have indicated that lead ions tend to accumulate on cell surfaces, thereby possibly affecting surface receptors and cell-to-cell communication. A study by Koller and Brauner (1977) indicated that lead does alter C3b binding to its cell surface receptor.

12.8.8 Summary

Lead renders animals highly susceptible to endotoxins and infectious agents. Host susceptibility and the humoral immune system appear to be particularly sensitive. As postulated in recent studies, the macrophage may be the primary immune target cell of lead. Lead-induced immunosuppression occurs at low dosages that induce no evident toxicity and, therefore, may be detrimental to the health of animals and perhaps of humans. The data accumulated to date provide good evidence that lead affects immunity, but additional studies are necessary to elucidate the actual mechanism by which lead exerts its immunosuppressive action. Knowledge of lead effects of lead on the immune system of man is lacking and must be properly ascertained in order to determine permissible levels for human exposure. However, since this chemical affects immunity in laboratory animals and is immunosuppressive at very low dosages, its potential serious effects in man should be carefully considered.

12.9 EFFECTS OF LEAD ON OTHER ORGAN SYSTEMS

12.9.1 The Cardiovascular System

Since the best understood pathophysiologic mechanisms of hypertension in humans are those resulting from renal disease, the clinical evidence for a relationship between lead and hypertension is reviewed in Section 12.5.3.5 above. Under conditions of long-term lead exposure at high levels, arteriosclerotic changes have been demonstrated in the kidney. Dingwall-Fordyce and Lane (1963) reported a marked increase in the cerebrovascular mortality rate among heavily exposed lead workers as compared with the expected rate. These workers were exposed to lead during the first quarter of this century when working conditions were quite bad. There has been no similar increase in the mortality rate for men employed in recent times.

There are conflicting reports regarding whether lead can cause atherosclerosis in experimental animals. Scroczynski et al. (1967) observed increased serum lipoprotein and cholesterol levels and cholesterol deposits in the aortas of rats and rabbits receiving large doses of lead. On the other hand, Prerovská (1973), using similar doses of lead given over an even longer period of time, did not produce atherosclerotic lesions in rabbits.

Structural and functional changes have been noted in the myocardium of children with acute lead poisoning, but to date the extent of such studies has been limited. Cases have been described in adults and in children, always with clinical signs of poisoning. There is, of course, the possibility that the coexistence of lead poisoning and myocarditis is coincidental. In many cases in which encephalopathy is present, the electrocardiographic abnormalities disappeared with chelation therapy, suggesting that lead may have been the original etiological factor (Freeman, 1965; Myerson and Eisenhauer, 1963; Silver and Rodriguez-Torres, 1968). Silver and Rodriguez-Torres (1968) noted abnormal electrocardiograms in 21 of 30 children (70 percent) having symptoms of lead toxicity. After chelation therapy, the electrocardiograms remained abnormal in only four (13 percent) of the patients. Electron microscopy of the myocardium of lead-intoxicated rats (Asokan, 1974) and mice (Khan et al., 1977) have shown diffuse degenerative changes. Kopp and coworkers have demonstrated depression of contractility, isoproterenol responsiveness, and cardiac protein phosphorylation (Kopp et al., 1980a), as well as high energy phosphate levels (Kopp et al., 1980b) in hearts of lead-fed rats. Similarly, persistent increased susceptibility to norepinephrine-induced arrhythmias has been observed in rats fed lead during the first three weeks of life (Hejtmancik and Williams, 1977, 1978, 1979a,b; Williams et al., 1977a,b).

In a review of five fatal cases of lead poisoning in young children, degenerative changes in heart muscle were reported to be the proximate cause of death (Kline, 1960). It is not clear that such morphological changes are a specific response to lead intoxication. Kósmider and Petelnz (1962) examined 38 adults over 46 years of age with chronic lead poisoning. They

found that 66 percent had electrocardiographic changes, a rate that was four times the expected rate for that age group.

The cardiovascular effects of lead in conjunction with cadmium have been studied in rats following chronic low level exposure by Perry and coworkers (Perry and Erlanger, 1978; Kopp et al., 1980a,b). Perry and Erlanger (1978) exposed female weanling Long-Evans rats to cadmium, lead, or cadmium plus lead (as acetate salts) at concentrations of 0.1, 1.0, or 5.0 ppm in deionized drinking water for up to 18 months. These authors reported statistically significant increases in systolic blood pressure for both cadmium and lead in the range of 15-20 mm Hg. Concomitant exposure to both cadmium and lead usually doubled the pressor effects of either metal alone. A subsequent study (Kopp et al., 1980a) using weanling female Long-Evans rats exposed to 5.0 ppm cadmium, lead, or lead plus cadmium in deionized drinking water for 15 or 20 months showed similar pressor effects of these two metals alone or in combination on systolic blood pressure. Electrocardiograms performed on these rats demonstrated statistically significant prolongation of the mean PR interval. Bundle electrograms also showed statistically significant prolongations. Other parameters of cardiac function were not markedly affected. Phosphorus-31 nuclear magnetic resonance (NMR) studies conducted on perchloric acid extracts of liquid nitrogen-frozen cardiac tissue from these animals disclosed statistically significant reductions in adenosine triphosphate (ATP) levels and concomitant increases in adenosine diphosphate (ADP) levels. Cardiac glycerol 3-phosphoryl-choline (GPC) were also found to be significantly reduced using this technique, indicating a general reduction of tissue high-energy phosphates by lead or cadmium. Pulse-labeling studies using ^{32}P demonstrated decreased incorporation of this isotope into myosin light-chain (LC-2) in all lead or cadmium treatment groups relative to controls. The results of these studies indicate that prolonged low-dose exposure to lead (and/or cadmium) reduces tissue concentrations of high-energy phosphates in rat hearts and suggest that this effect may be responsible for decreased myosin LC-2 phosphorylation and subsequent reduced cardiac contractility. Other studies by these authors (Kopp et al., 1980b) were also conducted on isolated perfused hearts of weanling female Long-Evans rats exposed to cadmium, lead, or lead plus cadmium in deionized drinking water at concentrations of 50 ppm for 3-15 months. Incorporation of ^{32}P into cardiac proteins was studied following perfusion on inotropic perfusate containing isoproterenol at a concentration of 7×10^{-7} M. Data from these studies showed a statistically significant reduction in cardiac active tension in hearts from cadmium- or lead-treated rats. Phosphorus-32 incorporation was also found to be signficantly reduced in myosin LC-2 proteins. The authors suggested that the observed decrease in LC-2 phosphorylation could be involved in the observed decrease in cardiac active tension in lead- or cadmium-treated rats.

Makasev and Krivdina (1972) observed a two-phase change in the permeability of blood vessels (first increased, then decreased permeability) in rats, rabbits, and dogs that received a solution of lead acetate. A phase change in the content of catecholamines in the myocardium and in the blood vessels was observed in subacute lead poisoning in dogs (Mambeeva and Kobkova, 1969). This effect appears to be a link in the complex mechanism of the cardiovascular pathology of lead poisoning.

The susceptibility of the myocardium to toxic effects of lead was supported by in vitro studies in rat mitochondria by Parr and Harris (1976). These investigators found that the rate of Ca^{2+} removal by rat heart mitochondria is decreased by 1 nmol Pb/mg protein.

12.9.2 The Hepatic System

The effect of lead poisoning on liver function has not been extensively studied. In a study of 301 workers in a lead-smelting and refining facility, Cooper et al. (1973) found an increase in serum glutamic oxaloacetic transaminase (SGOT) activity in 11.5 percent of subjects with blood lead levels below 70 μg/dl, in 20 percent of those with blood lead levels of about 70 μg/dl, and in 50 percent of the workers with blood lead levels of about 100 μg/dl. The correlation ($r = 0.18$) between blood lead levels and SGOT was statistically significant. However, there must also have been exposure to other metals, e.g., cadmium, since there was a zinc plant in the smelter. In lead workers with moderate effects on the hematopoietic system and no obvious renal signs, SGOT was not increased compared with controls on repeated examinations (Hammond et al., 1980). In most studies on lead workers, tests for liver function are not included.

The liver is the major organ for the detoxification of drugs. In Section 12.3.1.3 it is mentioned that exposure to lead may cause altered drug detoxification rates as a result of interference with the formation of heme-containing cytochrome P-450, which is part of the hepatic mixed function oxidase system. This enzyme system is involved in the hepatic biotransformation of medicaments, hormones, and many environmental chemicals (Remmer et al., 1966). Whereas a decrease in drug-metabolizing activity clearly has been demonstrated in experimental animals given large doses of lead resulting in acute toxicity, the evidence for effects of that type in humans is less consistent. Alvares et al. (1975) studied the effect of lead exposure on drug metabolism in children. There were no differences between two normal children and eight children with biochemical signs of lead toxicity in their capacities to metabolize two test drugs, antipyrine and phenylbutazone. In two acutely poisoned children in whom blood levels of lead exceeded 60 μg/dl, antipyrine half-lives were significantly longer than normal, and therapy with EDTA led to biochemical remission of the disease and restoration of deranged drug metabolism toward normal. One of the "normal" children in this

study had a blood lead level of 40 μg/dl, but normal ALA-D and EP values. No data were given on the analytical methods used for indices of lead exposure. Furthermore, the age of the children varied from 1 to 7.5 years, which is significant because, as pointed out by the authors, drug detoxification is age-dependent.

Meredith et al. (1977) demonstrated enhanced hepatic metabolism of antipyrine in lead-exposed workers (PbB: 77-195 μg/dl) following chelation therapy. The significance of this evidence of restored hepatic mixed oxidase function is, however, unclear because the pretreatment antipyrine biologic half-life and clearance were not significantly different in lead-exposed and control subjects. Moreover, there were more heavy smokers among the lead-exposed workers than controls. Smoking increases the drug-metabolizing capacity and may thus counteract the effects of lead. Also, the effect of chelation on antipyrine metabolism in non-exposed control subjects was not determined.

Hepatic drug metabolism in eight adult patients showing marked effects of chronic lead intoxication on the erythropoietic system was studied by Alvares et al. (1976). The plasma elimination rate of antipyrine, which, as noted above, is a drug primarily metabolized by hepatic microsomal enzymes, was determined in eight subjects prior to and following chelation therapy. In seven of eight subjects, chelation therapy shortened the antipyrine half-lives, but the effect was minimal. The authors concluded that chronic lead exposure results in significant inhibition of the heme biosynthetic pathway without causing significant changes in enzymatic activities associated with hepatic cytochrome P-450.

A confounding factor in the above three studies may be that treatment with EDTA causes an increase in the glomerular filtration rate (GFR) if it has been reduced by lead (Section 12.5.3.3). This may cause a decrease in the half-times of drugs. There are, however, no data on the effect of chelating agents on GFR in children or adults with moderate signs of lead toxicity.

In 11 children with blood lead levels between 43 and 52 μg/dl, Saenger et al. (1981) found a decrease in 24-hour urinary 6-beta-hydroxycortisol excretion that correlated closely ($r = 0.85$, $p < 0.001$) with a standardized EDTA lead-mobilization test (1000 mg EDTA/m^2 body surface area). This glucocorticoid metabolite is produced by the same hepatic microsomal mixed function oxidase system that hydroxylates antipyrine. The authors suggest that the depression of 6-beta-hydroxylation of cortisol in the liver may provide a non-invasive method for assessing body lead stores in children (Saenger et al., 1981).

In a few animal studies special attention has been paid to morphological effects of lead on the liver. White (1977) gave eight beagle dogs oral doses of lead carbonate, 50-100 mg Pb/kg b.w., for 3-7 weeks. Lead concentrations were not measured in blood or tissues. In two dogs exposed from 5 weeks of age to 50 mg/kg, morphological changes were noted. Changes in

enzyme activities were noted in most exposed animals; for example, some dehydrogenases showed increased activity after short exposure and decreased activity after longer exposures, mainly in animals with weight losses. The small number of animals and the absence of data on lead concentrations makes it impossible to use these results for risk evaluations.

Hoffmann et al. (1974) noted moderate to marked morphological changes in baboon livers after a single intravenous injection of large doses of lead acetate (25 mg/kg b.w.). It can be concluded that effects on the liver may be expected to occur only at high exposure levels. If effects on more sensitive systems, viz., the nervous and hematopoietic systems, are prevented, no adverse effects should be noted in the liver.

12.9.3 The Endocrine System

The effects of lead on the endocrine or hormonal system are not well defined at the present time, but some evidence exists for such effects, at least at high levels of lead exposure. Lead is thought, for example, to decrease thyroid function in man and experimental animals. Porritt (1931) suggested that lead dissolved from lead pipes by soft water was the cause of hypothyroidism in individuals living in southwest England. Later, Kremer and Frank (1955) reported the simultaneous occurrence of myxedema and plumbism in a house painter. Monaenkova (1957) observed impaired concentration of ^{131}I by thyroid glands in 10 of 41 patients with industrial plumbism. Subsequently, Zel'tser (1962) showed that *in vivo* ^{131}I uptake and thyroxine synthesis by rat thyroid were decreased by lead when doses of 2 and 5 percent lead acetate solution were administered. Uptake of ^{131}I, sometimes decreased in men with lead poisoning, can be offset by treatment with thyroid-stimulating hormone (TSH) (Sandstead et al., 1969; Sandstead and Galloway, 1967). Lead may act to depress thyroid function by inhibiting thiol groups or by displacing iodine in a protein sulfonyl iodine carrier (Sandstead and Galloway, 1967), and the results suggest that excessive lead may act at both the pituitary and the thyroid gland itself to impair thyroid function. None of these effects on the thyroid system, however, have been demonstrated to occur in humans at blood lead levels below 30-40 μg/dl.

Sandstead et al. (1970a) studied the effects of lead intoxication on pituitary and adrenal function in man and found that it may produce clinically significant hypopituitarism in some. The effects of lead on adrenal function were less consistent, but some of the patients showed a decreased responsiveness to an inhibitor (metapyrone) of 11-beta-hydroxylation in the synthesis of cortisol. This suggests a possible impact of lead on pituitary-adrenal hormonal functions. That excessive oral ingestion of lead may in fact result in pathological changes in the pituitary-adrenal axis is also supported by other reports of lead-induced decreased metapyrone responsiveness, a depressed pituitary reserve, and decreased immunoreactive ACTH

(Murashov, 1966; Pines, 1965). These same events may also affect adrenal gland function as much as decreased urinary excretion of 17-hydroxy-corticosteroids was observed in these patients. Also, suppression of responsiveness to exogenous ACTH in the zona fasciculata of the adrenal cortex has been reported in lead-poisoned subjects (Makotchenko, 1965), and impairment of the zona glomerulosa of the adrenal cortex has also been suggested (Sandstead et al., 1970b). Once again, however, none of these effects on adrenal hormone function have been shown to occur at blood lead levels as low as 30-40 μg/dl.

Other studies provide evidence suggestive of lead exposure effects on endocrine systems controlling reproductive functions (see also Section 12.6). For example, evidence of abnormal luteinizing hormone (LH) secretory dynamics was found in secondary lead smelter workers (Braunstein et al., 1978). Reduced basal serum testosterone levels with normal basal LH levels but a diminished rise in LH following stimulation indicated suppression of hypothalamic-pituitary function. Testicular biopsies in two lead-poisoned workmen showed peritubular fibrosis suggesting direct toxic effects of lead in the testes as well as effects at the hypothalamic-pituitary level. Lancranjan et al. (1975) also reported lead-related interference with male reproductive functions. Moderately increased lead absorption (blood lead mean = 52.8 μg/dl) among a group of 150 workmen who had long-term exposure to lead in varying degrees was said to result in gonadal impairment. The effects on the testes were believed to be direct, however, in that tests for hypothalamic-pituitary influence were negative.

In regard to effects of lead on ovarian function in human females, Panova (1972) reported a study of 140 women working in a printing plant for 1 to 2 months, where ambient air lead levels were <7 μg/m^3. Using a classification of various age groups (20-25, 26-35, and 36-40 yr) and type of ovarian cycle (normal, anovular, and disturbed lutein phase), Panova claimed that statistically significant differences existed between the lead-exposed and control groups in the age range 20-25 years. It should be noted that the report does not show the age distribution, the level of significance, or the data on specificity of the method used for classification. Also, Zielhuis and Wibowo (1976), in a critical review of the above study, concluded that the design of the study and presentation of data are such that it is difficult to evaluate the author's conclusion that chronic exposure to low air lead levels leads to disturbed ovarian function. Moreover, no consideration was given to the dust levels of lead, an important factor in print shops. Unfortunately, little else besides the above report exists in the literature in regard to assessing lead effects on human ovarian function or other factors affecting human female fertility. Studies offering firm data on maternal variables, e.g., hormonal state, that are known to affect the ability of the pregnant woman to carry the fetus full-term are also lacking, although certain studies do indicate that at least high-level lead exposure induces stillbirths and abortions (see Section 12.6).

One animal study (Petrusz et al., 1979) indicates that orally administered lead can exert effects on pituitary and serum gonadotropins, which may represent one mechanism by which lead affects reproductive functions. The blood lead levels at which alterations in serum and pituitary follicle stimulating hormone were observed in neonatal rats, however, were well in excess of 100 μg/dl.

12.9.4 The Gastrointestinal System

Colic is usually a consistent early symptom of lead poisoning, warning of much more serious effects that are likely to occur with continued or more intense lead exposure. Although most commonly seen in industrial exposure cases, colic is also a lead-poisoning symptom present in infants and young children.

Beritic (1971) examined 64 men suffering from abdominal colic due to lead intoxication through occupational exposure. The diagnosis of lead colic was based on the occurrence of severe attacks of spasmodic abdominal pain accompanied by constipation, abnormally high coproporphyrinuria, excessive basophilic stippling, reticulocytosis, and some degree of anemia (all clinical signs of lead poisoning). Thirteen of the 64 patients had blood lead levels of 40-80 μg/dl upon admission. However, the report did not indicate how recently the patients' exposures had been terminated or provide other details of their exposure histories.

A more recent report by Dahlgren (1978) focused on the gastrointestinal symptoms of lead smelter workers whose blood lead levels were determined within two weeks of the termination of their work exposure. Of 34 workers with known lead exposure, 27 (79 percent) complained of abdominal pain, abnormal bowel movements, and nausea. Fifteen of the 27 had abdominal pain for more than 3 months after removal from the exposure to lead. The mean (and SD) blood lead concentration for this group of 15 was 70 (± 4) μg/dl. There was, however, no correlation between severity of symptoms and blood lead levels, as those experiencing stomach pain for less than 3 months averaged 68 (± 9) μg/dl and the remaining 7 workers, reporting no pain at all, averaged 76 (± 9) μg/dl.

Hänninen et al. (1979) assessed the incidence of gastrointestinal symptoms in 45 workers whose blood lead levels had been regularly monitored throughout their exposure and had never exceded 69 μg/dl. A significant association between gastrointestinal symptoms (particularly epigastric pain) and blood lead level was reported. This association was more pronounced in subjects whose maximal blood lead levels had reached 50-69 μg/dl, but was also noted in those whose blood lead levels were below 50 μg/dl.

Other occupational studies have also suggested a relationship between lead exposure and gastrointestinal symptoms (Lilis et al., 1977; Irwig et al., 1978; Fischbein et al., 1979, 1980). For demonstrating such a relationship, however, the most useful measure of internal

exposure has not necessarily been blood lead concentrations. Fischbein et al. (1980) surveyed a cross-section of New York City telephone cable splicers exposed to lead in the process of soldering cables. Of the 90 workers evaluated, 19 (21 percent) reported gastrointestinal symptoms related to lead colic. The difference between mean blood lead levels in those reporting GI symptoms and those not reporting such symptoms (30 vs. 27 μg/dl) was not statistically significant. However, mean zinc protoporphyrin concentrations (67 vs. 52 μg/dl) were significantly different ($p < 0.02$)

Although gastrointestinal symptoms of lead exposure are clinically evident in frank lead intoxication and may even be present when blood lead levels approach the 30-80 μg/dl range, there is currently insufficient information to establish a clear dose-effect relationship for the general population at ambient exposure levels.

12.10 CHAPTER SUMMARY

12.10.1 Introduction

Lead has diverse biological effects in humans and animals. Its effects are seen at the subcellular level of organellar structures and processes as well as at the overall level of general functioning that encompasses all systems of the body operating in a coordinated, interdependent fashion. The present chapter not only categorizes and describes the various biological effects of lead but also attempts to identify the exposure levels at which such effects occur and the mechanisms underlying them. The dose-response curve for the entire range of biological effects exerted by lead is rather broad, with certain biochemical changes occurring at relatively low levels of exposure and perturbations in other systems, such as the endocrine, becoming detectable only at relatively high exposure levels. In terms of relative vulnerability to deleterious effects of lead, the developing organism generally appears to be more sensitive than the mature individual. A more detailed and quantitative examination of overall exposure-effect relationships for lead is presented in Chapter 13.

12.10.2 Subcellular Effects of Lead

The biological basis of lead toxicity is its ability to bind to ligating groups in biomolecular substances crucial to various physiological functions, thereby interfering with these functions by, for example, competing with native essential metals for binding sites, inhibiting enzyme activity, and inhibiting or otherwise altering essential ion transport. These effects are modulated by: 1) the inherent stability of such binding sites for lead; 2) the compartmentalization kinetics governing lead distribution among body compartments, among tissues, and within cells; and 3) the differences in biochemical organization across cells and

tissues due to their specific functions. Given the complexities introduced by items 2 and 3, it is not surprising that no single unifying mechanism of lead toxicity across all tissues in humans and experimental animals has yet been demonstrated.

In so far as effects of lead on activity of various enzymes are concerned, many of the available studies concern in vitro behavior of relatively pure enzymes with marginal relevance to various effects in vivo. On the other hand, certain enzymes are basic to the effects of lead at the organ or organ system level, and discussion is best reserved for such effects in the summary sections below dealing with lead effects on particular organ systems. This section is mainly concerned with organellar effects of lead, expecially those which provide some rationale for lead toxicity at higher levels of biological organization. Particular emphasis is placed on the mitochondrion, because this organelle is not only affected by lead in numerous ways but has also provided the most data bearing on the subcellular effects of lead.

The critical target organelle for lead toxicity in a variety of cell and tissue types clearly is the mitochondrion, followed probably by cellular and intracellular membranes. The mitochondrial effects take the form of structural changes and marked disturbances in mitochondrial function within the cell, particularly in energy metabolism and ion transport. These effects in turn are associated with demonstrable accumulation of lead in mitochondria, both in vivo and in vitro. Structural changes include mitochondrial swelling in a variety of cell types as well as distortion and loss of cristae, which occur at relatively moderate lead levels. Similar changes have also been documented in lead workers across a range of exposures.

Uncoupled energy metabolism, inhibited cellular respiration using both succinate and nicotinamide adenine dinucleotide (NAD)-linked substrates, and altered kinetics of intracellular calcium have been demonstrated in vivo using mitochondria of brain and non-neural tissue. In some cases, the lead exposure level associated with such changes has been relatively low. Several studies document the relatively greater sensitivity of this organelle in young vs. adult animals in terms of mitochondrial respiration. The cerebellum appears to be particularly sensitive, providing a connection between mitochondrial impairment and lead encephalopathy. Impairment by lead of mitochondrial function in the developing brain has also been consistently associated with delayed brain development, as indexed by content of various cytochromes. In the rat pup, ongoing lead exposure from birth is required for this effect to be expressed, indicating that such exposure must occur before, and is inhibitory to, the burst of oxidative metabolism activity that occurs in the young rat at 10 to 21 days postnatally.

In vivo lead exposure of adult rats also markedly inhibits cerebral cortex intracellular calcium turnover in a cellular compartment that appears to be the mitochondrion. The effect was seen at a brain lead level of 0.4 ppm. These results are consistent with a separate study showing increased retention of calcium in the brain of lead-dosed guinea pigs. Numerous

reports have described the in vivo accumulation of lead in mitochondria of kidney, liver, spleen, and brain tissue, with one study showing that such uptake was slightly more than occurred in the cell nucleus. These data are not only consistent with deleterious effects of lead on mitochondria but are also supported by other investigations in vitro.

Significant decreases in mitochondrial respiration in vitro using both NAD-linked and succinate substrates have been observed for brain and non-neural tissue mitochondria in the presence of lead at micromolar levels. There appears to be substrate specificity in the inhibition of respiration across different tissues, which may be a factor in differential organ toxicity. Also, a number of enzymes involved in intermediary metabolism in isolated mitochondria have been observed to undergo significant inhibition of activity with lead.

Of particular interest regarding lead effects on isolated mitochondria are ion transport effects, especially in regard to calcium. Lead movement into brain and other tissue mitochondria involves active transport, as does calcium. Recent sophisticated kinetic analyses of desaturation curves for radiolabeled lead or calcium indicate that there is striking overlap in the cellular metabolism of calcium and lead. These studies not only establish the basis of lead's easy entry into cells and cell compartments, but also provide a basis for lead's impairment of intracellular ion transport, particularly in neural cell mitochondria, where the capacity for calcium transport is 20-fold higher than even in heart mitochondria.

Lead is also selectively taken up in isolated mitochondria in vitro, including the mitochondria of synaptosomes and brain capillaries. Given the diverse and extensive evidence of lead's impairment of mitochondrial structure and function as viewed from a subcellular level, it is not surprising that these derangements are logically held to be the basis of dysfunction of heme biosynthesis, erythropoiesis, and the central nervous system. Several key enzymes in the heme biosynthetic pathway are intramitochondrial, particularly ferrochelatase. Hence, it is to be expected that entry of lead into mitochondria will impair overall heme biosynthesis, and in fact this appears to be the case in the developing cerebellum. Furthermore, lead levels associated with entry of lead into mitochondria and expression of mitochondrial injury can be relatively moderate.

Lead exposure provokes a typical cellular reaction in human and other species that has been morphologically characterized as a lead-containing nuclear inclusion body. While it has been postulated that such inclusions constitute a cellular protection mechanism, such a mechanism is an imperfect one. Other organelles, e.g., the mitochondrion, also take up lead and sustain injury in the presence of nuclear inclusion formations.

In theory, the cell membrane is the first organelle to encounter lead and it is not surprising that cellular effects of lead can be ascribed to interactions at cellular and intracellular membranes in the form of distrubed ion transport. The inhibition of membrane

(Na^+,K^+)-ATPase of erythrocytes as a factor in lead-impaired erythropoiesis is noted elsewhere. Lead also appears to interfere with the normal processes of calcium transport across membranes of different tissues. In peripheral cholinergic synaptosomes, lead is associated with retarded release of acetylcholine owing to a blockade of calcium binding to the membrane, while calcium accumulation within nerve endings can be ascribed to inhibition of membrane (Na^+,K^+)-ATPase.

Lysosomes accumulate in renal proximal convoluted tubule cells of rats and rabbits given lead over a range of dosing. This also appears to occur in the kidneys of lead workers and seems to represent a disturbance in normal lysosomal function, with the accumulation of lysosomes being due to enhanced degradation of proteins because of the effects of lead elsewhere within the cell.

12.10.3. Effects of Lead on Heme Biosynthesis, Erythropoiesis, and Erythrocyte Physiology in Humans and Animals

The effects of lead on heme biosynthesis are well known both because of their prominence and the large number of studies of these effects in humans and experimental animals. The process of heme biosynthesis starts with glycine and succinyl-coenzyme A, proceeds through formation of protoporphyrin IX, and culminates with the insertion of divalent iron into the porphyrin ring, thus forming heme. In addition to being a constituent of hemoglobin, heme is the prosthetic group of numerous tissue hemoproteins having variable functions, such as myoglobin, the P-450 component of the mixed function oxygenase system, and the cytochromes of cellular energetics. Hence, disturbance of heme biosynthesis by lead poses the potential for multiple-organ toxicity.

The steps in the heme synthesis pathway that have been best studied in regard to lead effects involve three enzymes: (1) stimulation of mitochondrial delta-aminolevulinic acid synthetase (ALA-S), which mediates formation of delta-aminolevulinic acid (ALA); (2) direct inhibition of the cytosolic enzyme, delta-aminolevulinic acid dehydrase (ALA-D), which catalyzes formation of porphobilinogen from two units of ALA; and (3) inhibition of insertion of iron (II) into protoporphyrin IX to form heme, a process mediated by the enzyme ferrochelatase.

Increased ALA-S activity has been documented in lead workers as well as lead-exposed animals, although the converse, an actual decrease in enzyme activity, has also been observed in several experimental studies using different exposure methods. It would appear, then, that enzyme activity increase via feedback derepression or that activity inhibition may depend on the nature of the exposure. In an *in vitro* study using rat liver cells in culture, ALA-S activity could be stimulated at levels as low as 5.0 μM or 1.0 μg Pb/g preparation. In the same study, increased activity was seen to be due to biosynthesis of more enzyme. The threshold for lead stimulation of ALA-S activity in humans, based upon a study using leukocytes

from lead workers, appears to be about 40 μg Pb/dl. The generality of this threshold level to other tissues is dependent upon how well the sensitivity of leukocyte mitochondria mirrors that in other systems. It would appear that the relative impact of ALA-S activity stimulation on ALA accumulation at lower levels of lead exposure is considerably less than the effect of ALA-D activity inhibition: at 40 μg/dl blood lead, ALA-D activity is significantly depressed, while ALA-S activity only begins to be affected.

Erythrocyte ALA-D activity is very sensitive to lead inhibition, which is reversed by reactivation of the sulfhydryl group with agents such as dithiothreitol, zinc, or zinc plus glutathione. The zinc levels employed to achieve reactivation, however, are well above normal physiological levels. Although zinc appears to offset the inhibitory effects of lead observed in human erythrocytes in vitro and in animal studies, lead workers exposed to both zinc and lead do not show significant changes in the relationship of ALA-D activity to blood lead concentration when compared to workers exposed only to lead. By contrast, zinc deficiency in animals has been shown to significantly inhibit ALA-D activity, with concomitant accumulation of ALA in urine. Since zinc deficiency has also been associated with increased lead absorption in experimental studies, the possibility exists for a dual effect of such deficiency on ALA-D activity: (1) a direct effect on activity due to reduced zinc availability, as well as (2) the effect of increased lead absorption leading to further inhibition of such activity.

The activity of erythrocyte ALA-D appears to be inhibited at virtually all blood lead levels measured so far, and any threshold for this effect in either adults or children remains to be determined. A further measure of this enzyme's sensitivity to lead comes from a report noting that rat bone marrow suspensions show inhibition of ALA-D activity by lead at a level of 0.1 μg/g suspension. Inhibition of ALA-D activity in erythrocytes apparently reflects a similar effect in other tissues. Hepatic ALA-D activity was inversely correlated in lead workers with both the erythrocyte activity as well as blood lead. Of significance are the experimental animal data showing that (1) brain ALA-D activity is inhibited with lead exposure and (2) inhibition appears to occur to a greater extent in the brain of developing vs. adult animals. This presumably reflects greater retention of lead in developing animals. In the avian brain, cerebellar ALA-D activity is affected to a greater extent than that of the cerebrum and, relative to lead concentration, shows inhibition approaching that occurring in erythrocytes.

The inhibition of ALA-D activity by lead is reflected in increased levels of its substrate, ALA, in blood, urine, and tissues. In one investigation, the increase in urinary ALA was seen to be preceded by a rise in circulating levels of the metabolite. Blood ALA levels were elevated at all corresponding blood lead values down to the lowest value determined (18 μg/dl), while urinary ALA was seen to rise exponentially with blood ALA. Numerous independent

studies have documented that there is a direct correlation between blood lead and the logarithm of urinary ALA in adult humans and children, and that the threshold is commonly accepted as being 40 μg/dl. Several studies of lead workers also indicate that the correlation of urinary ALA with blood lead continues below this value. Furthermore, one report has demonstrated that the slope of the dose-effect curve in lead workers is dependent upon the level of exposure.

The health significance of lead-inhibited ALA-D activity and accumulation of ALA at low levels of exposure has been an issue of some controversy. One view is that the "reserve capacity" of ALA-D activity is such that only high accumulations of the enzyme's substrate, ALA, in accessible indicator media would result in significant inhibition of activity. One difficulty with this view is that it is not possible to quantify at lower levels of lead exposure the relationship of urinary ALA to levels in target tissues nor to relate the potential neurotoxicity of ALA at any level of build-up to levels in indicator media; i.e., the threshold for potential neurotoxicity of ALA in terms of blood lead may be different from the level associated with urinary accumulation.

Accumulation of protoporphyrin in the erythrocytes of individuals with lead intoxication has been recognized since the 1930s, but it has only recently been possible to quantitatively assess the nature of this effect via the development of specific, sensitive microanalysis methods. Accumulation of protoporphyrin IX in erythrocytes is the result of impaired placement of iron (II) in the porphyrin moiety to form heme, an intramitochondrial process mediated by the enzyme ferrochelatase. In lead exposure, the porphyrin acquires a zinc ion in lieu of native iron, thus forming zinc protoporphyrin (ZPP), and is tightly bound in available heme pockets for the life of the erythrocytes. This tight sequestration contrasts with the relatively mobile non-metal, or free, erythrocyte protoporphyrin (FEP) accumulated in the congenital disorder erythropoietic protoporphyria.

Elevation of erythrocyte ZPP has been extensively documented as being exponentially correlated with blood lead in children and adult lead workers and is presently considered one of the best indicators of undue lead exposure. Accumulation of ZPP only occurs in erythrocytes formed during lead's presence in erythroid tissue, resulting in a lag of at least several weeks before such build-up can be measured. It has been shown that the level of such accumulation in erythrocytes of newly-employed lead workers continues to increase when blood lead has already reached a plateau. This would influence the relative correlation of ZPP and blood lead in workers with a short exposure history. In individuals removed from occupational exposure, the ZPP level in blood declines much more slowly than blood lead, even years after removal from exposure or after a drop in blood lead. Hence, ZPP level would appear to be a more reliable indicator of continuing intoxication from lead resorbed from bone.

The measurable threshold for lead-induced ZPP accumulation is affected by the relative spread of blood lead and corresponding ZPP values measured. In young children (under four years old) the ZPP elevation typically associated with iron-deficiency anemia should be taken into account. In adults, several studies indicate that the threshold for ZPP elevation with respect to blood lead is approximately 25-30 μg/dl. In children 10-15 years old the threshold is about 16 μg/dl; in this age group, iron deficiency is not a factor. In one report, it was noted that children over four years old showed the same threshold, 15.5 μg/dl, as a second group under four years old, indicating that iron deficiency was not a factor in the study. Fifty percent of the children were found to have significantly elevated EP levels (2 standard deviations above reference mean EP) or a dose-response threshold level of 25 μg/dl.

Within the blood lead range considered "normal," i.e., below 30-40 μg/dl, any assessment of the ZPP-blood lead relationship is strongly influenced by the relative analytical proficiency for measurement of both blood lead and EP. The types of statistical treatments employed in analyzing the data are also important. In a recent detailed statistical study involving 2004 children, 1852 of whom had blood lead values below 30 μg/dl, segmental line and probit analysis techniques were employed to assess the dose-effect threshold and dose-response relationship. An average blood lead threshold for the effect using both statistical techniques yielded a value of 16.5 μg/dl for either the full group or those subjects with blood lead levels below 30 μg/dl. The effect of iron deficiency was tested for and removed. Of particular interest was the finding that the blood lead values corresponding to EP elevations more than 1 or 2 standard deviations above the reference mean in 50 percent of the children were 28.6 or 35.7 μg Pb/dl, respectively. Hence, fully half of the children were seen to have significant elevations of EP at blood lead levels around the currently accepted cut-off value for undue lead exposure, 30 μg/dl. From various reports, children and adult females appear to be more sensitive to the effects of lead on EP accumulation at any given blood lead level, with children being somewhat more sensitive than adult females.

Effects of lead on ZPP accumulation and reduced heme formation are not restricted to the erythropoietic system. Recent studies show that reduction of serum 1,25-dihydroxy vitamin D seen with even low level lead exposure is apparently the result of lead's inhibition of the activity of renal 1-hydroxylase, a cytochrome P-450 mediated enzyme. Cytochrome P-450, a heme-containing protein, is an integral part of the hepatic mixed function oxygenase system and is known to be affected in humans and animals by lead exposure, particularly acute intoxication. Reduced P-450 content has been found to be correlated with impaired activity of such detoxifying enzyme systems as aniline hydroxylase and aminopyrine demethylase.

Studies of organotypic chick dorsal root ganglion in culture show that the nervous system not only has heme biosynthetic capability but also such preparations elaborate porphyrinic material in the presence of lead. In the neonatal rat, chronic lead exposure resulting in

moderately elevated blood lead levels is associated with retarded growth in the hemoprotein cytochrome C and with disturbed electron transport in the developing rat cerebral cortex. These data parallel the effect of lead on ALA-D activity and ALA accumulation in neural tissue. When these effects are viewed in the toxicokinetic context of increased retention of lead in both developing animals and children, there is an obvious, serious potential for impaired heme-based metabolic function in the nervous system of lead-exposed children.

As can be seen from the above discussion, the health significance of ZPP accumulation rests with the fact that such build-up is evidence of impaired heme and hemoprotein formation in tissues, particularly the nervous system, arising from entry of lead into mitochondria. Such evidence for reduced heme synthesis is consistent with a diverse body of data documenting lead-associated effects on mitochondria, including impairment of ferrochelatase activity. As a mitochondrial enzyme, ferrochelatase activity may be inhibited either directly by lead or indirectly by impairment of iron transport to the enzyme.

The relative value of the lead-ZPP relationship in erythropoietic tissue as an index of this effect in other tissues hinges on the relative sensitivity of the erythropoietic system compared with other systems. For example, one study of rats exposed to low levels of lead over their lifetime demonstrated that protoporphyrin accumulation in renal tissue was already significant at levels of lead exposure where little change was seen in erythrocyte porphyrin levels. The issue of sensitivity is obviously distinct from the question of which system is most accessible to measurement of the effect.

Other steps in the heme biosynthesis pathway are also known to be affected by lead, although these have not been studied as much on a biochemical or molecular level. Levels of coproporphyrin are increased in urine, reflecting active lead intoxication. Lead also affects the activity of the enzyme uroporphyrinogen-I-synthetase, resulting in an accumulation of its substrate, porphobilinogen. The erythrocyte enzyme is much more sensitive to lead than the hepatic species and presumably accounts for much of the accumulated substrate.

Anemia is a manifestation of chronic lead intoxication, being characterized as mildly hypochromic and usually normocytic. It is associated with reticulocytosis, owing to shortened cell survival, and the variable presence of basophilic stippling. Its occurrence is due to both decreased production and increased rate of destruction of erythrocytes. In children under four years old, the anemia of iron deficiency is exacerbated by lead, and vice versa. Hemoglobin production is negatively correlated with blood lead levels in young children, where iron deficiency may be a confounding factor, as well as in lead workers. In one study, blood lead values that were usually below 80 μg/dl were inversely correlated with hemoglobin content. In these subjects, iron deficiency was found to be absent. The blood lead threshold for reduced hemoglobin content is about 50 μg/dl in adult lead workers and somewhat lower in children, around 40 μg/dl.

The mechanism of lead-associated anemia appears to be a combination of reduced hemoglobin production and shortened erythrocyte survival because of direct cell injury. Effects of lead on hemoglobin production involve disturbances of both heme and globin biosynthesis. The hemolytic component to lead-induced anemia appears to be due to increased cell fragility and increased osmotic resistance. In one study using rats, it was noted that the reduced cell deformability and consequent hemolysis associated with vitamin E deficiency is exacerbated by lead exposure. The molecular basis for increased cell destruction rests with inhibition of (Na^+, K^+)-ATPase and pyrimidine-5'-nucleotidase. Inhibition of the former enzyme leads to cell "shrinkage," and inhibition of the latter results in impaired pyrimidine nucleotide phosphorolysis and disturbance of the activity of the purine nucleotides necessary for cellular energetics.

Tetraethyl lead and tetramethyl lead, components of leaded gasoline, undergo transformation *in vivo* to the neurotoxic trialkyl metabolites as well as further conversion to inorganic lead. Hence, one might anticipate that exposure to such agents may show effects commonly associated with inorganic lead in terms of heme synthesis and erythropoiesis. Various surveys and case reports make it clear that leaded-gasoline sniffing is associated with chronic lead intoxication in children from socially deprived backgrounds in rural or remote areas. Notable in these subjects is evidence of impaired heme biosynthesis as indexed by significantly reduced ALA-D activity. In several case reports of frank lead toxicity from habitual sniffing of leaded gasoline, such effects as basophilic stippling in erythrocytes and significantly reduced hemoglobin have also been noted.

Lead-associated disturbances of heme biosynthesis as a possible factor underlying neurological effects of lead are of considerable interest because of (1) the recognized similarity between the classical signs of lead neurotoxicity and numerous neurological components of the congenital disorder known as acute intermittent porphyria, as well as (2) some unusual aspects of lead neurotoxicity. There are two possible points of connection between lead effects on both heme biosynthesis and the nervous system. Concerning the similarity of lead neurotoxicity to acute intermittent porphyria, there is the common feature of excessive systemic accumulation and excretion of ALA. Second, lead neurotoxicity reflects, to some degree, impaired synthesis of heme and hemoproteins involved in crucial cellular functions. Available information indicates that ALA levels are elevated in the brain of lead-exposed animals, arising via *in situ* inhibition of brain ALA-D activity or via transport to the brain after formation in other tissues. ALA is known to traverse the blood-brain barrier. Hence, ALA is accessible to, or formed within, the brain during lead exposure and may express its neurotoxic potential.

Based on various *in vitro* and *in vivo* data obtained in the context of neurochemical studies of lead neurotoxicity, it appears that ALA can readily affect GABAergic function, particularly inhibiting release of the neurotransmitter GABA from presynaptic receptors, where

ALA appears to be very potent even at low levels. In an in vitro study, agonist behavior by ALA was demonstrated at levels as low as 1.0 μM ALA. This in vitro observation supports results of a study using lead-exposed rats in which there was reported inhibition of both resting and K^+-stimulated preloaded ^{3}H-GABA. Further evidence for an effect of some agent other than lead acting directly is the observation that in vivo effects of lead on neurotransmitter function cannot be duplicated with in vitro preparations to which lead is added. Human data on lead-induced associations between disturbed heme synthesis and neurotoxicity, while limited, also suggest that ALA may function as a neurotoxicant.

The connection between impaired heme and hemoprotein synthesis in the brain of the neonatal rat was noted earlier. In these studies there was reduced cytochrome C production and impaired operation of the cytochrome C respiratory chain. Hence, one might expect that such impairment would be most prominent in areas of relatively greater cellularization, such as the hippocampus. As noted in Chapter 10, these are also regions where selective lead accumulation appears to occur.

12.10.4 Neurotoxic Effects of Lead

An assessment of the impact of lead on human and animal neurobehavioral function raises a number of issues. Among the key points addressed here are: (1) the internal exposure levels, as indexed by blood lead levels, at which various neurotoxic effects occur; (2) the persistence or reversibility of such effects; and (3) populations that appear to be most susceptible to neural damage. In addition, the question arises as to the utility of using animal studies to draw parallels to the human condition.

12.10.4.1 Internal Lead Levels at which Neurotoxic Effects Occur. Markedly elevated blood lead levels are associated with the most serious neurotoxic effects of lead exposure (including severe, irreversible brain damage as indexed by the occurrence of acute or chronic encephalopathic symptoms, or both) in both humans and animals. For most adult humans, such damage typically does not occur until blood lead levels exceed 120 μg/dl. Evidence does exist, however, for acute encephalopathy and death occurring in some human adults at blood lead levels below 120 μg/dl. In children, the effective blood lead level for producing encephalopathy or death is lower, starting at approximately 80-100 μg/dl. It should be emphasized that, once encephalopathy occurs, death is not an improbable outcome, regardless of the quality of medical treatment available at the time of acute crisis. In fact, certain diagnostic or treatment procedures themselves may exacerbate matters and push the outcome toward fatality if the nature and severity of the problem are not diagnosed or fully recognized. It is also crucial to note the rapidity with which acute encephalopathic symptoms can develop or death can occur in apparently asymptomatic individuals or in those apparently only

mildly affected by elevated lead body burdens. Rapid deterioration often occurs, with convulsions or coma suddenly appearing with progression to death within 48 hours. This strongly suggests that even in apparently asymptomatic individuals, rather severe neural damage probably exists at high blood lead levels even though it is not yet overtly manifested in obvious encephalopathic symptoms. This conclusion is further supported by numerous studies showing that overtly lead intoxicated children with high blood lead levels, but not observed to manifest acute encephalopathic symptoms, are permanently cognitively impaired, as are most children who survive acute episodes of frank lead encephalopathy.

Recent studies show that overt signs and symptoms of neurotoxicity (indicative of both CNS and peripheral nerve dysfunction) are detectable in some human adults at blood lead levels as low as 40-60 μg/dl, levels well below the 60 or 80 μg/dl criteria previously discussed as being "safe" for adult lead exposures. In addition, certain electrophysiological studies of peripheral nerve function in lead workers, indicate that slowing of nerve conduction velocities in some peripheral nerves are associated with blood lead levels as low as 30-50 μg/dl (with no clear threshold for the effect being evident). These results are indicative of neurological dysfunctions occurring at relatively low lead levels in non-overtly lead intoxicated adults.

Other evidence tends to confirm that neural dysfunctions exist in apparently asymptomatic children, at similar or even lower levels of blood lead. The body of studies on low-or moderate-level lead effects on neurobehavioral functions in non-overtly lead intoxicated children, as summarized in Table 12-1, presents an array of data pointing to that conclusion. Several well-controlled studies have found effects that are clearly statistically significant, whereas other have found nonsignificant but borderline effects. Even some studies reporting generally nonsignificant findings at times contain data confirming some statistically significant effects, which the authors attribute to various extraneous factors. It should also be noted that, given the apparent nonspecific nature of some of the behavioral or neural effects probable at low levels of lead exposure, one would not expect to find striking differences in every instance. The lowest observed blood lead levels associated with significant neurobehavioral deficits indicative of CNS dysfunction, both in apparently asymptomatic children and in developing rats and monkeys generally appear to be in the range of 30-50 μg/dl. However, other types of neurotoxic effects, e.g., altered EEG patterns, have been reported at lower levels, supporting a continuous dose-response relationship between lead and neurotoxicity. Such effects, when combined with adverse social factors (such as low parental IQ, low socioeconomic status, poor nutrition, and poor quality of the caregiving environment) can place children, especially those below the age of three years, at significant risk. However, it must be acknowledged that nutritional covariates, as well as demographic social factors, have

been poorly controlled in many of the human studies reviewed. Socioeconomic status also is a crude measure of parenting and family structure that requires further assessment as a possible contributor to observed results of neurobehavioral studies.

Timing, type, and duration of exposure are important factors in both animal and human studies. It is often uncertain whether observed blood lead levels represent the levels that were responsible for observed behavioral deficits or electrophysiological changes. Monitoring of lead exposures in human subjects in all cases has been highly intermittent or nonexistent during the period of life preceding neurobehavioral assessment. In most human studies, only one or two blood lead values are provided per subject. Tooth lead may be an important cumulative exposure index, but its modest, highly variable correlation to blood lead or FEP and to external exposure levels makes findings from various studies difficult to compare quantitatively. The complexity of the many important covariates and their interaction with dependent variable measures of modest validity, e.g., IQ tests, may also account for many of the discrepancies among the different studies.

12.10.4.2 Early Development and the Susceptibility to Neural Damage. On the question of early childhood vulnerability, the neurobehavioral data are consistent with morphological and biochemical studies of the susceptibility of the heme biosynthetic pathway to perturbation by lead. Various lines of evidence suggest that the order of susceptibility to lead's effects is: (1) young > adults and (2) female > male. Animal studies also have pointed to the perinatal period of ontogeny as a particularly critical time for a variety of reasons: (1) it is a period of rapid development of the nervous system; (2) it is a period where good nutrition is particularly critical; and (3) it is a period where the caregiver environment is vital to normal development. However, the precise boundaries of a critical period are not yet clear and may vary depending on the species and function or endpoint that is being assessed. Nevertheless, there is general agreement that human infants and toddlers below the age of three years are at special risk because of *in utero* exposure, increased opportunity for exposure because of normal mouthing behavior, and increased rates of lead absorption due to various factors, e.g., nutritional deficiences.

12.10.4.3 The Question of Irreversibility. Little research on humans is available on persistence of effects. Some work suggests that mild forms of peripheral neuropathy in lead workers may be reversible after termination of lead exposure, but little is known regarding the reversibility of lead effects on central nervous system function in humans. A recent two-year follow-up study of 28 children of battery factory workers found a continuing relationship between blood lead levels and altered slow wave voltage of cortical slow wave potentials indicative of persisting CNS effects of lead. Current population studies, however, will have to be supplemented by prospective longitudinal studies of the effects of lead on development in

order to address the issue of reversibility or persistence of lead neurotoxic effects in humans more satisfactorily.

Various animal studies provide evidence that alterations in neurobehavioral function may be long-lived, with such alterations being evident long after blood lead levels have returned to control levels. These persistent effects have been demonstrated in monkeys as well as rats under a variety of learning performance test paradigms. Such results are also consistent with morphological, electrophysiological, and biochemical studies on animals that suggest lasting changes in synaptogenesis, dendritic development, myelin and fiber tract formation, ionic mechanisms of neurotransmission, and energy metabolism.

12.10.4.4 Utility of Animal Studies in Drawing Parallels to the Human Condition. Animal models are used to shed light on questions where it is impractical or ethically unacceptable to use human subjects. This is particularly true in the case of exposure to environmental toxins such as lead. In the case of lead, it has been effective and convenient to expose developing animals via their mothers' milk or by gastric gavage, at least until weaning. In many studies, exposure was continued in the water or food for some time beyond weaning. This approach simulates at least two features commonly found in human exposure: oral intake and exposure during early development. The preweaning period in rats and mice is of particular relevance to in terms of parallels with the first two years or so of human brain development.

However, important questions exist concerning the comparability of animal models to humans. Given differences between humans, rats, and monkeys in heme chemistry, metabolism, and other aspects of physiology and anatomy, it is difficult to state what constitutes an equivalent internal exposure level (much less an equivalent external exposure level). For example, is a blood lead level of 30 μg/dl in a suckling rat equivalent to 30 μg/dl in a three-year-old child? Until an answer is available to this question, i.e., until the function describing the relationship of exposure indices in different species is available, the utility of animal models for deriving dose-response functions relevant to humans will be limited.

Questions also exist regarding the comparability of neurobehavioral effects in animals with human behavior and cognitive function. One difficulty in comparing behavioral endpoints such as locomotor activity is the lack of a consistent operational definition. In addition to the lack of standardized methodologies, behavior is notoriously difficult to "equate" or compare meaningfully across species because behavioral analogies do not demonstrate behavioral homologies. Thus, it is improper to assume, without knowing more about the responsible underlying neurological structures and processes, that a rat's performance on an operant conditioning schedule or a monkey's performance on a stimulus discrimination task corresponds to a child's performance on a cognitive function test. Still deficits in performance on such tasks are indicative of altered CNS function which is likely to parallel some type of altered human CNS function as well.

In terms of morphological findings, there are reports of hippocampal lesions in both lead-exposed rats and humans that are consistent with a number of behavioral findings suggesting an impaired ability to respond appropriately to altered contingencies for rewards. That is, subjects tend to persist in certain patterns of behavior even when changed conditions make the behavior inappropriate. Other morphological findings in animals, such as demyelination and glial cell decline, are comparable to human neuropathologic observations mainly at relatively high exposure levels.

Another neurobehavioral endpoint of interest in comparing human and animal neurotoxicity of lead is electrophysiological function. Alterations of electroencephalographic patterns and cortical slow wave voltage have been reported for lead-exposed children, and various electrophysiological alterations both in vivo (e.g., in rat visual evoked response) and in vitro (e.g., in frog miniature endplate potentials) have also been noted in laboratory animals. At this time, however, these lines of work have not converged sufficiently to allow for strong conclusions regarding the electrophysiological aspects of lead neurotoxicity.

Biochemical approaches to the experimental study of leads effects on the nervous system have generally been limited to laboratory animal subjects. Although their linkage to human neurobehavioral function is at this point somewhat speculative, such studies do provide insight to possible neurochemical intermediaries of lead neurotoxicity. No single neurotransmitter system has been shown to be particularly sensitive to the effects of lead exposure; lead-induced alterations have been demonstrated in various neurotransmitters, including dopamine, norepinephrine, serotonin, and gamma-aminobutyric acid. In addition, lead has been shown to have subcellular effects in the central nervous system at the level of mitochondrial function and protein synthesis.

Given the above-noted difficulties in formulating a comparative basis for internal exposure levels among different species, the primary value of many animal studies, particularly in vitro studies, may be in the information they can provide on basic mechanisms involved in lead neurotoxicity. A number of in vitro studies show that significant, potentially deleterious effects on nervous system function occur at in situ lead concentrations of 5 μM and possibly lower, suggesting that no threshold may exist for certain neurochemical effects of lead on a subcellular or molecular level. The relationship between blood lead levels and lead concentrations at such extra- or intracellular sites of action, however, remains to be determined. Despite the problems in generalizing from animals to humans, both the animal and the human studies show great internal consistency in that they support a continuous dose-response functional relationship between lead and neurotoxic biochemical, morphological, electrophysiological, and behavioral effects.

12.10.5 Effects of Lead on the Kidney

It has been known for more than a century that kidney disease can result from lead poisoning. Identifying the contributing causes and mechanisms of lead-induced nephropathy has been difficult, however, in part because of the complexities of human exposure to lead and other nephrotoxic agents.

Nevertheless, it is possible to estimate at least roughly lead exposure ranges associated with detectable renal dysfunction in both human adults and children. More specifically, numerous studies of occupationally exposed workers have provided evidence for lead-induced chronic nephropathy being associated with blood lead levels ranging from 40 to more than 100 μg/dl, and some are suggestive of renal effects possibly occurring even at levels as low as 30 μg/dl. Similarly, in children, the relatively sparse evidence available points to the manifestation of renal dysfunction, as indexed for example by generalized aminoaciduria, at blood lead levels across the range of 40 to more than 100 μg/dl. The current lack of evidence for renal dysfunction at lower blood lead levels in children may simply reflect the greater clinical concern with neurotoxic effects of lead intoxication in children. The persistence of lead-induced renal dysfunction in children also remains to be more fully investigated, although a few studies indicate that children diagnosed as being acutely lead poisoned experience lead nephropathy effects lasting throughout adulthood.

Parallel results from experimental animal studies reinforce the findings in humans and help illuminate the mechanisms underlying such effects. For example, a number of transient effects in human and animal renal function are consistent with experimental findings of reversible lesions such as nuclear inclusion bodies, cytomegaly, swollen mitochondria, and increased numbers of iron-containing lysosomes in proximal tubule cells. Irreversible lesions such as interstitial fibrosis are also well documented in both humans and animals following chronic exposure to high doses of lead. Functional renal changes observed in humans have also been confirmed in animal model systems with respect to increased excretion of amino acids and elevated serum urea nitrogen and uric acid concentrations. The inhibitory effects of lead exposure on renal blood flow and glomerular filtration rate are currently less clear in experimental model systems; further research is needed to clarify the effects of lead on these functional parameters in animals. Similarly, while lead-induced perturbation of the renin-angiotensin system has been demonstrated in experimental animal models, further research is needed to clarify the exact relationships among lead exposure (particularly chronic low-level exposure), alteration of the renin-angiotensin system, and hypertension in both humans and animals.

On the biochemical level, it appears that lead exposure produces changes at a number of sites. Inhibition of membrane marker enzymes, decreased mitochondrial respiratory function/

cellular energy production, inhibition of renal heme biosynthesis, and altered nucleic acid synthesis are the most marked changes to have been reported. The extent to which these mitochondrial alterations occur is probably mediated in part by the intracellular bioavailability of lead, which is determined by its binding to high affinity kidney cytosolic binding proteins and deposition within intranuclear inclusion bodies.

Recent studies in humans have indicated that the EDTA lead-mobilization test is the most reliable technique for detecting persons at risk for chronic nephropathy. Blood lead measurements are a less satisfactory indicator because they may not accurately reflect cumulative absorption some time after exposure to lead has terminated.

A number of major questions remain to be more definitively answered concerning the effect of lead on the kidney. Can a distinctive lead-induced renal lesion be identified either in functional or histologic terms? What biologic measurements are most reliable for the prediction of lead-induced nephropathy? What is the incidence of lead nephropathy in the general population as well as among specifically defined subgroups with varying exposure? What is the natural history of treated and untreated lead nephropathy? What is the mechanism of lead-induced hypertension and renal injury? What are the contributions of environmental and genetic factors to the appearance of renal injury due to lead? At what level of lead in blood can the kidneys be affected? Is there a threshold for renal effects of lead? The most difficult question to answer may well be to determine the contribution of low levels of lead exposure to renal disease of non-lead etiologies.

12.10.6 Effects of Lead on Reproduction and Development

Data from human and animal studies indicate that lead may exert gametotoxic, embryotoxic, and (according to some animal studies) teratogenic effects that may influence the survival and development of the fetus and newborn. Prenatal viability and development, it appears, may also be affected indirectly, contributing to concern for unborn children and, therefore, pregnant women or women of childbearing age being group at special risk for lead effects. Early studies of quite high dose lead exposure in pregnant women indicate toxic--but not teratogenic--effects on the conceptus. Effects on reproductive performance in women at lower exposure levels are not well documented. Unfortunately, currently available human data regarding lead effects on the fetus during development generally do not lend themselves to accurate estimation of lowest observed or no-effect levels. However, some studies have shown that fetal heme synthesis is affected at maternal and fetal blood lead levels as low as approximately 15 μg/dl, as indicated by urinary ALA levels and ALA-D activity. This observed effect level is consistant with lowest observed effect levels for indications of altered heme synthesis seen at later ages for preschool and older children.

There are currently no reliable data pointing to adverse effects in human offspring following paternal exposure to lead, but industrial exposure of men to lead at levels resulting in blood lead values of 40-50 μg/dl appear to have resulted in altered testicular function. Also, another study provided evidence of effects of prostatic and seminal vesicle functions at 40-50 μg/dl blood lead levels in lead workers.

The paucity of human exposure data force an examination of the animal studies for indications of threshold levels for effects of lead on the conceptus. It must be noted that the animal data are almost entirely derived from rodents. Based on these rodent data, it seems likely that fetotoxic effects have occurred in animals at chronic exposures to 600-1000 ppm lead in the diet. Subtle effects on fetal physiology and metabolism appear to have been observed in rats after chronic maternal exposure to 10 ppm lead in drinking water, while similar effects of inhaled lead have been seen at chronic levels of 10 mg/m^3. With acute exposure by gavage or by injection, the values are 10-16 mg/kg and 16-30 mg/kg, respectively. Since humans are most likely to be exposed to lead in their diet, air, or water, the data from other routes of exposure are of less value in estimating harmful exposures. Indeed, it seems likely that teratogenic effects occur only when the maternal dose is given by injection.

Although human and animal responses may be dissimilar, the animal evidence does document a variety of effects of lead exposure on reproduction and development. Measured or apparent changes in production of or response to reproductive hormones, toxic effects on the gonads, and toxic or teratogenic effects on the conceptus have all been reported. The animal data also suggest subtle effects on such parameters as metabolism and cell structure that should be monitored in human populations. Well designed human epidemiological studies involving large numbers of subjects are still needed. Such data could clarify the relationship of exposure levels and durations to blood lead values associated with significant effects, and are needed for estimation of no-effect levels.

Given that the most clear-cut data concerning the effects of lead on reproduction and development are derived from studies employing high lead doses in laboratory animals, there is still a need for more critical research to evaluate the possible subtle toxic effects of lead on the fetus, using biochemical, ultrastructural, or neurobehavioral endpoints. An exhaustive evaluation of lead-associated changes in offspring will require consideration of possible additional effects due to paternal lead burden. Neonatal lead intake via consumption of milk from lead-exposed mothers may also be a factor at times. Also, it must be recognized that lead effects on reproduction may be exacerbated by other environmental factors (e.g., dietary influences, maternal hyperthermia, hypoxia, and co-exposure to other toxins).

12.10.7. Genotoxic and Carcinogenic Effects of Lead

It is difficult to conclude what role lead may play in the induction of human neoplasia. Epidemiological studies of lead-exposed workers provide no definitive findings. However, statistically significant elevations in cancer of the respiratory tract and digestive system in workers exposed to lead and other agents warrant some concern. Since it is clear that lead acetate can produce renal tumors in some experimental animals, it seems reasonable to conclude that at least that particular lead compound should be regarded as a carcinogen and prudent to treat it as if it were also human carcinogen (as per IARC conclusions and recommendations). However, this statement is qualified by noting that lead has been seen to increase tumorogenesis rates in animals only at relatively high concentrations, and therefore does not seem to be an extremely potent carcinogen. In vitro studies further support the genotoxic and carcinogenic role of lead, but also indicate that lead is not extremely potent in these systems.

12.10.8. Effects of Lead on the Immune System

Lead renders animals highly susceptible to endotoxins and infectious agents. Host susceptibility and the humoral immune system appear to be particularly sensitive. As postulated in recent studies, the macrophage may be the primary immune target cell of lead. Lead-induced immunosuppression occurs at low lead exposures (blood lead levels in the 20-40 μg/dl range) that, although they induce no overt toxicity, may nevertheless be detrimental to health. Available data provide good evidence that lead affects immunity, but additional studies are necessary to elucidate the actual mechanisms by which lead exerts its immunosuppressive action. Knowledge of lead effects on the human immune system is lacking and must be ascertained in order to determine permissible levels for human exposure. However, in view of the fact that lead affects immunity in laboratory animals and is immunosuppressive at very low dosages, its potential for serious effects in humans should be carefully considered.

12.10.9 Effects of Lead on Other Organ Systems

The cardiovascular, hepatic, endocrine, and gastrointestional systems generally show signs of dysfunction mainly at relatively high lead exposure levels. Consequently, in most clinical and experimental studies attention has been primarily focused on more sensitive and vulnerable target organs, such as the hematopoietic and nervous systems. However, it should be noted that overt gastrointestinal symptoms associated with lead intoxication have been observed in some recent studies to occur in lead workers at blood lead levels as low as 40-60 μg/dl, suggesting that effects on the gastrointestinal and the other above organ systems may occur at relatively low exposure levels but remain to be demonstrated by future scientific investigations.

12.11 REFERENCES

Abbott, W. S. (1925) A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

Abdulla, M.; Haeger-Aronsen, B.; Svensson, S. (1976) Effect of ethanol and zinc on ALA-dehydratase activity in red blood cells. Enzyme 21: 248-252.

Adams, N.; Boice, R. (1981) Mouse (Mus) burrows: effects of age, strain, and domestication. Anim. Learn. Behav. 9: 140-144.

Adebonojo, F. O. (1974) Hematologic status of urban black children in Philadelphia: emphasis on the frequency of anemia and elevated blood lead levels. Clin. Pediatr. (Philadelphia) 13: 874-888.

Agerty, H. A. (1952) Lead poisoning in children. Med. Clin. North Am. 36: 1587-1597.

Ahlberg, J.; Ramel, C.; Wachtmeister, C. A. (1972) Organolead compounds shown to be genetically active. Ambio 1: 29-31.

Albahary, C. (1972) Lead and hemopoiesis: the mechanism and consequences of erythropathy of occupational lead poisoning. Am. J. Med. 52: 367-378.

Albahary, C.; Truhant, R.; Boudene, C.; Desoille, H. (1961) Le dépistage de l'imprégnation saturnine par un test de mobilisation du plomb. [A case study of lead uptake from a test for lead mobilization.] Presse Med. 69: 2121-2123.

Albahary, C.; Richet, G.; Guillaume, J.; Morel-Maroger, L. (1965) Le rein dans le saturnisme professionel. [The kidney during occupational lead poisoning.] Arch. Mal. Prof. Med. Trav. Secur. Soc. 26: 5-19.

Albert, R. E.; Shore, R. E.; Sayers, A. J.; Strehlow, C.; Kneip, T. J.; Pasternack, B. S.; Freidhoff, A. J.; Covan, F.; Cimino, J. A. (1974) Follow-up of children overexposed to lead. Environ. Health Perspect. 7: 33-40.

Alessio, L.; Bertazzi, P. A.; Monelli, O.; Toffoletto, F. (1976a) Free erythrocyte protoporphyrin as an indicator of the biological effect of lead in adult males. III: Behavior of free erythrocyte protoporphyrin in workers with past lead exposure. Int. Arch. Occup. Environ. Health 37: 77-86.

Alessio, L.; Bertazzi, P. A.; Monelli, O.; Foa, V. (1976b) Free erythrocyte protoporphyrin as an indicator of the biological effect of lead in adult males. II: Comparison between free erythrocyte protoporphyrin and other indicators of effect. Int. Arch. Occup. Environ. Health 37: 89-105.

Alessio, L.; Castoldi, M. R.; Monelli, O.; Toffoletto, F.; Zocchetti, C. (1979) Indicators of internal dose in current and past exposure to lead. Int. Arch. Occup. Environ. Health 44: 127-132.

Alexander, F. W. (1982) Lead levels and abnormal births. Presented at: International symposium on low level lead exposure and its effects on human beings; May; London, United Kingdom. London, United Kingdom: Clear Charitable Trust.

Alexander, F. W.; Delves, H. T. (1981) Blood lead levels during pregnancy. Int. Arch. Occup. Environ. Health 48: 35-39.

Alfano, D. P.; Petit, T. L. (1982) Neonatal lead exposure alters the dendritic development of hippocampal dentate granule cells. Exp. Neurol. 75: 275-288.

Alfano, D. P.; LeBoutillier, J. C.; Petit, T. L. (1982) Hippocampal mossy fiber pathway development in normal and postnatally lead-exposed rats. Exp. Neurol. 75: 308-319.

Allen, J. R.; McWey, P. J.; Suomi, S. J. (1974) Pathobiological and behavioral effects of lead intoxication in the infant Rhesus monkey. Environ. Health Perspect. 7: 239-246.

Alvares, A. P.; Leigh, S.; Cohn, J.; Kappas, A. (1972) Lead and methyl mercury: effects of acute exposure on cytochrome P-450 and the mixed function oxidase system in the liver. J. Exp. Med. 135: 1406-1409.

Alvares, A. P.; Kapelner, S.; Sassa, S.; Kappas, A. (1975) Drug metabolism in normal children, lead-poisoned children, and normal adults. Clin. Pharmacol. Ther. 17: 179-183.

Alvares, A. P.; Fischbein, A.; Sassa, S.; Anderson, K. E.; Kappas, A. (1976) Lead intoxication: effects on cytochrome P-450-mediated hepatic oxidations. Clin. Pharmacol. Ther. 19: 183-190.

Angell, N. F.; Weiss, B. (1982) Operant behavior of rats exposed to lead before or after weaning. Toxicol. Appl. Pharmacol. 63: 62-71.

Angle, C. R.; McIntire, M. S. (1964) Lead poisoning during pregnancy: fetal tolerance of calcium disodium EDTA. Am. J. Dis. Child. 108: 436-439.

Angle, C. R.; McIntire, M. S. (1978) Low level lead and inhibition of erythrocyte pyrimidine nucleotidase. Environ. Res. 17: 296-302.

Angle, C. R.; McIntire, M. S. (1982) Children, the barometer of environmental lead. Adv. Pediatr. 27: 3-31.

Angle, C. R.; McIntire, M. S.; Swanson, M. S.; Stohs, S. J. (1982) Erythrocyte nucleotides in children - increased blood lead and cytidine triphosphate. Pediatr. Res. 16: 331-334.

Anku, V. D.; Harris, J. W. (1974) Peripheral neuropathy and lead poisoning in a child with sickle-cell anemia. J. Pediatr. (St. Louis) 85: 337-340.

Anonymous. (1966) Nephropathy in chronic lead poisoning [Editorial]. J. Am. Med. Assoc. 197: 722.

Araki, S.; Honma, T. (1976) Relationships between lead absorption and peripheral nerve conduction velocities in lead workers. Scand. J. Work. Environ. Health 4: 225-231.

Araki, S.; Honma, T.; Yanagihara, S.; Ushio, K. (1980) Recovery of slowed nerve conduction velocity in lead-exposed workers. Int. Arch. Occup. Environ. Health 46: 151-157.

Araki, S.; Mutata, K.; Yanagihara, S.; Ushio, K. (1982) High medical consultation rates of lead workers after industrial dispute over lead effects. Int. Arch. Environ. Health 49: 241-250.

Arnvig, E.; Grandjean, P.; Beckmann, J. (1980) Neurotoxic effects of heavy lead exposure determined with psychological tests. Toxicol. Lett. 5: 399-404.

Ashby, J. A. S. (1980) A neurological and biochemical study of early lead poisoning. Br. J. Ind. Med. 37: 133-140.

Asokan, S. K. (1974) Experimental lead cardiomyopathy: myocardial structural changes in rats given small amounts of lead. J. Lab. Clin. Med. 84: 20-25.

Aub, J. C.; Reznikoff, P. (1924) Lead studies. III: The effects of lead in red blood cells. Part 3: A chemical explanation of the reaction of lead with red blood cells. J. Exp. Med. 40: 189-208.

Aub, J. C.; Fairhall, L. T., Minot, A. S.; Reznikoff, P.; Hamilton, A. (1925) Lead poisoning. Medicine (Baltimore) 4: 250. Also see: Baltimore, MD: The Williams and Wilkins Company. (Medicine monographs: v. 7). (1926).

Averill, D. R.,, Jr.; Needleman, H. L. (1980) Neonatal lead exposure retards cortical synaptogenesis in the rat. In: Needleman, H. L., ed. Low level lead exposure: the clinical implications of current research. New York, NY: Raven Press; pp. 201-210.

Aviv, A.; John, E.; Bernstein, J.; Goldsmith, D. I.; Spitzer, A. (1980) Lead intoxication during development: its late effects on kidney function and blood pressure. Kidney Int. 17: 430-437.

Azar, A.; Trochimowicz, H. J.; Maxfield, M. E. (1973) Review of lead studies in animals carried out at Haskell Laboratory: two year feedings study and response to hemmorhage study. In: Barth, D.; Berlin, A.; Engel, R.; Recht, P.; Smeets, J., eds. Environmental health aspects of lead: proceedings, international symposium; October 1972; Amsterdam, The Netherlands. Luxembourg: Commission of the European Communities, Centre for Information and Documentation; pp. 199-210.

Bach, F. H.; Bach, M. L.; Sondel, P. M. (1976) Differential function of major histocompatibility complex antigens in T-lymphocyte activation. Nature (London) 259: 273-281.

Baker, E. L., Jr.; Landrigan, P. J.; Barbour, A. G.; Cox, D. H.; Folland, D. S.; Ligo, R. N.; Throckmorton, J. (1979) Occupational lead poisoning in the United States: clinical and biochemical findings related to blood lead levels. Br. J. Ind. Med. 36: 314-322.

Baker, E. L.; Goyer, R. A.; Fowler, B. A.; Khettry, U.; Bernard, D. B.; Adler, S.; White, R. D.; Babayan, R.; Feldman, R. G. (1980) Occupational lead exposure, nephropathy, and renal cancer. Am. J. Ind. Med. 1: 139-148.

Baldwin, R. W.; Cunningham. G. J.; Pratt, D. (1964) Carcinogenic action of motor engine oil additives. Br. J. Cancer 18: 503-507.

Ball, G. V.; Sorensen, L. B. (1969) Pathogensis of hyperuricemia in saturnine gout. N. Engl. J. Med. 280: 1199-1202.

Balo, J.; Batjai, A.; Szende, B. (1965) Experimental adenomas of the kidney produced by chronic administration of lead phosphate. Magyar Onkol. 9: 144-151.

Baloh, R.; Sturm, R.; Green, B.; Gleser, G. (1975) Neuropsychological effects of chronic asymptomatic increased lead absorption: a controlled study. Arch. Neurol. 32: 326-330.

Barlow, K. A.; Beilin, L. J. (1968) Renal disease in primary gout. Q. J. Med. 37: 79-98.

Barltrop, D. (1966) The prevalence of pica. Am. J. Dis. Child. 112: 116-123.

Barltrop, D.; Barrett, A. J.; Dingle, J. T. (1971) Subcellular distribution of lead in the rat. J. Lab. Clin. Med. 77: 705-712.

Barrett, J.; Livesey, P. J. (1982) The acetic acid component of lead acetate: its effect on rat weight and activity. Neurobehav. Toxicol. Teratol. 4: 105-108.

Barry, P. S. I. (1975) A comparison of concentrations of lead in human tissues. Br. J. Ind. Med. 32: 119-139.

Barthalmus, G. T.; Leander, J. D.; McMillan, D. E.; Mushak, P.; Krigman, M. R. (1977) Chronic effects of lead on schedule-controlled pigeon behavior. Toxicol. Appl. Pharmacol. 42: 271-284.

Barton, J. C.; Conrad, M. E.; Nuby, S.; Harrison, L. (1978) Effects of iron on the absorption and retention of lead. J. Lab. Clin. Med. 92: 536-547.

Batuman, V.; Maesaka, J. K.; Haddad, B.; Tepper, E.; Landry, E.; Wedeen, R. P. (1981) The role of lead in gout nephropathy. N. Engl. J. Med. 304: 520-523.

Batuman, V.; Landy, E.; Maesaka, J. K.; Wedeen, R. P. (1983) Contribution of lead to hypertension with renal impairment. N. Engl. J. Med. 309: 17-21.

Bauchinger, M.; Schmid, E. (1972) Chromosomenanalysen in Zellkulturen des chinesischen Hamsters nach Applikation von Bleiacetat. [Chromosome analysis in Chinese hamster cell cultures after treatment with lead acetate.] Mutat. Res. 14: 95-100.

Bauchinger, M.; Schmid, E.; Schmidt, D. (1972) Chromosomenanalyse bei Verkehrspolizisten mit erhöhter Bleilast. [Chromosome analysis of policemen with increased blood lead level.] Mutat. Res. 16: 407-412.

Bauchinger, M.; Schmid, E.; Einbrodt, H. J.; Dresp, J. (1976) Chromosome aberrations in lymphocytes after occupational exposure to lead and cadmium. Mutat. Res. 40: 57-62.

Bauchinger, M.; Dresp, J.; Schmid, E.; Englert, N.; Krause, Chr. (1977) Chromosome analyses of children after ecological lead exposure. Mutat. Res. 56: 75-80.

Beattie, A. D.; Moore, M. R.; Goldberg, A.; Finlayson, M. J. W.; Graham, J. F.; Mackie, E. M.; Main, J. C.; McLaren, D. A.; Murdoch, K. M.; Stewart, G. T. (1975) Role of chronic low-level lead exposure in the aetiology of mental retardation. Lancet 1(7907): 589-592.

Bedford, J. M.; Calvin, H. I. (1974) Changes in -S-S- linked structures of the sperm tail during epididymal maturation, with comparative observations in sub-mammalian species. J. Exp. Zool. 187: 181-204.

Beek, B.; Obe, G. (1974) Effect of lead acetate on human leukocyte chromosomes in vitro. Experientia 30: 1006-1007.

Beek, B.; Obe, G. (1975) The human leukocyte test system. VI: The use of sister chromatid exhanges as possible indicators for mutagenic activities. Humangenetik 29: 127-134.

Beevers, D. G.; Cruickshank, J. K.; Yeoman, W. B.; Carter, G. F.; Goldberg, A.; Moore, M. R. (1980) Blood-lead and cadmium in human hypertension. J. Environ. Pathol. Toxicol. 4: 251-260.

Beevers, D. G.; Erskine, E.; Robertson, M.; Beattie, A. D.; Campbell, B. C.; Goldberg, A.; Moore, M. R.; Hawthorne, V. M. (1976) Blood-lead and hypertension. Lancet 2(7975): 1-3.

Belknap, E. L. (1936) Clinical studies on lead absorption in the human. III: Blood pressure observations. J. Ind. Hyg. Toxicol. 18: 380-390.

Bellinger, D. C.; Needleman, H. L. (1983) Lead and the relationship between maternal and child intelligence. J. Pediatr. (St. Louis) 102: 523-527.

Benignus, V. A.; Otto, D. A.; Muller, K. E.; Seiple, K. J. (1981) Effects of age and body lead burden on CNS function in young children. II: EEG spectra. Electroencephalogr. Clin. Neurophysiol. 52: 240-248.

Beritić, T. (1971) Lead concentration found in human blood in association with lead colic. Arch. Environ. Health 23: 289-291.

Berk, P. D.; Tschudy, D. P.; Shepley, L. A.; Waggoner, J. G.; Berlin, N. I. (1970) Hematologic and biochemical studies in a case of lead poisoning. Am. J. Med. 48: 137-144.

Betts, P. R.; Astley, R.; Raine, D. N. (1973) Lead intoxication in children in Birmingham. Br. Med. J. 1(5850): 402-406.

Biagini, G.; Caudarella, R.; Vangelista, A. (1977) Renal morphological and functional modification in chronic lead poisoning. In: Brown, S. S., ed. Clinical chemistry and chemical toxicology of metals. New York, NY: Elsevier/North-Holland Biomedical Press; pp. 123-126.

Björklund, H.; Olson, L.; Seiger, Å.; Hoffer, B. (1980) Chronic lead and brain development: intraocular brain grafts as a method to reveal regional and temporal effects in the central nervous system. Environ. Res. 22: 224-236.

Blackfan, K. D. (1917) Lead poisoning in children with especial reference to lead as a cause of convulsions. Am. J. Med. Sci. 153: 877-887.

Blackman, S. S., Jr. (1936) Intranuclear inclusion bodies in the kidney and liver caused by lead poisoning. Bull. Johns Hopkins Hosp. 58: 384-402.

Blackman, S. S., Jr. (1937) The lesions of lead encephalopathy in children. Bull. Johns Hopkins Hosp. 61: 1-43.

Blakley, B. R.; Archer, D. L. (1981) The effect of lead acetate on the immune response in mice. Toxicol. Appl. Pharmacol. 61: 18-26.

Blakley, B. R.; Archer, D. L. (1982) Mitogen stimulation of lymphocytes exposed to lead. Toxicol. Appl. Pharmacol. 62: 183-189.

Blakley, B. R.; Sisodia, C. S.; Mukkur, T. K. (1980) The effect of methylmercury, tetraethyl lead, and sodium arsenite on the humoral immune response in mice. Toxicol. Appl. Pharmacol. 52: 245-254.

Blakley, B. R.; Archer, D. L.; Osborne, L. (1982) The effect of lead on immune and viral interferon production. Can. J. Comp. Med. 46: 43-46.

Blanksma, L. A.; Sachs, H. K.; Murray, E. F.; O'Connell, M. J. (1969) Failure of the urinary Δ-aminolevulinic acid (ALA) test to detect pediatric lead poisoning. Am. J. Clin. Pathol. 52: 96.

Bluestone, R.; Waisman, J.; Klinenberg, J. R. (1977) The gouty kidney. Semin. Arthritis Rheum. 7: 97-112.

Boeckx, R. L.; Posti, B.; Coodin, F. J. (1977) Gasoline sniffing and tetraethyl lead poisoning in children. Pediatrics 60: 140-145.

Bondy, S. C.; Agrawal, A. K. (1980) The inhibition of cerebral high affinity receptor sites by lead and mercury compounds. Arch. Toxicol. 46: 249-256.

Bondy, S. C.; Anderson, C. L.; Harrington, M. E.; Prasad, K. N. (1979a) The effects of organic and inorganic lead and mercury on neurotransmitter high-affinity transport and release mechanisms. Environ. Res. 19: 102-111.

Bondy, S. C.; Harrington, M. E.; Anderson, C. L.; Prasad, K. N. (1979b) The effect of low concentrations of an organic lead compound on the transport and release of putative neurotransmitters. Toxicol. Lett. 3: 35-41.

Border, E. A.; Cantrell, A. C.; Kilroe-Smith, T. A. (1976) The in vitro effect of zinc on the inhibition of human δ-aminolevulinic acid dehydratase by lead. Br. J. Ind. Med. 33: 85-87.

Bordo, B. M.; Filippini, G.; Massetto, N.; Musicco, M.; Boeri, R. (1982) Electrophysiological study of subjects occupationally exposed to lead poisoning. Scand. J. Work Environ. Health 8 (Suppl. 1): 142-147.

Bornschein, R. L., ed. (1983) Proceedings of the international conference on prospective studies of lead exposure in children; September 1981; Cincinnati, OH. J. Neurotoxicol.: (in press)

Bornschein, R.; Pearson, D.; Reiter, L. (1980) Behavioral effects of moderate lead exposure in children and animal models. Part 2: Animal studies. CRC Crit. Rev. Toxicol. 8: 101-152.

Borová, J.; Ponka, P.; Neuwirt, J. (1973) Study of intracellular iron distribution in rabbit reticulocytes with normal and inhibited heme synthesis. Biochim. Biophys. Acta 320: 143-156.

Boscolo, P.; Galli, G.; Iannaccone, A.; Martino, F.; Porcelli, G.; Troncone, L. (1981) Plasma renin activity and urinary kallikrein excretion in lead-exposed workers as related to hypertension and nephropathy. Life Sci. 28: 175-184.

Bouldin, T. W.; Mushak, P.; O'Tuama, L. A.; Krigman, M. R. (1975) Blood-brain barrier dysfunction in acute lead encephalopathy: a reappraisal. Environ. Health Perspect. 12: 81-88.

Bouley, G.; Dubreuil, A.; Arsac, F.; Boudene, C. (1977) Effet du plomb microparticulaire, introduit dans l'appareil respiratoire, sur la sensibilité de la souris à l'infection par aérosol de Pasteurella multocida. [Effect of microparticulate lead, introduced through respiratory apparatus, on the resistance of mice to infection by aerosolized Pasteurella multocida.] C. R. Hebd. Seances Acad. Sci. Ser. D 285: 1553-1556.

Bowie, E. A.; Simmonds, H. A.; North, J. D. K. (1967) Allopurinol in treatment of patients with gout and chronic renal failure. N.Z. Med. J. 66: 606-611.

Boyland, E.; Dukes, C. E.; Grover, P. L.; Mitchley, B. C. V. (1962) The induction of renal tumours by feeding lead acetate to rats. Br. J. Cancer 16: 283-288.

Bradley, J. E.; Baumgartner, R. J. (1958) Subsequent mental development of children with lead encephalopathy, as related to type of treatment. J. Pediatr. (St. Louis) 53: 311-315.

Bradley, J. E.; Powell, A. E.; Niermann, W.; McGrady, K. R.; Kaplan, E. (1956) The incidence of abnormal blood levels of lead in a metropolitan pediatric clinic: with observation on the value of coproporphyrinuria as a screening test. J. Pediatr. (St. Louis) 49: 1-6.

Brashear, C. W.; Kopp, V. J.; Krigman, M. R. (1978) Effect of lead on the developing peripheral nervous system. J. Neuropathol. Exp. Neurol. 37: 414-425.

Braunstein, G. D.; Dahlgren, J.; Loriaux, D. L. (1978) Hypogonadism in chronically lead-poisoned men. Infertility 1: 33-51.

Brennan, M. J. W.; Cantrill, R. C. (1979) δ-Aminolaevulinic acid is a potent agonist for GABA autoreceptors. Nature (London) 280: 514-515.

Brieger, H.; Rieders, F. (1959) Chronic lead and mercury poisoning: contemporary views on ancient occupational diseases. J. Chronic Dis. 9: 177-184.

Briney, W. G.; Ogden, D.; Bartholomew, B.; Smyth, C. J. (1975) The influence of allopurinol on renal function in gout. Arthritis Rheum. (Suppl.) 18: 877-881.

Broman, S. H.; Nichols, P. L.; Kennedy, W. A. (1975) Preschool IQ: prenatal and early developmental correlates. Hillsdale, NJ: Lawrence Erlbaum Associates, pp. 1-16.

Brown, D. R. (1975) Neonatal lead exposure in the rat: decreased learning as a function of age and blood lead concentrations. Toxicol. Appl. Pharmacol. 32: 628-637.

Brown, J.; Mallory, G. K. (1950) Renal changes in gout. N. Engl. J. Med. 243: 325-329.

Brown, S.; Dragann, N.; Vogel, W. H. (1971) Effects of lead acetate on learning and memory in rats. Arch. Environ. Health 22: 370-372.

Bruce, W. R.; Heddle, J. A. (1979) The mutagenic activity of 61 agents as determined by the micronucleus, Salmonella, and sperm abnormality assays. Can. J. Genet. Cytol. 21: 319-334.

Bryce-Smith, D.; Deshpande, R. R.; Hughes, J.; Waldron, H. A. (1977) Lead and cadmium levels in stillbirths. Lancet 1(8022): 1159.

Buc, H. A.; Kaplan, J. C. (1978) Red-cell pyrimidine 5'-nucleotidase and lead poisoning. Clin. Chim. Acta 87: 49-55.

Buchet, J-P.; Roels, H.; Hubermont, G.; Lauwerys, R. (1976) Effect of lead on some parameters of the heme biosynthetic pathway in rat tissues in vivo. Toxicology 6: 21-34.

Buchet, J-P.; Roels, H.; Bernard, A.; Lauwerys, R. (1980) Assessment of renal function of workers exposed to inorganic lead, cadmium or mercury vapor. J. Occup. Med. 22: 741-750.

Buchthal, F.; Behse, F. (1979) Electrophysiology and nerve biopsy in men exposed to lead. Br. J. Ind. Med. 36: 135-147.

Buchthal, F.; Behse, F. (1981) Nerve conduction and nerve biopsy in men exposed to lead. In: Lynam, D. R.; Piantanida, L. G.; Cole, J. R., eds. Environmental lead. New York, NY: Academic Press; pp. 69-94.

Bull, R. J. (1977) Effects of trace metals and their derivatives on the control of brain energy metabolism. In: Lee, S. D., ed. Biochemical effects of environmental pollutants. Ann Arbor, MI: Ann Arbor Science; pp. 425-440.

Bull, R. J. (1980) Lead and energy metabolism. In: Singhal, P. L.; Thomas, J. A., eds. Lead toxicity. Baltimore, MD: Urban and Schwarzenberg, Inc.; pp. 119-168.

Bull, R. J.; Stanaszek, P. M.; O'Neill, J. J.; Lutkenhoff, S. D. (1975) Specificity of the effects of lead on brain energy metabolism for substrates donating a cytoplasmic reducing equivalent. Environ. Health Perspect. 12: 89-95.

Bull, R. J.; Lutkenhoff, S. D.; McCarty, G. E.; Miller, R. G. (1979) Delays in the postnatal increase of cerebral cytochrome concentrations in lead-exposed rats. Neuropharmacology 18: 83-92.

Bulsma, J. B.; De France, H. F. (1976) Cytogenetic investigations in volunteers ingesting inorganic lead. Int. Arch. Occup. Environ. Health 38: 145-148.

Burchfiel, J. L.; Duffy, F. H.; Bartels, P. H.; Needleman, H. L. (1980) The combined discriminating power of quantitative electroencephalography and neuropsychologic measures in evaluating central nervous system effects of lead at low levels. In: Needleman, H. L., ed. Low level lead exposure: the clinical implications of current research. New York, NY: Raven Press; pp. 75-90.

Bushnell, P. J. (1978) Behavioral toxicology of lead in the infant Rhesus monkey. Madison, WI: University of Wisconsin-Madison. Available from: University Microfilms, Ann Arbor, MI; publication no. 78-15,037. Dissertation.

Bushnell, P. J.; Bowman, R. E. (1979a) Reversal learning deficits in young monkeys exposed to lead. Pharmacol. Biochem. Behav. 10: 733-742.

Bushnell, P. J.; Bowman, R. E. (1979b) Persistence of impaired reversal learning in young monkeys exposed to low levels of dietary lead. J. Toxicol. Environ. Health 5: 1015-1023.

Bushnell, P. J.; Bowman, R. E. (1979c) Effects of chronic lead ingestion on social development in infant Rhesus monkeys. Neurobehav. Toxicol. 1: 207-219.

Bushnell, P. J.; Bowman, R. E.; Allen, J. R.; Marlar, R. J. (1977) Scotopic vision deficits in young monkeys exposed to lead. Science (Washington D.C.) 196: 333-335.

Butt, E. M.; Pearson, H. E.; Simonsen, D. G. (1952) Production of meningoceles and craniochisis in chick embryos with lead nitrate. Proc. Soc. Exp. Biol. Med. 79: 247-249.

Byers, R. K.; Lord, E. E. (1943) Late effects of lead poisoning on mental development. Am. J. Dis. Child. 66: 471-494.

Cadman, H. C. (1905) Lead as an abortifacient [Letter]. Br. Med. J. 1: 653.

Calvin, H. I.; Bedford, J. M. (1971) Formation of disulphide bonds in the nucleus and accessory structures of mammalian spermatozoa during maturation in the epididymis. J. Reprod. Fertil. Suppl. 13: 65-75.

Campbell, B. A.; Lytle, L. D.; Fibiger, H. C. (1969) Ontogeny of adrenergic arousal and cholinergic inhibitory mechanisms in the rat. Science (Washington D.C.) 166: 635-637.

Campbell, B. C.; Beattie, A. D.; Moore, M. R.; Goldberg, A.; Reid, A. G. (1977) Renal insufficiency associated with excessive lead exposure. Br. Med. J. 1(6059): 482-485.

Campbell, B. C.; Moore, M. R.; Goldberg, A.; Hernandez, L. A.; Dick, W. C. (1978) Subclinical lead exposure: a possible cause of gout. Br. Med. J. 2(6149): 1403.

Campbell, B. C.; Beattie, A. D.; Elliott, H. L.; Goldberg, A.; Moore, M. R.; Beevers, D. G.; Tree, M. (1979) Occupational lead exposure and renin release. Arch. Environ. Health 34: 439-443.

Campbell, J. B.; Woolley, D. E.; Vijakan, V. K.; Overmann, S. R. (1982) Morphometric effects of postnatal lead exposure on hippocampal development of the 15-day-old rat. Dev. Brain Res. 3: 595-612.

Cannon, P. J.; Stason, W. B.; Demartini, F. E.; Sommers, S. C.; Laragh, J. H. (1966) Hyperuricemia in primary and renal hypertension. N. Engl. J. Med. 275: 457-464.

Cantarow, A.; Trumper, M. (1944) Lead poisoning. Baltimore, MD: Williams and Wilkins.

Capel, I. D.; Pinnock, M. H.; Dorrell, H. M.; Williams, D. C.; Grant, E. C. G. (1981) Comparison of concentrations of some trace, bulk, and toxic metals in the hair of normal and dyslexic children. Clin. Chem. (Winston Salem N.C.) 27: 879-881.

Capobianco, S.; Hamilton, L. W. (1976) Effects of interruption of limbic system pathways on different measures of activity. Physiol. Behav. 17: 65-72.

Carpenter, S. J.; Ferm, V. H. (1977) Embryopathic effects of lead in the hamster: a morphologic analysis. Lab. Invest. 37: 369-385.

Carroll, K. G.; Spinelli, F. R.; Goyer, R. A. (1970) Electron probe microanalyzer localization of lead in kidney tissue of poisoned rats. Nature (London) 227: 1056.

Castellino, N.; Aloj, S. (1969) Intracellular distribution of lead in the liver and kidney of the rat. Br. J. Ind. Med. 26: 139-143.

Casto, B. C.; Meyers, J.; Dipaolo, J. A. (1979) Enhancement of viral transformation for evaluation of the carcinogenic or mutagenic potential of inorganic metal salts. Cancer Res. 39: 193-198.

Castranova, V.; Bowman, L.; Reasor, M. J.; Miles, P. R. (1980) Effects of heavy metal ions on selected oxidative metabolic processes in rat alveolar macrophages. Toxicol. Appl. Pharmacol. 53: 14-23.

Cerottini, J. C.; Brunner, K. T. (1974) Cell-mediated cytotoxicity, allograft rejection, and tumor immunity. Adv. Immunol. 18: 67-132.

Chang, L. W.; Wade, P. R.; Lee, G. W. (1981) An ultrastructural reevaluation of lead-induced pathology in the kidney. Environ. Res. 26: 136-151.

Charcot; Gombault. (1881) Note relative a l'étude anatomique de la néphrite saturnine expérimentale. [Note on the anatomical study of experimental lead nephritis.] Arch. Physiol. 8: 126-154.

Charcot, J. M. (1868) Lecons sur les maladies vieillards et les maladies chroniques. Paris, France: Adrien Delahye.

Chisolm, J. J., Jr. (1962) Aminoaciduria as a manifestation of renal tubular injury in lead intoxication and a comparison with patterns of aminoaciduria seen in other diseases. J. Pediatr. (St. Louis) 60: 1-17.

Chisolm, J. J., Jr. (1965) Chronic lead intoxication in children. Dev. Med. Child Neurol. 7: 529-536.

Chisolm, J. J., Jr. (1968) The use of chelating agents in the treatment of acute and chronic lead intoxication in childhood. J. Pediatr. (St. Louis) 73: 1-38.

Chisolm, J. J., Jr. (1973) Management of increased lead absorption and lead poisoning in children. N. Engl. J. Med. 289: 1016-1018.

Chisolm, J. J., Jr. (1980) Poisoning from heavy metals (mecury, lead, and cadmium). Pediatr. Ann. 9: 458-468.

Chisolm, J. J., Jr. (1981) Dose-effect relationships for lead in young children: evidence in children for interactions among lead, zinc and iron. In: Lynam, D. R.; Piantanida, L. G; Cole, J. R., eds. Environmental lead: proceedings of the second international symposium on environmental lead research; December 1978; Cincinnati, OH. New York, NY: Academic Press; pp. 1-7.

Chisolm, J. J., Jr.; Barltrop, D. (1979) Recognition and management of children with increased lead absorption. Arch. Dis. Child. 54: 249-262.

Chisolm, J. J., Jr.; Brown, D. H. (1979) Micromethod for zinc protoporphyrin in erythrocytes: including new data on the absorptivity of zinc protoporphyrin and new observations in neonates and sickle cell disease. Biochem. Med. 22: 214-237.

Chisolm, J. J., Jr.; Harrison, H. E. (1956) The exposure of children to lead. Pediatrics 18: 943-958.

Chisolm, J. J., Jr.; Harrison, H. C.; Eberlein, W. R.; Harrison, H. E. (1955) Amino-aciduria, hypophosphatemia, and rickets in lead poisoning: study of a case. Am. J. Dis. Child. 89: 159-168.

Chisolm, J. J., Jr.; Mellits, E. D.; Barrett, M. B. (1976) Interrelationships among blood lead concentration, quantitative daily ALA-U and urinary lead output following calcium EDTA. In: Nordberg, G. F.; ed. Proceedings of third meeting of the subcommittee on the toxicology of metals under the Permanent Commission and International Association on Occupational Health; November 1974; Tokyo, Japan. Amsterdam, The Netherlands: Elsevier Publishing Co.; pp. 416-433.

Choie, D. D.; Richter, G. W. (1972a) Cell proliferation in rat kidney induced by lead acetate and effects of uninephrectomy on the proliferation. Am. J. Pathol. 66: 265-275.

Choie, D. D.; Richter, G. W. (1972b) Cell proliferation in rat kidneys after prolonged treatment with lead. Am. J. Pathol. 68: 359-370.

Choie, D. D.; Richter, G. W. (1974a) Cell proliferation in mouse kidney induced by lead. I: Synthesis of deoxyribonucleic acid. Lab. Invest. 30: 647-651.

Choie, D. D., Richter, G. W. (1974b) Cell proliferation in mouse kidney induced by lead. II: Synthesis of ribonucleic acid and protein. Lab. Invest. 30: 652-656.

Choie, D. D.; Richter, G. W.; Young, L. B. (1975) Biogenesis of intranuclear lead-protein inclusions in mouse kidney. Beitr. Pathol. 155: 197-203.

Chow, C. P.; Cornish, H. H. (1978) Effects of lead on the induction of hepatic microsomal enzymes by phenobarbital and 3,4-benzpyrene. Toxicol. Appl. Pharmacol. 43: 219-228.

Chruściel, H. (1975) Wplyw toksycznych czynników środowiska pracy na powstawanie leukoplakii u hutnikow cynku i olowiu. [The effect of toxic environmental products on the development of leucoplakia in workers in zinc and lead processing plants.] Czas. Stomatol. 28: 103-110.

Cihák, A.; Seifertová, M. (1976) Simulated DNA synthesis in livers and kidneys induced to proliferate associated with unchanged thymidine and thymidylate kinase activities. Chem. Biol. Interact. 13: 141-149.

Clark, A. R. L. (1977) Placental transfer of lead and its effects on the newborn. Postgrad. Med. J. 53: 674-678.

Clarkson, T. W.; Kench, J. E. (1956) Urinary excretion of amino acids by men absorbing heavy metals. Biochem. J. 62: 361-372.

Cohen, D. J.; Johnson, W. T.; Caparulo, B. K. (1976) Pica and elevated blood lead level in autistic and atypical children. Am. J. Dis. Child. 130: 47-48.

Cohen, G. J.; Ahrens, W. E. (1959) Chronic lead poisoning: a review of seven years' experience at the Children's Hospital, District of Columbia. J. Pediatr. (St. Louis) 54: 271-284.

Cohen, J.; Cohen, P. (1975) Applied multiple regressional/correlation analysis for the behavioral sciences. Hillsdale, NJ: Lawrence Erlbaum Associates; pp. 123-167.

Cole, L. J.; Bachhuber, L. J. (1914) Effects of lead on the germ cells of the male rabbit and fowl as indicated by their progeny. Proc. Soc. Exp. Biol. Med. 42: 24.

Cole, R.; Cole, J. (1976) Correlations between disturbed haem synthesis and fetal malformation. Lancet 2(7986): 640.

Colle, A.; Grimaud, J. A.; Boucherat, M.; Manuel, Y. (1980) Lead poisoning in monkeys: functional and histopathological alterations of the kidneys. Toxicology 18: 145-158.

Conradi, S.; Ronnevi, L-O.; Vesterberg, O. (1976) Abnormal tissue distribution of lead in amyotrophic lateral sclerosis. J. Neurol. Sci. 29: 259-265.

Conradi, S.; Ronnevi, L-O.; Stibler, H. (1978a) Serum protein binding of lead in vitro in amyotrophic lateral sclerosis patients and controls. J. Neurol. Sci. 37: 95-105.

Conradi, S.; Ronnevi, L-O.; Vesterberg, O. (1978b) Lead concentration in skeletal muscle in amyotrophic lateral sclerosis patients and control subjects. J. Neurol. Neurosurg. Psychiatry 41: 1001-1004.

Conradi, S.; Ronnevi, L-O.; Nise, G.; Vesterberg, O. (1980) Abnormal distribution of lead in amyotrophic lateral sclerosis: reestimation of lead in the cerebrospinal fluid. J. Neurol. Sci. 48: 413-418.

Cook, J. A.; Marconi, E. A.; Di Luzio, N. R. (1974) Lead, cadmium, endotoxin interaction: effect on mortality and hepatic function. Toxicol. Appl. Pharmacol. 28: 292-302.

Cook, J. A.; Hoffman, E. O.; Di Luzio, N. R. (1975) Influence of lead and cadmium on the susceptibility of rats to bacterial challenge (39117). Proc. Soc. Exp. Biol. Med. 150: 741-747.

Cooley, W. W.; Lohnes, P. R. (1971) Multivariate statistical analysis. New York, NY: John Wiley & Sons, Inc.; pp. 3-28.

Cooper, G. P.; Manalis, R. S. (1974) Effects of polyvalent cations on synaptic transmission in frog neuromuscular junction and frog sympathetic ganglion. In: Xintaras, C.; Johnson, B. L.; de Groot, I., eds. Behavioral toxicology: early detection of occupational hazards. Cincinnati, OH: U.S. Department of Health, Education, and Welfare, National Institute for Occupational Safety and Health; DHEW (NIOSH) publication no. 74-126; pp. 267-276.

Cooper, G. P.; Steinberg, D. (1977) Effects of cadmium and lead on adrenergic neuromuscular transmission in the rabbit. Am. J. Physiol. 232: C128-C131.

Cooper, G. P.; Fox, D. A.; Howell, W. E.; Laurie, R. D.; Tsang, W.; Lewkowski, J. P. (1980) Visual evoked responses in rats exposed to heavy metals. In: Merigan, W. H.; Weiss, B., eds. Neurotoxicity of the visual system. New York, NY: Raven Press; pp. 203-218.

Cooper, R. A.; Arner, E. C.; Wiley, J. S.; Shattil, S. J. (1975) Modification of red cell membrane structure by cholesterol-rich lipid dispersions: a model for the primary spur cell defect. J. Clin. Invest. 55: 115-126.

Cooper, W. C. (1976) Cancer mortality patterns in the lead industry. Ann. N.Y. Acad. Sci. 271: 250-259.

Cooper, W. C. (1981) Mortality in employees of lead production facilities and lead battery plants, 1971-1975. In: Lynam, D. R.; Piantanida, L. G.; Cole, J. F., eds. Environmental lead. Proceedings of the second international symposium on environmental lead research; December 1978; Cincinnati, OH. New York, NY: Academic Press; pp. 111-142.

Cooper, W. C.; Gaffey, W. R. (1975) Mortality of lead workers. J. Occup. Med. 17: 100-107.

Cooper, W. C.; Tabershaw, I. R.; Nelson, K. W. (1973) Laboratory studies of workers in lead smelting and refining. In: Barth, D.; Brlin, A.; Engel, R.; Recht, P.; Smeets, J., eds. Environmental health aspects of lead: proceedings, international symposium; October 1972; Amsterdam, The Netherlands, Luxembourg: Commission of the European Communities, Centre for Information and Documentation; pp. 517-529

Cory-Slechta, D. A.; Thompson, T. (1979) Behavioral toxicity of chronic postweaning lead exposure in the rat. Toxicol. Appl. Pharmacol. 47: 151-159.

Cory-Slechta, D. A.; Bissen, S. T.; Young, A. M.; Thompson, T. (1981) Chronic postweaning lead exposure and response duration performance. Toxicol. Appl. Pharmacol. 60: 78-84.

Coss, R. G.; Glohus, A. (1978) Spine stems on tectal interneurons in jewel fish are shortened by social stimulation. Science (Washington D.C.) 100: 787-790.

Costa, M. (1980) Metal carcinogensis testing: principles and in vitro methods. Clifton, NJ: Humana Press, Inc.

Cowan, L. D.; Leviton, A. (1980) Epidemiologic considerations in the study of the sequelae of low level lead exposure. In: Needleman, H. L., ed. Low level lead exposure: the clinical implications of current research. New York, NY: Raven Press; pp. 91-119.

Cramer, K.; Dahlberg, L. (1966) Incidence of hypertension among lead workers: a follow-up study based on regular control over 20 years. Br. J. Ind. Med. 23: 101-104.

Cramer, K.; Goyer, R. A.; Jagenburg, R.; Wilson, M. H. (1974) Renal ultrastructure, renal function, and parameters of lead toxicity in workers with different periods of lead exposure. Br. J. Ind. Med. 31: 113-127.

Crofton, K. M.; Taylor, D. H.; Bull, R. J.; Sivulka, D. J.; Lutkenhoff, S. D. (1980) Developmental delays in exploration and locomotor activity in male rats exposed to low level lead. Life Sci. 26: 823-831.

Cumings, J. N. (1959) Heavy metals and the brain. Part 3: Lead. Springfield, IL: Thomas; pp. 93-155.

Cutler, M. G. (1977) Effects of exposure to lead on social behaviour in the laboratory mouse. Psychopharmacology 52: 279-282.

Dagg, J. H.; Goldberg, A.; Lochhead, A.; Smith, J. A. (1965) The relationship of lead poisoning to acute intermittent porphyria. Q. J. Med. 34: 163-175.

Dahlgren, J. (1978) Abdominal pain in lead workers. Arch. Environ. Health 33: 156-159.

Danilović, V. (1958) Chronic nephritis due to ingestion of lead-contaminated flour. Br. Med. J. 1(5061): 27-28.

Danjoy, D'L. (1864) De l'albuminurie dans l'encephalopathie et l'amaurose saturnines. [On albuminuria in encephalopathy and lead amaurosis.] Arch. Gen. Med. 1: 402-423.

David, O.; Clark, J.; Voeller, K. (1972) Lead and hyperactivity. Lancet 2(7783): 900-903.

David, O. J.; Hoffman, S. P.; Sverd, J.; Clark, J.; Voeller, K. (1976) Lead and hyperactivity: behavioral response to chelation: a pilot study. Am. J. Psychiatry 133: 1155-1158.

David, O. J.; Clark, J.; Hoffman, S. (1979a) Childhood lead poisoning: a re-evaluation. Arch. Environ. Health 34: 106-111.

David, O. J.; Hoffman, S.; Kagey, B. (1979b) Subclinical lead levels and behavior in children. In: Hemphill, D. D., ed. Trace substances in environmental health-XIII: [proceedings of University of Missouri's 13th annual conference on trace substances in environmental health]; June; Columbia, MO. Columbia, MO: University of Missouri-Columbia; pp. 52-58.

David, O. J.; Wintrob, H. L.; Arcoleo, C. G. (1982a) Blood lead stability. Arch. Environ. Health 37: 147-150.

David, O. J.; Grad, G.; McGann, B.; Koltun, A. (1982b) Mental retardation and "nontoxic" lead levels. Am. J. Psychiatry 139: 806-809.

David, O. J.; Hoffman, S.; Clark, J.; Grad, G.; Sverd, J. (1983) Penicillamine in the treatment of hyperactive children with moderately elevated lead levels. In: Rutter, M.; Russell Jones, R., eds. Lead versus health: sources and effects of low level lead exposure. New York, NY: John Wiley & Sons; pp. 297-317.

Davis, J. M. (1982) Ethological approaches to behavioral toxicology. In: Mitchell, C. L., ed. Nervous system toxicology. New York, NY: Raven Press; pp. 29-44. (Dixon, R. L., ed. Target organ toxicology series).

Davis, J. R.; Abrahams, R. H.; Fischbein, W. I.; Fabrega, E. A. (1968) Urinary delta-aminolevulinic acid (ALA) levels in lead poisoning. II: Correlation of ALA values with clinical findings in 250 children with suspected lead ingestion. Arch. Environ. Health 17: 164-171.

Davison, A. N. (1977) The biochemistry of brain development and mental retardation. Br. J. Psychiatry 131: 565-574.

De Gennaro, L. D. (1978) The effects of lead nitrate on the central nervous system of the chick embryo. I: Observations of light and electron microscopy. Growth 42: 141-155.

De la Burde, B.; Choate, M. S., Jr. (1972) Does asymptomatic lead exposure in children have latent sequelae? J. Pediatr. (St. Louis) 81: 1088-1091.

De la Burde, B.; Choate, M. S., Jr. (1975) Early asymptomatic lead exposure and development at school age. J. Pediatr. (St. Louis) 87: 638-642.

Deknudt, G.; Deminatti, M. (1978) Chromosome studies in human lymphocytes after in vitro exposure to metal salts. Toxicology 10: 67-75.

Deknudt, G.; Gerber, G. B. (1979) Chromosomal aberrations in bone-marrow cells of mice given a normal or a calcium-deficient diet supplemented with various heavy metals. Mutat. Res. 68: 163-168.

Deknudt, G.; Leonard, A.; Ivanov, B. (1973) Chromosome aberrations observed in male workers occupationally exposed to lead. Environ. Physiol. Biochem. 3: 132-138.

Deknudt, G.; Colle, A.; Gerber, G. B. (1977a) Chromosomal abnormalities in lymphocytes from monkeys poisoned with lead. Mutat. Res. 45: 77-83.

Deknudt, G.; Manuel, Y.; Gerber, G. B. (1977b) Chromosomal aberrations in workers professionally exposed to lead. J. Toxicol. Environ. Health 3: 885-891.

Der, R.; Fahim, Z.; Hilderbrand, D.; Fahim, M. (1974) Combined effect of lead and low protein diet on growth, sexual development, and metabolism in female rats. Res. Commun. Chem. Pathol. Pharmacol. 9: 723-738.

Dieter, M. P.; Finley, M. T. (1979) δ-Aminolevulinic acid dehydratase enzyme activity in blood, brain, and liver of lead-dosed ducks. Environ. Res. 19: 127-135.

Dietz, D. D.; McMillan, D. E.; Grant, L. D.; Kimmel, C. A. (1978) Effects of lead on temporally-spaced responding in rats. Drug Chem. Toxicol. 1: 401-419.

Dietz, D. D.; McMillan, D. E.; Mushak, P. (1979) Effects of chronic lead administration on acquisition and performance of serial position sequences by pigeons. Toxicol. Appl. Pharmacol. 47: 377-384.

Dilts, P. V., Jr.; Ahokas, R. A. (1979) Effects of dietary lead and zinc on pregnancy. Am. J. Obstet. Gynecol. 135: 940-946.

Dilts, P. V., Jr.; Ahokas, R. A. (1980) Effects of dietary lead and zinc on fetal organ growth. Am. J. Obstet. Gynecol. 136: 889-896.

Dingwall-Fordyce, I.; Lane, R. E. (1963) A follow-up study of lead workers. Br. J. Ind. Med. 20: 313-315.

Dipaolo, J. A.; Nelson, R. L.; Casto, B. C. (1978) In vitro neoplastic transformation of Syrian hamster cells by lead acetate and its relevance to environmental carcinogenesis. Br. J. Cancer 38: 452-455.

Dobbing, J. (1970) Undernutrition and the developing brain. In: Himwich, W. A., ed. Developmental neurobiology. Springfield, IL: Charles C. Thomas Co.; pp. 241-261.

Dolinsky, Z. S.; Burright, R. G.; Donovick, P. J.; Glickman, L. T.; Babish, J.; Summers, B.; Cypess, R. H. (1981) Behavioral effects of lead and Toxocara canis in mice. Science (Washington D.C.) 213: 1142-1144.

Donald, J. M.; Cutler, M. G.; Moore, M. R.; Bradley, M. (1981) Development and social behaviour in mice after prenatal and postnatal administration of low levels of lead acetate. Neuropharmacology 20: 1097-1104.

Donovan, M. P.; Schein, L. G.; Thomas, J. A. (1980) Inhibition of androgen-receptor interaction in mouse prostate gland cytosol by divalent metal ions. Mol. Pharmacol. 17: 156-162.

Dresner, D. L.; Ibrahim, N. G.; Mascarenhas, B. R.; Levere, R. D. (1982) Modulation of bone marrow heme and protein synthesis by trace elements. Environ. Res. 28: 55-66.

Drew, W. G.; Kostas, J.; McFarland, D. J.; De Rossett, S. E. (1979) Effects of neonatal lead exposure on apomorphine-induced aggression and stereotypy in the rat. Pharmacology 18: 257-262.

Driscoll, J. W.; Stegner, S. E. (1976) Behavioral effects of chronic lead ingestion on laboratory rats. Pharmacol. Biochem. Behav. 4: 411-417.

Driscoll, J. W.; Stegner, S. E. (1978) Lead-produced changes in the relative rate of open field activity of laboratory rats. Pharmacol. Biochem. Behav. 8: 743-747.

Dubas, T. C.; Hrdina, P. D. (1978) Behavioural and neurochemical consequences of neonatal exposure to lead in rats. J. Environ. Pathol. Toxicol. 2: 473-484.

Dubas, T. C.; Stevenson, A.; Singhal, R. L.; Hrdina, P. D. (1978) Regional alterations of brain biogenic amines in young rats following chronic lead exposure. Toxicology 9: 185-190.

Dyck, P. J.; Windebank, A. J.; Low, P. A.; Baumann, W. J. (1980) Blood nerve barrier in rat and cellular mechanisms of lead-induced segmental demyelination. J. Neuropathol. Exp. Neurol. 39: 700-709.

Eales, W. (1905) Lead as an abortifacient [Letter]. Br. Med. J. 1:653.

Edens, F. W.; Benton, E.; Bursian, S. J.; Morgan, G. W. (1976) Effect of dietary lead on reproductive performance in Japanese quail, Coturnix coturnix japonica. Toxicol. Appl. Pharmacol. 38: 307-314.

Elardo, R.; Bradley, R.; Caldwell, B. M. (1975) The relation of infants' home environments to mental test performance from six to thirty-six months: a longitudinal analysis. Child Develop. 46: 71-76.

Ely, D. L.; Mostardi, R. A.; Woebkenberg, N.; Worstell, D. (1981) Aerometric and hair trace metal content in learning-disabled children. Environ. Res. 25: 325-339.

Emmerson, B. T. (1963) Chronic lead nephropathy: the diagnostic use of calcium EDTA and the association with gout. Australas. Ann. Med. 12: 310-324.

Emmerson, B. T. (1968) The clinical differentiation of lead gout from primary gout. Arthritis Rheum. 11: 623-634.

Emmerson, B. T. (1973) Chronic lead nephropathy. Kidney Int. 4: 1-5.

Emmerson, B. T. (1980) Uricosuric diuretics. Kidney Int. 18: 677-685.

Emmerson, B. T.; Mirosch, W.; Douglas, J. B. (1971) The relative contributions of tubular reabsorption and secretion to urate excretion in lead nephropathy. Aust. N.Z. J. Med. 4: 353-362.

Englert, N. (1980) Periphere motorische Nervenleitgeschwindigkeit bei Probanden mit beruflicher Blei-Exposition. [Peripheral motor nerve conduction velocity in test subjects occupationally exposed to lead.] Arbeitsmed. Sozialmed. Praeventivmed. 15: 254-260.

Ennis, J. M.; Harrison, H. E. (1950) Treatment of lead encephalopathy with BAL (2,3-dimercaptopropanol). Pediatrics 5: 853-868.

Epstein, S. S.; Mantel, N. (1968) Carcinogenicity of tetraethyl lead. Experientia 24: 580-581.

Erenberg, G.; Rinsler, S. S.; Fish, B. G. (1974) Lead neuropathy and sickle cell disease. Pediatrics 54: 438-441.

Ernhart, C. B.; Landa, B.; Schell, N. B. (1981) Subclinical levels of lead and developmental deficit - a multivariate follow-up reassessment. Pediatrics 67: 911-919.

Eskew, A. E.; Crutcher, J. C.; Zimmerman, S. L.; Johnston, G. W.; Butz, W. C. (1961) Lead poisoning resulting from illicit alcohol consumption. J. Forensic Sci. 6: 337-350.

Ewers, U.; Erbe, R. (1980) Effects of lead, cadmium and mercury on brain adenylate cyclase. Toxicology 16: 227-237.

Ewers, U.; Stiller-Winkler, R.; Idel, H. (1982) Serum immunoglobulin, complement C3, and salivary IgA levels in lead workers. Environ. Res. 29: 351-357.

Exon, J. H.; Koller, L. D.; Kerkvliet, N. I. (1979) Lead-cadmium interaction: effects on viral-induced mortality and tissue residues in mice. Arch. Environ. Health 34: 469-475.

Eyden, B. P.; Maisin, J. R.; Mattelin, G. (1978) Long-term effects of dietary lead acetate on survival, body weight and seminal cytology in mice. Bull. Environ. Contam. Toxicol. 19: 266-272.

Fahim, M. S.; Fahim, Z.; Hall, D. G. (1976) Effects of subtoxic lead levels on pregnant women in the state of Missouri. Res. Commun. Chem. Pathol. Pharmacol. 13: 309-331.

Faith, R. E.; Luster, M. I.; Kimmel, C. A. (1979) Effect of chronic developmental lead exposure on cell-mediated immune functions. Clin. Exp. Immunol. 35: 413-420.

Fejerman, N.; Gimenez, E. R.; Vallejo, N. E.; Medina, C. S. (1973) Lennox's syndrome and lead intoxication. Pediatrics 52: 227-234.

Feldman, R. G.; Haddow, J.; Chisolm, J. J. (1973) Chronic lead intoxication in urban children: motor nerve conduction velocity studies. In: Desmedt, J.; Karger, S., eds. New developments in electromyography and clinical neurophysiology; v. 2. Basel, Switzerland: S. Karger; pp. 313-317.

Feldman, R. G.; Haddow, J.; Kopito, L.; Schwachman, H. (1973) Altered peripheral nerve conduction velocity. Am. J. Dis. Child 125: 39-41.

Feldman, R. G.; Hayes, M. K.; Younes, R.; Aldrich, F. D. (1977) Lead neuropathy in adults and children. Arch. Neurol. 34: 481-488.

Ferm, V. H. (1969) The synteratogenic effect of lead and cadmium. Experientia 25: 56-57.

Ferm, V. H.; Carpenter, S. J. (1967) Developmental malformations resulting from the administration of lead salts. Exp. Mol. Pathol. 7: 208-213.

Ferm, V. H.; Ferm, D. W. (1971) The specificity of the teratogenic effect of lead in the golden hamster. Life Sci. 10: 35-39.

Fessel, W. J. (1979) Renal outcomes of gout and hyperuricemia. Am. J. Med. 67: 74-82.

Filkins, J. P. (1970) Bioassay of endotoxin inactivation in the lead-sensitized rat (34844). Proc. Soc. Exp. Biol. Med. 134: 610-612.

Filkins, J. P.; Buchanan, B. J. (1973) Effects of lead acetate on sensitivity to shock, intravascular carbon and endotoxin clearances, and hepatic endotoxin detoxification. Proc. Soc. Exp. Biol. Med. 142: 471-475.

Fineberg, S. K.; Altschul, A. (1956) The nephropathy of gout. Ann. Intern. Med. 44: 1182-1194.

Finelli, V. N.; Klauder, D. S.; Karaffa, M. A.; Petering, H. G. (1975) Interaction of zinc and lead on δ-aminolevulinate dehydratase. Biochem. Biophys. Res. Commun. 65: 303-311.

Fischbein, A.; Alvares, A. P.; Anderson, K. E.; Sassa, S.; Kappas, A. (1977) Lead intoxication among demolition workers: the effect of lead on the hepatic cytochrome P-450 system in humans. J. Toxicol. Environ. Health 3: 431-437.

Fischbein, A.; Rice, C.; Sarkozi, L.; Kon, S. H.; Petrocci, M.; Selikoff, I. J. (1979) Exposure to lead in firing ranges. J. Am. Med. Assoc. 241: 1141-1144.

Fischbein, A.; Thornton, J.; Blumberg, W. E.; Bernstein, J.; Valciukas, J. A.; Moses, M.; Davidow, B.; Kaul, B.; Sirota, M.; Selikoff, I. J. (1980) Health status of cable splicers with low-level exposure to lead: results of a clinical survey. Am. J. Public Health 70: 697-700.

Flatmark, T.; Romslo, I. (1975) Energy-dependent accumulation of iron by isolated rat liver mitochondria. J. Biol. Chem. 250: 6433-6438.

Fleischer, N.; Mouw, D. R.; Vander, A. J. (1980) Chronic effects of lead on renin and renal sodium excretion. J. Lab. Clin. Med. 95: 759-770.

Flynn, J. C.; Flynn, E. R.; Patton, J. H. (1979) Effects of pre- and post-natal lead on affective behavior and learning in the rat. Neurobehav. Toxicol. 1 (Suppl. 1): 93-103.

Fonzi, S.; Penque, L.; Raddi, R. (1967a) Processi immunitari nella intossicazione sperimentale da piombo. Nota II. Comportamento delle globuline anticorpali in corso di intossicazione, prima e durante stimolo vaccinico. [Immunitary processes in experimental lead poisoning. Note II. Behavior of anti-body globulins before and during active immunization.] Lav. Um. 19: 200-205.

Fonzi, S.; Penque, L.; Raddi, R. (1967b) Processi immunitari nella intossicazione sperimentale da piombo. Nota VI. Variazioni degli anticorpi antitifici (completi ed incompleti) in corso di immunizzazione attiva specifica. [Immunitary processes in experimental lead poisoning. Note. VI. The behavior of antithyphus antibodies (complete and incomplete) during active immunization.] Lav. Um. 19: 324-328.

Forni, A.; Cambiaghi, G.; Secchi, G. C. (1976) Initial occupational exposure to lead: chromosome and biochemical findings. Arch. Environ. Health 31: 73-78.

Forni, A.; Sciamé, A.; Bertazzi, P. A.; Alessio, L. (1980) Chromosome and biochemical studies in women occupationally exposed to lead. Arch. Environ. Health 35: 139-145.

Fouts, P. J.; Page, I. H. (1942) The effect of chronic lead poisoning on arterial blood pressure in dogs. Am. Heart J. 24: 329-331.

Fowler, B. A. (1978) General subcellular effects of lead, mercury, cadmium and arsenic. Environ. Health Perspect. 22: 37-41.

Fowler, B. A.; Kimmel, C. A.; Woods, J. S.; McConnell, E. E.; Grant, L. D. (1980) Chronic low-level lead toxicity in the rat. III: An integrated assessment of long-term toxicity with special reference to the kidney. Toxicol. Appl. Pharmacol. 56: 59-77.

Fowler, B. A.; Squibb, K. S.; Oskarsson, A.; Taylor, J. A.; Carver, G. T. (1981a) Lead-induced alteration of renal mitochondrial membrane structure and function. Toxicologist 1: 19.

Fowler, B. A.; Squibb, K. S.; Oskarsson, A. (1981b) Mitochondrial membrane potential ($\Delta\Psi$) and energy-linked membrane transformation: inhibition by Pb binding in vitro. J. Cell Biol. 91: 287a.

Fox, D. A.; Sillman, A. J. (1979) Heavy metals affect rod, but not cone, photoreceptors. Science (Washington D.C.) 206: 78-80.

Fox, D. A.; Wright, A. A. (1982) Evidence that low-level developmental lead exposure produces toxic amblyopia. Soc. Neurosci. Abstr. 8: 81.

Fox, D. A.; Lewkowski, J. P.; Copper, G. P. (1977) Acute and chronic effects of neonatal lead exposure on development of the visual evoked response in rats. Toxicol. Appl. Pharmacol. 40: 449-461.

Fox, D. A.; Overmann, S. R.; Woolley, D. E. (1978) Early lead exposure and ontogeny of seizure responses in the rat. Toxicol. Appl. Pharmacol. 45: 270.

Fox, D. A.; Overmann, S. R.; Woolley, D. E. (1979) Neurobehavioral ontogeny of neonatally lead-exposed rats. II: Maximal electroshock seizures in developing and adult rats. Neurotoxicology 1: 149-170.

Freedman, M. L.; Rosman, J. (1976) A rabbit reticulocyte model for the role of hemin-controlled repressor in hypochromic anemias. J. Clin. Invest. 57: 594-599.

Freeman, R. (1965) Reversible myocarditis due to chronic lead poisoning in childhood. Arch. Dis. Child. 40: 389-393.

Fujita, H.; Orii, Y.; Sano, S. (1981) Evidence of increased synthesis of δ-aminolevulinic acid dehydratase in experimental lead-poisoned rats. Biochim. Biophys. Acta 678: 39-50.

Fullerton, P. M. (1966) Chronic peripheral neuropathy produced by lead poisoning in guinea-pigs. J. Neuropathol. Exp. Neurol. 25: 214-236.

Furst, A.; Schlauder, M.; Sasmore, D. P. (1976) Tumorigenic activity of lead chromate. Cancer Res. 36: 1779-1783.

Gainer, J. H. (1973) Activation of the Rauscher leukemia virus by metals. J. Natl. Cancer Inst. (U.S.) 51: 609-613.

Gainer, J. H. (1974) Lead aggravates viral disease and represses the antiviral activity of interferon inducers. Environ. Health Perspect. 7: 113-119.

Gainer, J. H. (1977a) Effects on interferon of heavy metal excess and zinc deficiency. Am. J. Vet. Res. 38: 863-867.

Gainer, J. H. (1977b) Effects of heavy metals and of deficiency of zinc on mortality rates in mice infected with encephalomyocarditis virus. Am. J. Vet. Res. 38: 869-872.

Gale, T. F. (1978) A variable embryotoxic response to lead in different strains of hamsters. Environ. Res. 17: 325-333.

Gallagher, K.; Matarazzo, W. J.; Gray, I. (1979) Trace metal modification of immunocompetence. II: Effect of Pb^{2+}, Cd^{2+}, and Cr^{3+} on RNA turnover, hexokinase activity, and blastogenesis during B-lymphocyte transformation in vitro. Clin. Immunol. Immunopathol. 13: 369-377.

Galle, P.; Morel-Maroger, L. (1965) Les lésions rénales du saturnisme humain et expérimental. [Renal lesions of clinical and experimental lead poisoning.] Nephron 2: 273-286.

Gant, V. A. (1938) Lead poisoning. Ind. Med. 7: 679-699.

Garcia-Cañero, R.; Kapoor, S. C.; Rabinowitz, M.; van Rossum, G. D. V. (1981) Effect of Pb on O_2 consumption and substrate transport in isolated renal proximal tubules and mitochondria. Pharmacologist 23: 190.

Garrod, A. B. (1859) Changes in the kidneys of gouty subjects. In: The nature and treatment of gout and rheumatic gout. London, United Kingdom: Walton and Maberly; pp. 236-249.

Garza-Chapa, R.; Leal-Garza, C. H. and Molina-Ballesteros, G. (1977) Analisis cromosomico en personas profesionalmente expuestas a contaminacion con plomo. [Chromosome analysis in subjects exposed professionally to lead contamination.] Arch. Invest. Med. 8: 11-20.

Gath, J.; Theiss, A. M. (1972) Chromosome studies in chemical workers. Zentralbl. Arbeitsmed. 22: 357-362.

Gaworski, C. L.; Sharma, R. P. (1978) The effects of heavy metals on [^{3}H]Thymidine uptake in lymphocytes. Toxicol. Appl. Pharmacol. 46: 305-313.

Geist, C. R.; Balko, S. W. (1980) Effects of postnatal lead acetate exposure on activity and emotionality in developing laboratory rats. Bull. Psychon. Soc. 15: 288-290.

Geist, C. R.; Mattes, B. R. (1979) Behavioral effects of postnatal lead acetate exposure in developing laboratory rats. Physiol. Psychol. 7: 399-402.

Geist, C. R.; Praed, J. E. (1982) Chronic lead exposure of rats: open-field performance. Percept. Mot. Skills 55: 487-490.

Gepper, T. (1882) Chronische Nephritis nach Bleivergiftung. [Chronic nephritis following lead poisoning.] Dtsch. Med. Wochenschr. 8: 241-242.

Gerber, G. B.; Maes, J. (1978) Heme synthesis in the lead-intoxicated mouse embryo. Toxicology 9: 173-179.

Gerber, G. B.; Maes, J.; Gilliavod, N.; Casale, G. (1978) Brain biochemistry of infant mice and rats exposed to lead. Toxicol. Lett. 2: 51-63.

Gerhardt, R. E.; Crecelius, E. A.; Hudson, J. B. (1980) Trace element content of moonshine. Arch. Environ. Health 35: 332-334.

Giannattasio, R. C.; Bedo, A. V.; Pirozzi, M. J. (1952) Lead poisoning: observations in fourteen cases. Am. J. Dis. Child. 84:316-321.

Giavini, E.; Prati, M.; Vismara, C. (1980) Effects of cadmium, lead and copper on rat preimplantation embryos. Bull. Environ. Contam. Toxicol. 25: 702-705.

Gibson, J. L.; Love, W.; Hardie, D.; Bancroft, P.; Turner, A. J. (1893) Notes on lead-poisoning as observed among children in Brisbane. In: Huxtable, L. R., ed. Intercolonial medical congress of Australasia: transactions of the 3rd session; September 1892; Sydney, Australia. Sydney, Australia: Charles Potter; pp. 76-83.

Gibson, S. L. M.; Goldberg, A. (1970) Defects in haem synthesis in mammalian tissues in experimental lead poisoning and experimental porphyria. Clin. Sci. 38: 63-72.

Gibson, T.; Simmonds, H. A.; Potter, C.; Jeyarajah, N.; Highton, J. (1978) Gout and renal function. Eur. J. Rheumatol. Inflammation 1: 79-85.

Gibson, T.; Highton, J.; Potter, C.; Simmonds, H. A. (1980a) Renal impairment and gout. Ann. Rheum. Dis. 39: 417-423.

Gibson, T.; Simmonds, H. A.; Potter, C.; Rogers, V. (1980b) A controlled study of the effect of long term allopurinol treatment on renal function in gout. In: Rapado, A.; Watts, R. W. E.; DeBruyn, C. H. M. M., eds. Purine metabolism in man. III: Clinical and therapeutic aspects. New York, NY: Plenum Press; pp. 257-262.

Gilani, S. H. (1973a) Congenital anomalies in lead poisoning. Obstet. Gynecol. 41: 265-269.

Gilani, S. H. (1973b) Congenital cardiac anomalies in lead poisoning. Pathol. Microbiol. (Basel) 39: 85-90.

Gmerek, D. E.; McCafferty, M. R.; O'Neill, K. J.; Melamed, B. R.; O'Neill, J. J. (1981) Effect of inorganic lead on rat brain mitochondrial respiration and energy production. J. Neurochem. 36: 1109-1113.

Goddard, G. A.; Robinson, J. D. (1976) Uptake and release of calcium by rat brain synaptosomes. Brain Res. 110: 331-350.

Goldberg, A. (1968) Lead poisoning as a disorder of heme biosynthesis. Semin. Hematol. 5: 424-433.

Goldberg, A. M.; Meredith, P. A.; Miller, S.; Moore, M. R.; Thompson, G. G. (1978) Hepatic drug metabolism and haem biosynthesis in lead-poisoned rats. Br. J. Pharmacol. 62: 529-536.

Goldman, D.; Hejtmancik, M. R., Jr.; Williams, B. J.; Ziegler, M. G. (1980) Altered noradrenergic systems in the lead-exposed neonatal rat. Neurobehav. Toxicol. 2: 337-343.

Goldman, J. M.; Vander, A. J.; Mouw, D. R.; Keiser, J.; Nicholls, M. G. (1981) Multiple short-term effects of lead on the renin-angiotensin system. J. Lab. Clin. Med. 97: 251-263.

Goldstein, G. W.; Wolinksy, J. S.; Csejtey, J. (1977) Isolated brain capillaries: a model for the study of lead encephalopathy. Ann. Neurol. 1: 235-239.

Goldstein, G. W.; Asbury, A. K.; Diamond, I. (1974) Pathogenesis of lead encephalopathy: uptake of lead and reaction of brain capillaries. Arch. Neurol. (Chicago) 31: 382-389.

Golter, M.; Michaelson, I. A. (1975) Growth, behavior, and brain catecholamines in lead-exposed neonatal rats: a reappraisal. Science (Washington D.C.) 187: 359-361.

Golubovich, E. Ya.; Avkhimenko, M. M.; Chirkova, E. M. (1968) Biochemical and morphological changes in the testicles of rats induced by small doses of lead. Toksikol. Nov. Prom. Khim. Veschestv 10: 63-73.

Gonick, H. C.; Rubini, M. E.; Gleason, I. O.; Sommers, S. C. (1965) The renal lesion in gout. Ann. Intern. Med. 62: 667-674.

Gonzalez, J. J.; Werk, E. E.; Thrasher, K.; Loadholdt, C. B. (1978) The renin aldosterone system and potassium levels in chronic lead intoxication. Clin. Res. 26: 47A.

Govoni, S.; Montefusco, O.; Spano, P. F.; Trabucchi, M. (1978a) Effect of chronic lead treatment on brain dopamine synthesis and serum prolactin release in the rat. Toxicol. Lett. 2: 333-337.

Govoni, S.; Spano, P. F.; Trabucchi, M. (1978b) Neurochemical changes induced by dietary lead chronic assumption. Dev. Toxicol. Environ. Sci. 3: 381-385.

Govoni, S.; Memo, M.; Spano. P. F.; Trabucchi, M. (1979) Chronic lead treatment differentially affects dopamine synthesis in various rat brain areas. Toxicology 12: 343-349.

Govoni, S.; Memo, M.; Lucchi, L.; Spano, P. F.; Trabucchi, M. (1980) Brain neurotransmitter systems and chronic lead intoxication. Pharmacol. Res. Commun. 12: 447-460.

Goyer, R. A. (1968) The renal tubule in lead poisoning. I: Mitochondrial swelling and aminoaciduria. Lab. Invest. 19: 71-77.

Goyer, R. A. (1971) Lead toxicity: a problem in environmental pathology. Am. J. Pathol. 64: 167-181.

Goyer, R. A.; Krall, A. R. (1969a) Further observations on the morphology and biochemistry of mitochondria from kidneys of normal and lead-intoxicated rats. Fed. Proc. Fed. Am. Soc. Exp. Biol. 28: 619A.

Goyer, R. A.; Krall, A. R. (1969b) Ultrastructural transformation in mitochondria isolated from kidneys of normal and lead-intoxicated rats. J. Cell Biol. 41: 393-400.

Goyer, R. A.; Moore, J. F. (1974) Cellular effects of lead. Adv. Exp. Med. Biol. 48: 447-462.

Goyer, R. A.; Rhyne, B. C. (1973) Pathological effects of lead. Int. Rev. Exp. Pathol. 12: 1-77.

Goyer, R. A.; Wilson, M. H. (1975) Lead-induced inclusion bodies: results of ethylenediaminetetraacetic acid treatment. Lab. Invest. 32: 149-156.

Goyer, R. A.; Leonard, D. L.; Moore, J. F.; Rhyne, B.; Krigman, M. R. (1970a) Lead dosage and the role of the intranuclear inclusion body: an experimental study. Arch. Environ. Health 20: 705-711.

Goyer, R. A.; Leonard, D. L.; Bream, P. R.; Irons, T. G. (1970b) Aminoaciduria in experimental lead poisoning. (35140). Proc. Soc. Exp. Biol. Med. 135: 767-771.

Goyer, R. A.; Tsuchiya, K.; Leonard, D. L.; Kahyo, H. (1972) Aminoaciduria in Japanese workers in the lead and cadmium industries. Am. J. Clin. Pathol. 57: 635-642.

Granahan, P.; Huber, A. M. (1978) Effect of ingested lead on prenatal development and trace element deposition in the rat. Fed. Proc. Fed. Am. Soc. Exp. Biol. 37: 895.

Grandjean, P. (1979) Occupational lead exposure in Denmark: screening with the haematofluorometer. Br. J. Ind. Med. 36: 52-58.

Grandjean, P.; Lintrup, J. (1978) Erythrocyte-Zn-protoporphyrin as an indicator of lead exposure. Scand. J. Clin. Lab. Invest. 38: 669-675.

Grandjean, P.; Nielsen, O. V.; Shapiro, I. M. (1978) Lead retention in ancient Nubian and contemporary populations. J. Environ. Pathol. Toxicol. 2: 781-787.

Granick, J. L.; Sassa, S.; Granick, S.; Levere, R. D.; Kappas, A. (1973) Studies in lead poisoning. II: Correlation between the ratio of activated and inactivated δ-aminolevulinic acid dehydratase of whole blood and the blood lead level. Biochem. Med. 8: 149-159.

Granick, J. L.; Sassa, S.; Kappas, A. (1978) Some biochemical and clinical aspects of lead intoxication. Adv. Clin. Chem. 20: 287-339.

Grant, L. D.; Kimmel, C. A.; West, G. L.; Martinez-Vargas, C. M.; Howard, J. L. (1980) Chronic low-level lead toxicity in the rat. II: Effects on postnatal physical and behavioral development. Toxicol. App. Pharmacol. 56: 42-58.

Gray, L. E., Jr.; Reiter, L. (1977) Lead-induced developmental and behavioral changes in the mouse. Toxicol. Appl. Pharmacol. 41: 140.

Greenbaum, D.; Ross, J. H.; Steinberg, V. L. (1961) Renal biopsy in gout. Br. Med. J. 1(5238): 1502-1504.

Greenfield, I.; Gray, I. (1950) Lead poisoning. IX: The failure of lead poisoning to affect the heart and blood vessels. Am. Heart J. 39: 430-435.

Greengard, J.; Adams, B.; Berman, E. (1965) Acute lead encephalopathy in young children: evaluation of therapy with a corticosteroid and moderate hypothermia. J. Pediatr. (St. Louis) 66: 707-711.

Gross-Selbeck, E.; Gross-Selbeck, M. (1981) Changes in operant behavior of rats exposed to lead at the accepted no-effect level. Clin. Toxicol. 18: 1247-1256.

Gross, S. B. (1981) Human oral and inhalation exposures to lead: summary of Kehoe balance experiments. J. Toxicol. Environ. Health 8: 333-377.

Gross, S. B.; Pfitzer, E. A.; Yeager, D. W.; Kehoe, R. A. (1975) Lead in human tissues. Toxicol. Appl. Pharmacol. 32: 638-651.

Grundt, I. K.; Neskovic, N. M. (1980) Comparison of the inhibition by methylmercury and triethyllead of galactolipid accumulation in rat brain. Environ. Res. 23: 282-291.

Grundt, I. K.; Ammitzbøll, T.; Clausen, J. (1981) Triethyllead treatment of cultured brain cells. Neurochem. Res. 6: 193-201.

Gudbrandsson, T.; Hansson, L.; Herlitz, H.; Lindholm, L.; Nilsson, L. A. (1981) Immunological changes in patients with previous malignant essential hypertension. Lancet 1(8217): 406-408.

Gull, W. W.; Sutton, H. G. (1872) On the pathology of the morbid state commonly called chronic Bright's disease with contracted kidney ("arterio-capillary fibrosis"). Medico Clin. Tr. 55: 18, 274-326.

Haas, T.; Wieck, A. G.; Schaller, K. H.; Mache, K.; Valentin, H. (1972) Die usuelle Bleibelastung bei Neugeborenen und ihren Müttern. [The usual lead load in new-born infants and their mothers.] Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1: Orig. Reihe B 155: 341-349.

Hackett, P. L.; Hess, J. O.; Sikov, M. R. (1978) Lead distribution and effects during development in the rat. In: Mahlum, D. D.; Sikov, M. R.; Hackett, P. L.; Andrew, F. D., eds. Developmental toxicology of energy-related pollutants: proceedings of the 17th annual Hanford biology symposium; October 1977; Richland, WA. Available from: NTIS, Springfield, VA; CONF-771017.

Hackett, P. L.; Hess, J. O.; Sikov, M. R. (1982a) Effect of dose level and pregnancy on the distribution and toxicity of intravenous lead in rats. J. Toxicol. Environ. Health 9: 1007-1020.

Hackett, P. L.; Hess, J. O.; Sikov, M. R. (1982b) Distribution and effects of intravenous lead in the fetoplacental unit of the rat. J. Toxicol. Environ. Health 9: 1021-1032.

Haeger-Aronsen, B. (1960) Studies on urinary excretion of δ-aminolaevulic acid and other haem precursors in lead workers and lead-intoxicated rabbits. Scand. J. Clin. Lab. Invest. 12 (Suppl. 47) 1-128.

Haeger, B. (1957) Increased content of a δ-aminolaevulic acid-like substance in urine from workers in lead industry. Scand. J. Clin. Lab. Invest. 9: 211-212.

Haenninen, H.; Hernberg, S.; Mantere, P.; Vesanto, R.; Jalkanen, M. (1978) Psychological performance of subjects with low exposure to lead. J. Occup. Med. 20: 683-689.

Haenninen, H.; Mantere, P.; Hernberg, S.; Seppäläinen, A. M.; Kock, B. (1979) Subjective symptoms in low-level exposure to lead. Neurotoxicology 1: 333-347.

Hall, A.; Cantab, M. D. (1905) The increasing use of lead as an abortifacient: a series of thirty cases of plumbism. Br. Med. J. 1: 584-587.

Hamilton, D. L. (1978) Interrelationships of lead and iron retention in iron-deficient mice. Toxicol. Appl. Pharmacol. 46: 651-661.

Hammond, P. B.; Lerner, S. I.; Gartside, P. S.; Hanenson, I. B.; Roda, S. B.; Foulkes, E. C.; Johnson, D. R.; Pesce, A. J. (1980) The relationship of biological indices of lead exposure to the health status of workers in a secondary lead smelter. J. Occup. Med. 22: 475-484.

Hammond, P. B. (1971) The effects of chelating agents on the tissue distribution and excretion of lead. Toxicol. Appl. Pharmacol. 18: 296-310.

Hammond, P. B.; Hong, C. D.; O'Flaherty, E. J.; Lerner, S. I.; Hanenson, I. B. (1982) The rat as an animal model of lead nephropathy. In: Porter, G., ed. Nephrotoxic mechanisms of drugs and environmental agents. New York, NY: Plenum Press; pp. 267-277.

Hansen, J. C.; Christensen, L. B.; Tarp, U. (1980) Hair lead concentration in children with minimal cerebral dysfunction. Dan. Med. Bull. 27: 259-262.

Hansen, K. S.; Sharp, F. R. (1978) Gasoline sniffing, lead poisoning, and myoclonus. J. Am. Med. Assoc. 240: 1375-1376.

Harris, J. W.; Greenberg, M. S. (1954) Erythrocyte fragilities in plumbism. Clin. Res. Proc. 2: 55.

Hart, D.; Graziano, J.; Piomelli, S. (1980) Red blood cell protoporphyrin accumulation in experimental lead poisoning. Biochem. Med. 23: 167-178.

Harvey, P.; Hamlin, M.; Kumar, R. (1983) The Birmingham blood lead study. Presented at: annual conference of the British Psychological Society, symposium on lead and health: some psychological data; April; University of York, United Kingdom. Available for inspection at: U.S. Environmental Protection Agency, Environmental Criteria and Assessment Office, Research Triangle Park, NC.

Hasan, J.; Vihko, V.; Hernberg, S. (1967) Deficient red cell membrane /Na^+ + K^+/-ATPase in lead poisoning. Arch. Environ. Health 14: 313-318.

Hass, G. M.; Brown, D. V. L.; Eisenstein, R.; Hemmens, A. (1964) Relations between lead poisoning in rabbit and man. Am. J. Pathol. 45: 691-727.

Hass, G. M.; McDonald, J. H.; Oyasu, R.; Battifora, H. A.; Palouchek, J. T. (1967) Renal neoplasia induced by combinations of dietary lead subacetate and N-2-fluorenulacetamide. In: King, J. S., Jr., ed. Renal neoplasia. Boston, MA: Little, Brown and Company; pp. 377-412.

Hastings, L.; Cooper, G. P.; Bornschein, R. L.; Michaelson, I. A. (1977) Behavioral effects of low level neonatal lead exposure. Pharmacol. Biochem. Behav. 7: 37-42.

Hastings, L.; Cooper, G. P.; Bornschein, R. L.; Michaelson, I. A. (1979) Behavioral deficits in adult rats following neonatal lead exposure. Neurobehav. Toxicol. 1: 227-231.

Havelda, C. J.; Sohi, G. S.; Richardson, C. E. (1980) Evaluation of lead, zinc, and copper excretion in chronic moonshine drinkers. South. Med. J. 73: 710-715.

Heck, J. D.; Costa, M. (1982a) In vitro assessment of the toxicity of metal compounds. I: Mammalian cell transformation. Biol. Trace Elem. Res. 4: 71-82.

Heck, J. D.; Costa, M. (1982b) In vitro assessment of the toxicity of metal compounds. II: Mutagenesis. Biol. Trace Elem. Res. 4: 319-330.

Heddle, J. A.; Bruce, W. R. (1977) Comparison of tests for mutagenicity or carcinogenicity using assays for sperm abnormalities, formation of micronuclei, and mutations in Salmonella. In: Hiatt, H. H.; Watson, J. D.; Winsten, J. A., eds. Origins of human cancer. Book C: Human risk assessment. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory; pp. 1549-1557. (Cold Spring Harbor conferences on cell proliferation: v. 4).

Hejtmancik, M., Jr.; Williams, B. J. (1977) Lead (Pb) exposure and norepinephrine (NE) cardiotoxicity: participation of the vagus nerve. Pharmacologist 19: 134.

Hejtmancik, M., Jr.; Williams, B. J. (1978) Neonatal lead (Pb) exposure and norepinephrine (NE) cardiotoxicity. Pharmacologist 20: 590.

Hejtmancik, M. R., Jr.; Williams, B. J. (1979a) Effect of chronic lead exposure on the direct and indirect components of the cardiac response to norepinephrine. Toxicol. Appl. Pharmacol. 51: 239-245.

Hejtmancik, M., Jr.; Williams, B. J. (1979b) Time and level of perinatal lead exposure for development of norepinephrine cardiotoxicity. Res. Commun. Chem. Pathol. Pharmacol. 24: 367-376.

Hemphill, F. E.; Kaeberle, M. L.; Buck, W. B. (1971) Lead suppression of mouse resistance to Salmonella typhimurium. Science (Washington D.C.) 172: 1031-1032.

Henck, P. S.; Vanzant, F. R.; Nomland, R. (1941) Basis for the early differential diagnosis of gout. J. Am. Med. Assoc. 116: 217-229.

Henderson, D. A. (1954) A follow-up of cases of plumbism in children. Australas. Ann. Med. 3: 219-224.

Henderson, D. A.; Inglis, J. A. (1957) The lead content of bone in chronic Bright's disease. Australas. Ann. Med. 6: 145-154.

Heptinstall, R. H. (1974) Pathology of the kidney. 2nd Ed., vol. 2. Boston, MA: Little, Brown and Company; pp. 1043-1122.

Herber, R. F. M. (1980) Estimation of blood lead values from blood porphyrin and urinary δ-aminolevulinic acid levels in workers. Int. Arch. Occup. Environ. Health 45: 169-179.

Hernberg, S.; Nikkanen, J. (1970) Enzyme inhibition by lead under normal urban conditions. Lancet 1(7637): 63-64.

Hernberg, S.; Nurminen, M.; Hasan, J. (1967a) Nonrandom shortening of red cell survival times in men exposed to lead. Environ. Res. 1: 247-261.

Hernberg, S.; Veikko, V.; Hasan, J. (1967b) Red cell membrane ATPases in workers exposed to inorganic lead. Arch. Environ. Health 14: 319-324.

Hesley, K. L.; Wimbish, G. H. (1981) Blood lead and zinc protoporphyrin in lead industry workers. Am. Ind. Hyg. Assoc. J. 42: 42-46.

Hicks, R. M. (1972) Air-borne lead as an environmental toxin: a review. Chem. Biol. Interact. 5: 361-390.

Hietanen, E.; Kilpiö, J.; Koivusaari, U.; Nevalainen, T.; Närhi, M.; Savolainen, H.; Vainio, H. (1980) Neurotoxicity of lead in rabbits. Dev. Toxicol. Environ. Sci. 8: 67-70.

Hilbelink, D. R. (1980) Caudal dysplasia: an animal model in the cadmium-lead treated golden hamster. Teratology 21: 44A.

Hilderbrand, D. C.; Der, R.; Griffin, W. T.; Fahim, M. S. (1973) Effect of lead acetate on reproduction. Am. J. Obstet. Gynecol. 115: 1058-1065.

Hill, A. B. (1966) Principles of medical statistics. 8th Ed. New York, NY: Oxford University Press.

Hinton, D. E.; Heatfield, B. M.; Lipsky, M. M.; Trump, B. F. (1980) Animal model: chemically induced renal tubular carcinoma in rats. Am. J. Pathol. 100: 317-320.

Hirano, A.; Kochen, J. A. (1973) Neurotoxic effects of lead in the chick embryo. Lab. Invest. 29: 659-668.

Hirsch, G. H. (1973) Effect of chronic lead treatment on renal function. Toxicol. Appl. Pharmacol. 25: 84-93.

Hoffman, D. J.; Niyogi, S. K. (1977) Metal mutagens and carcinogens affect RNA synthesis rates in a distinct manner. Science (Washington D.C.) 198: 513-514.

Hoffmann, E. O.; Trejo, R. A.; Di Luzio, N. R.; Lamberty, J. (1972) Ultrastructural alterations of liver and spleen following acute lead administration in rats. Exp. Mol. Pathol. 17: 159-170.

Hoffmann, E. O.; Di Luzio, N. R.; Holper, K.; Brettschneider, L.; Coover, J. (1974) Ultrastructural changes in the liver of baboons following lead and endotoxin administration. Lab. Invest. 30: 311-319.

Hole, K.; Dahle, H.; Klove, H. (1979) Lead intoxication as an etiologic factor in hyperkinetic behavior in children: a negative report. Acta Paediatr. Scand. 68: 759-760.

Holmes, E. W.; Kelley, W. N. (1974) Reevaluation of the pyrazinamide suppression test. In: Sperling, O.; De Vries, A.; Wyngaarden, J. B.; eds. Purine metabolism in man: biochemistry and pharmacology of uric acid metabolism. Proceedings of the international symposium on purine metabolism in man; June ±973; Tel Aviv, Israel. New York: Plenum Press; pp. 739-744. (Advances in experimental medicine and biology, v. 41.)

Holtzman, D.; Shen Hsu, J. (1976) Early effects of inorganic lead on immature rat brain mitochondrial respiration. Pediatr. Res. 10: 70-75.

Holtzman, D.; Shen Hsu, J.; Mortell, P. (1977) Effects of inorganic lead on isolated rat brain mitochondrial respiration. Pediatr. Res. 11: 407.

Holtzman, D.; Shen Hsu, J.; Mortell, P. (1978a) The pathogenesis of lead encephalopathy in the rat pup: effects of maternal $PbCO_3$ feedings from birth. Pediatr. Res. 12: 1077-1082.

Holtzman, D.; Shen Hsu, J.; Mortell, P. (1978b) In vitro effects of inorganic lead on isolated rat brain mitochondrial respiration. Neurochem. Res. 3: 195-206.

Holtzman, D.; Herman, M. M.; Shen Hsu, J.; Mortell, P. (1980a) The pathogenesis of lead encephalopathy: effects of lead carbonate feedings on morphology, lead content, and mitochondrial respiration in brains of immature and adult rats. Virchows Arch. A: Pathol. Anat. Histol. 387: 147-164.

Holtzman, D.; Obana, K.; Olson, J. (1980b) Ruthenium red inhibition of in vitro lead effects on brain mitochondrial respiration. J. Neurochem. 34: 1776-1778.

Holtzman, D.; Shen Hsu, J.; Desautel, M. (1981) Absence of effects of lead feedings and growth-retardation on mitochondrial and microsomal cytochromes in the developing brain. Toxicol. Appl. Pharmacol. 58: 48-56.

Hong, C. D.; Hanenson, I. G.; Lerner, S.; Hammond, P. B.; Pesce, A. J.; Pollak, V. E. (1980) Occupational exposure to lead: effects on renal function. Kidney Int. 18: 489-493.

Horiguchi, S.; Teramoto, K.; Nakano, H.; Shinagawa, K.; Endo, G.; Kiyota, I. (1974) Osmotic fragility test of red blood cells of lead workers by coil planet centrifuge. Osaka City Med. J. 20: 51-53.

Hubermont, G.; Buchet, J-P.; Roels, H.; Lauwerys, R. (1976) Effect of short-term administration of lead to pregnant rats. Toxicology 5: 379-384.

Hunt, T. J.; Hepner, R.; Seaton, K. W. (1982) Childhood lead poisoning and inadequate child care. Am. J. Dis. Child. 136: 538-542.

Ibrahim, N. G.; Hoffstein, S. T.; Freedman, M. L. (1979) Induction of liver cell haem oxygenase in iron-overloaded rats. Biochem. J. 180: 257-263.

Imbus, C. E.; Warner, J.; Smith, E.; Pegelow, C. H.; Allen, J. P.; Powars, D. R. (1978) Peripheral neuropathy in lead-intoxicated sickle cell patients. Muscle Nerve 1: 168-171.

Impelman, D.; Lear, C. L.; Wilson, R.; Fox, D. A. (1982) Central effects of low level developmental lead exposure on optic nerve conduction and the recoverability of geniculocortical responses in hooded rats. Soc. Neurosci. Abstr. 8: 81.

Inglis, J. A.; Henderson, D. A.; Emerson. B. T. (1978) The pathology and pathogenesis of chronic lead nephropathy occurring in Queensland. J. Pathol. 124: 65-76

International Agency for Research on Cancer. (1980) Lead and lead compounds. In: IARC working group on the carcinogenic risk of chemicals to humans: some metals and metallic compounds; October 1979; Lyon, France. Geneva, Switzerland: World Health Organization/IARC; pp. 325-416. (IARC monographs on the carcinogenic risk of chemicals to humans: v. 23.)

Irwig, L. M.; Harrison, W. O.; Rocks, P.; Webster, I.; Andrew, M. (1978) Lead and morbidity: a dose-response relationship. Lancet 2(8079): 4-7.

Ivanans, T. (1975) Effect of maternal education and ethnic background on infant development. Arch. Dis. Child. 50: 454-457.

Ivanova-Chemishanska, L.; Antonov, G.; Khinkova, L.; Volcheva, Vl.; Khristeva, V. (1980) Deistvie na oloviniya atsetat v"rkhu reproduktsiyata na m"zhki beli pl"khove. [Effect of lead acetate on reproduction in male white rats.] Khig. Zdraveopaz. 23: 304-308.

Jacquet, P. (1976) Effets du plomb administré durante la gestation à des souris C57B1. [Effects of lead administered during the gestation period of mice C57B1.] C. R. Seances Soc. Biol. Ses Fil. 170: 1319-1322.

Jacquet, P. (1977) Early embryonic development in lead-intoxicated mice. Arch. Pathol. Lab. Med. 101: 641-643.

Jacquet, P. (1978) Influence de la progestérone et de l'estradiol exogènes sur le processus de l'implantation embryonnaire, chez la souris femelle intoxiquée par le plomb. [Influence of progesterone and exogenous estradiol on the process of embryonic implantation in a female mouse with lead intoxication.] C. R. Seances Soc. Biol. Ses Fil. 172: 1037-1040.

Jacquet, P.; Gerber, G. B. (1979) Teratogenic effects of lead in the mouse. Biomedicine 30: 223-229.

Jacquet, P.; Leonard, A.; Gerber, G. B. (1975) Embryonic death in mouse due to lead exposure. Experienta 31: 24-25.

Jacquet, P.; Leonard, A.; Gerber, G. B. (1976) Action of lead on early divisions of the mouse embryo. Toxicolgy 6: 129-132.

Jacquet, P.; Gerber, G. B.; Maes, J. (1977a) Biochemical studies in embryos after exposure of pregnant mice to dietary lead. Bull. Environ. Contam. Toxicol. 18: 271-277.

Jacquet, P.; Gerber, G. B.; Leonard, A.; Maes, J. (1977b) Plasma hormone levels in normal and lead-treated pregnant mice. Experientia 33: 1375-1377.

Jason, K. M.; Kellogg, C. K. (1980) Behavioral neurotoxicity of lead. In: Singhal, P. L.; Thomas, J. A., eds. Lead toxicity. Baltimore, MD: Urban and Schwarzenberg, Inc.; pp. 241-272.

Jason, K. M.; Kellogg, C. K. (1981) Neonatal lead exposure: effects on development of behavior and striatal dopamine neurons. Pharmacol. Biochem. Behav. 15: 641-649.

Johnson, B. L.; Burg, J. R.; Xintaras, C.; Handke, J. L. (1980) A neurobehavioral examination of workers from a primary nonferrous smelter. Neurotoxicology 1: 561-581.

Johnson, D. R.; Kleinman, L. I. (1979) Effects of lead exposure on renal function in young rats. Toxicol. Appl. Pharmacol. 48: 361-367.

Johnstone, R. T. (1964) Clinical inorganic lead intoxication. Arch. Environ. Health 8: 250-255.

Joselow, M. M.; Flores, J. (1977) Application of the zinc protoporphyrin (ZP) test as a monitor of occupational exposure to lead. Am. Ind. Hyg. Assoc. J. 38: 63-66.

Kammholz, L. P.; Thatcher, L. G.; Blodgett, F. M.; Good, T. A. (1972) Rapid protoporphyrin quantitation for detection of lead poisoning. Pediatrics 50: 625-631.

Kang, H. K.; Infante, P. F.; Carra, J. S. (1980) Occupational lead exposure and cancer [Letter]. Science (Washington D.C.) 207: 935-936.

Karai, I.; Fukumoto, K.; Horiguchi, S. (1981) Studies on osmotic fragility of red blood cells determined with a coil planet centrifuge for workers occupationally exposed to lead. Int. Arch. Occup. Environ. Health 48: 273-281.

Karp, W. B.; Robertson, A. F. (1977) Correlation of human placental enzymatic activity with trace metal concentration in placentas from three geographical locations. Environ. Res. 13: 470-477.

Kassenaar, A.; Morrell, H.; London, I. M. (1957) The incorporation of glycine into globin and the synthesis of heme in vitro in duck erythrocytes. J. Biol. Chem. 229: 423-435.

Kaufman, A. (1973) Gasoline sniffing among children in a Pueblo Indian village. Pediatrics 51: 1060-1064.

Kazantzis, G. (1970) Industrial hazards to the kidney and urinary tract. In: Slater, J. D. H., ed. Sixth symposium on advanced medicine: proceedings of a conference held at the Royal College of Physicians of London; February; London, United Kingdom. London, United Kingdom: Pitman Medical & Scientific Publishing Co. Ltd.; pp. 263-274.

Kehoe, R. A. (1961a) The metabolism of lead in man in health and disease: the normal metabolism of lead. (The Harben lectures, 1960). J. R. Inst. Public Health Hyg. 24: 81-97.

Kehoe, R. A. (1961b) The metabolism of lead in man in health and disease: the metabolism of lead under abnormal conditions. (The Harben lectures, 1960). J. R. Inst. Public Health Hyg. 24: 129-143.

Kehoe, R. A. (1961c) The metabolism of lead in man in health and disease: present hygienic problems relating to the absorption of lead. (The Harben lectures, 1960). J. R. Inst. Public Health Hyg. 24: 177-203.

Kendall, R. J.; Veit, H. P.; Scanlon, P. F. (1981) Histological effects and lead concentrations in tissues of adult male ringed turtle doves that ingested lead shot. J. Toxicol. Environ. Health 8: 649-658.

Kennedy, G. L.; Arnold, D. W.; Calandra, J. C. (1975) Teratogenic evaluation of lead compounds in mice and rats. Food Cosmet. Toxicol. 13: 629-632.

Kerkvliet, N. I.; Baecher-Steppan, L. (1982) Immunotoxicology studies on lead: effects of exposure on tumor growth and cell-mediated immunity after syngeneic or allogeneic stimulation. Immunopharmacology 4: 213-224.

Khan, M. Y.; Buse, M.; Louria, D. B. (1977) Lead cardiomyopathy in mice. Arch. Pathol. Lab. Med. 101: 89-94.

Khare, N.; Der, R.; Ross, G.; Fahim, M. (1978) Prostatic cellular changes after injection of cadmium and lead into rat prostate. Res. Commun. Chem. Pathol. Pharmacol. 20: 351-365.

Khera, A. K.; Wibberley, D. G.; Dathan, J. G. (1980) Placental and stillbirth tissue lead concentrations in occupationally exposed women. Br. J. Ind. Med. 37: 394-396.

Kim C. S.; O'Tuama, L. A.; Cookson, S. L.; Mann, J. D. (1980) The effects of lead poisoning on calcium transport by brain in 30-day-old albino rabbits. Toxicol. Appl. Pharmacol. 52: 491-496.

Kimmel, C. A. (1983) Critical periods of exposure and developmental effects of lead. Neurotoxicology (IN PRESS)

Kimmel, C. A.; Grant, L. D.; Sloan, C. S.; Gladen, B. C. (1980) Chronic low-level lead toxicity in the rat. Toxicol. Appl. Pharmacol. 56: 28-41.

Kimmel, C. A.; Buelke-Sam, J.; Adams, J.; Reiter, L. W.; Sobotka, T. J.; Tilson, H. A. (1982) Design considerations in the evaluation of standardized methods in a behavioral teratology protocol. Teratology 25: 54A.

Kincaid-Smith, P. (1982) Renal pathology in hypertension and the effects of treatment. Br. J. Clin. Pharmacol. 13: 107-115.

Kiremidjian-Schumacher, L.; Stotzky, G.; Dickstein, R. A.; Schwartz, J. (1981) Influence of cadmium, lead, and zinc on the ability of guinea pig macrophages to interact with macrophage migration inhibitory factor. Environ. Res. 24: 106-116.

Kirkconnell, S. C.; Hicks, L. E. (1980) Residual effects of lead poisoning on Denver developmental screening test scores. J. Abnorm. Child Psychol. 8: 257-267.

Klein, A. W.; Koch, T.; Lapinsky, P.; Schein, J.; Moda, L. (1978) The effects of critical periods and low levels of lead exposure on prenatal rat brain morphology. Anat. Rec. 190: 447.

Kline, T. S. (1960) Myocardial changes in lead poisoning. Am. J. Dis. Child. 99: 48-54.

Kobayashi, N.; Okamoto, T. (1974) Effects of lead oxide on the induction of lung tumors in Syrian hamsters. J. Natl. Cancer Inst. (U.S.) 53: 1605-1610.

Kohn, K. W.; Ewig, R. A. G.; Erickson, L. C.; Zwelling, L. A. (1981) Measurement of strand breaks and cross-links by alkaline elution. In: Friedberg, E. C.; Hanawalt, P. C., eds. DNA repair: a laboratory manual of research procedures: vol. I, part B. New York, NY: Marcel Dekker; pp. 379-401.

Koinuma, S. (1926) Impotence of workmen. J. Am. Med. Assoc. 86: 1924.

Kolber, A. R.; Krigman, M. R.; Morell, P. (1980) The effect of in vitro and in vivo lead intoxication on monosaccharide transport in isolated rat brain microvessels. Brain Res. 192: 513-522.

Koller, L. D. (1973) Immunosuppression produced by lead, cadmium, and mercury. Am. J. Vet. Res. 34: 1457-1458.

Koller, L. D.; Kovacic, S. (1974) Decreased antibody formation in mice exposed to lead. Nature (London) 250: 148-150.

Koller, L. D.; Brauner, J. A. (1977) Decreased B-lymphocyte response after exposure to lead and cadmium. Toxicol. Appl. Pharmacol. 42: 621-624.

Koller, L. D.; Roan, J. G. (1977) Effects of lead and cadmium on mouse peritoneal macrophages. J. Reticuloendothel. Soc. 21: 7-12.

Koller, L. D.; Roan, J. G. (1980a) Effects of lead, cadmium and methylmercury on immunological memory. J. Environ. Pathol. Toxicol. 4: 47-52.

Koller, L. D.; Roan, J. G. (1980b) Response of lymphocytes from lead, cadmium, and methylmercury exposed mice in the mixed lymphocyte culture. J. Environ. Pathol. Toxicol. 4: 393-398.

Koller, L. D.; Exon, J. H.; Roan, J. G. (1976) Humoral antibody response in mice after exposure to lead or cadmium (39205). Proc. Soc. Exp. Biol. Med. 151: 339-342.

Koller, L. D.; Roan, J. G.; Kerkvliet, N. I. (1979) Mitogen stimulation of lymphocytes in CBA mice exposed to lead and cadmium. Environ. Res. 19: 177-188.

Koller, L. D.; Exon, J. H.; Moore, S. A.; Watanabe, P. G. (1983) Evaluation of ELISA for detecting in vivo chemical immunomodulation. J. Toxicol. Environ. Health 11: 15-22.

Kolton, L.; Yaari, Y. (1982) Sites of action of lead on spontaneous transmitter release from motor nerve terminals. Isr. J. Med. Sci. 18: 165-170.

Konat, G.; Clausen, J. (1978) Protein composition of forebrain myelin isolated from triethyllead-intoxicated young rats. J. Neurochem. 30: 907-909.

Konat, G.; Clausen, J. (1980) Suppressive effect of triethyllead on entry of proteins into the CNS myelin sheath in vitro. J. Neurochem. 35: 382-387.

Konat, G.; Offner, H.; Clausen, J. (1978) Effect of triethyllead on protein synthesis in rat forebrain. Exp. Neurol. 59: 162-167.

Konat, G.; Offner, H.; Clausen, J. (1979) The effect of triethyllead on total and myelin protein synthesis in rat forebrain slices. J. Neurochem. 32: 187-190.

Kopp, S. J.; Glonek, T.; Erlanger, M.; Perry, E. F.; Perry, H. M., Jr.; Bárány, M. (1980a) Cadmium and lead effects on myocardial function and metabolism. J. Environ. Pathol. Toxicol. 4: 205-227.

Kopp, S. J.; Bárány, M.; Erlanger, M.; Perry, E. F.; Perry, H. M., Jr. (1980b) The influence of chronic low-level cadmium and/or lead feeding on myocardial contractility related to phosphorylation of cardiac myofibrillar proteins. Toxicol. Appl. Pharmacol. 54: 48-56.

Kostas, J.; McFarland, D. J.; Drew, W. G. (1976) Lead-induced hyperactivity: chronic exposure during the neonatal period in the rat. Pharmacology 14: 435-442.

Kostas, J.; McFarland, D. J.; Drew, W. G. (1978) Lead-induced behavioral disorders in the rat: effects of amphetamine. Pharmacology 16: 226-236.

Kostial, K.; Vouk, V. B. (1957) Lead ions and synaptic transmission in the superior cervical ganglion of the cat. Br. J. Pharmacol. 12: 219-222.

Kotok, D. (1972) Development of children with elevated blood levels: a controlled study. J. Pediatr. (St. Louis) 80: 57-61.

Kotok, D.; Kotok, R.; Heriot, T. (1977) Cognitive evaluation of children with elevated blood lead levels. Am. J. Dis. Child. 131: 791-793.

Kowalski, S.; Parker, G. H.; Persinger, M. A. (1982) Interactions of 2-ppm lead in the water supply with food deprivation upon maze-swimming behavior of mice. Percept. Mot. Skills 55: 515-519.

Krasovskii, G. N.; Vasukovich, L. Y.; Chariev, O. G. (1979) Experimental study of biological effects of lead and aluminum following oral administration. Environ. Health Perspect. 30: 47-51.

Krehbiel, D.; Davis, G. A.; LeRoy, L. M.; Bowman, R. E. (1976) Absence of hyperactivity in lead-exposed developing rats. Environ. Health Perspect. 18: 147-157.

Kremer, H. U.; Frank, M. N. (1955) Coexisting myxedema and chronic plumbism. Ann. Intern. Med. 42: 1130-1136.

Krigman, M. R. (1978) Neuropathology of heavy metal intoxication. Environ. Health Perspect. 26: 117-120.

Krigman, M. R.; Druse, M. J.; Traylor, T. D.; Wilson, M. N.; Newell, L. R.; Hogan, E. L. (1974a) Lead encephalopathy in the developing rat. I: Effect upon myelination. J. Neuropathol. Exp. Neurol. 33: 58-73.

Krigman, M. R.; Druse, M. J.; Trayler, T. D.; Wilson, M. N.; Newell, L. R.; Hogan, E. L. (1974b) Lead encephalopathy in the developing rat. II: Effect on cortical ontogenesis. J. Neuropathol. Exp. Neurol. 33: 671-686.

Krigman, M. R.; Traylor, D. T.; Hogan, E. L.; Mushak, P. (1974c) Subcellular distribution of lead in the brains of intoxicated and control rats. J. Neuropathol. Exp. Neurol. 33: 562.

Kristensen, B. O. (1978) Increased serum levels of immunoglobulins in untreated and treated essential hypertension. I: Relation to blood pressure. Acta. Med. Scand. 203: 49-54.

Kristensen, B. O.; Andersen, P. L. (1978) Autoantibodies in untreated and treated essential hypertension. Acta Med. Scand. 203: 55-59.

Kuhnert, P. M.; Erhard, P.; Kuhnert, B. R. (1977) Lead and δ-aminolevulinic acid dehydratase in RBC's of urban mothers and fetuses. Environ. Res. 14: 73-80.

Kurlander, H. M.; Patten, B. M. (1978) Metals in spinal cord tissue of patients dying of motor neuron disease. Ann. Neurol. 6: 21-24.

Kusell, M.; Lake, L.; Andersson, M.; Gerschenson, L. E. (1978) Cellular and molecular toxicology of lead. II: Effect of lead on δ-aminolevulinic acid synthetase of cultured cells. J. Toxicol. Environ. Health 4: 515-525.

Lamola, A-A.; Joselow, M.; Yamane, T. (1975a) Zinc protoporphyrin (ZPP): a simple, sensitive, fluorometric screening test for lead poisoning. Clin. Chem. (Winston Salem N.C.) 21: 93-97.

Lamola, A-A.; Piomelli, S.; Poh-Fitzpatrick, M. B.; Yamane, T.; Harber, L. C. (1975b) Erythropoietic protoporphyria and lead intoxication: the molecular basis for difference in cutaneous photosensitivity. II: Different binding of erythrocyte protoporphyrin to hemoglobin. J. Clin. Invest. 56: 1528-1535.

Lamon, J. M.; Frykholm, B. C.; Tschudy, D. P. (1979) Hematin administration to an adult with lead intoxication. Blood 53: 1007-1011.

Lampert, P. W.; Schochet, S. S., Jr. (1968) Demyelination and remyelination in lead neuropathy. J. Neuropathol. Exp. Neurol. 27: 527-545.

Lancereaux, E. (1862) Note relative a un cas de paralysie saturnine avec altération des cordons nerveux et des muscles paralysés. [Note on a case of saturnine paralysis with alteration of the paralyzed nerve funiculi and muscles.] Gaz. Med. Paris : 709-713.

Lancranjan, I.; Popescu, H. I.; Gavanescu, O.; Klepsch, I.; Serbanescu, M. (1975) Reproductive ability of workmen occupationally exposed to lead. Arch. Environ. Health 30: 396-401.

Landaw, S. A.; Schooley, J. C.; Arroyo, F. L. (1973) Decreased erythropoietin (ESF) synthesis and impaired erythropoiesis in acutely lead-poisoned rats. Clin. Res. 21: 559.

Landrigan, P. J.; Gehlbach, S. H.; Rosenblum, B. F.; Shoults, J. M.; Candelaria, R. M.; Barthel, W. F.; Liddle, J. A.; Smrek, A. L.; Staehling, N. W.; Sanders, J. F. (1975) Epidemic lead absorption near an ore smelter: the role of particulate lead. N. Engl. J. Med. 292: 123-129.

Landrigan, P. J.; Baker, E. L., Jr.; Feldman, R. G.; Cox, D. H.; Eden, K. V.; Orenstein, W. A.; Mather, J. A.; Yankel, A. J.; von Lindern, I. H. (1976) Increased lead absorption with anemia and slowed nerve conduction in children near a lead smelter. J. Pediatr. (St. Louis) 89: 904-910.

Landrigan, P. J.; Albrecht, W. N.; Watanabe, A.; Lee, S. (1982) Health hazard evaluation HETA 80-116-1034. Cincinnati, OH: National Institute of Occupational Safety and Health, Division of Standards Development and Technology Transfer, Public Dissemination; NIOSH report no. HETA 80-116-1034. Available from: NTIS, Springfield, VA; PB82-196833.

Lane, R. E. (1949) The care of the lead worker. Br. J. Ind. Med. 6: 125-143.

Lane, R. E. (1964) Health control in inorganic lead industries: a follow-up of exposed workers. Arch. Environ. Health 8: 243-250.

Lansdown, R. G.; Shepherd, J.; Clayton, B. E.; Delves, H. T.; Graham, P. J.; Turner, W. C. (1974) Blood-lead levels, behaviour, and intelligence: a population study. Lancet 1(7857): 538-541.

Lanthorn, T.; Isaacson, R. L. (1978) Effects of chronic lead ingestion in adult rats. Physiol. Psychol. 6: 93-95.

Lauwerys, R.; Buchet, J-P.; Roels, H. A.; Materne, D. (1974) Relationship between urinary δ-aminolevulinic acid excretion and the inhibition of red cell δ-aminolevulinate dehydratase by lead. Clin. Toxicol. 7: 383-388.

Lauwerys, R.; Buchet, J-P.; Roels, H.; Hubermont, G. (1978) Placental transfer of lead, mercury, cadmium, and carbon monoxide in women. I: Comparison of the frequency distributions of the biological indices in maternal and umbilical cord blood. Environ. Res. 15: 278-289.

Lawrence, D. A. (1981a) Heavy metal modulation of lymphocyte activities. I: In vitro effects of heavy metals on primary humoral immune responses. Toxicol. Appl. Pharmacol. 57: 439-451.

Lawrence, D. A. (1981b) Heavy metal modulation of lymphocyte activities. II: Lead, an in vitro mediator of B-cell activation. Int. J. Immunopharmacol. 3: 153-161.

Lawrence, D. A. (1981c) In vivo and in vitro effects of lead on humoral and cell-mediated immunity. Infect. Immun. 31: 136-143.

Leckie, W. J. H.; Tompsett, S. L. (1958) The diagnostic and therapeutic use of edathamil calcium disodium (EDTA,VERSENE) in excessive inorganic lead absorption. Q. J. Med. 27: 65-82.

Legge, T. M. (1901) Industrial lead poisoning. J. Hyg. 1: 96-108.

Leikin, S.; Eng, G. (1963) Erythrokinetic studies of the anemia of lead poisoning. Pediatrics 31: 996-1002.

Lejeune, E.; Tolot, F.; Meunier, P. (1969) Goutte et hyperuricémie au cours du saturnisme. [Gout and hyperuricemia in lead poisoning.] Rev. Rhum. Mal. Osteo-Articulaires 36: 161-173.

Léonard, A.; Linden, G.; Gerber, G. B. (1973) Étude, chez la souris, des effets génétiques et cytogénétiques d'une contamination par le plomb. [Study in the mouse of genetic and cytogenetic effects of lead contamination.] In: Barth, D.; Berlin, A.; Engel, R.; Recht, P.; Smeets, J., eds. Environmental health aspects of lead: proceedings, international symposium; October 1972; Amsterdam, The Netherlands. Luxembourg: Commission of the European Communities, Centre for Information and Documentation; pp. 303-309.

Léonard, A.; Deknudt, G.; Debackere, M. (1974) Cytogenetic investigations on leucocytes of cattle intoxicated with heavy metals. Toxicology 2: 269-273.

Levander, O. A.; Welsh, S. O.; Morris, V. C. (1980) Erythrocyte deformability as affected by vitamin E deficiency and lead toxicity. Ann. N.Y. Acad. Sci. 355: 227-239.

Levere, R. D.; Granick, S. (1967) Control of hemoglobin synthesis in the cultured chick blastoderm. J. Biol. Chem. 242: 1903-1911.

Levi, D. S.; Curran, A. S.; Alexander, S. D.; Davidow, B.; Piomelli, S. (1976) Physiological significance of erythrocyte protoporphyrin (EP) in moderate blood lead burden. Pediatr. Res. 10: 378.

Levin, N. W.; Abrahams, O. L. (1966) Allopurinol in patients with impaired renal function. Ann. Rheum. Dis. 25: 681-687.

Lewis, B. W.; Collins, R. J.; Wilsón, H. S. (1955) Seasonal incidence of lead poisoning in children in St. Louis. South. Med. J. 48: 298-301.

Lilis, R.; Dumitriu, C.; Roventa, A.; Nestorescu, B.; Pilat, L. (1967) Renal function in chronic lead poisoning. Med. Lav. 58: 506-512.

Lilis, R.; Gavrilescu, N.; Nestorescu, B.; Durimtsiu, C.; Roventa, A. (1968) Nephropathy in chronic lead poisoning. Br. J. Ind. Med. 25: 196-202.

Lilis, R.; Fischbein, A.; Eisinger, J.; Blumberg, W. E.; Diamond, S.; Anderson, H. A.; Rom, W.; Rice, C.; Sarkozi, L.; Kon, S.; Selikoff, I. J. (1977) Prevalence of lead disease among secondary lead smelter workers and biological indicators of lead exposure. Environ. Res. 14: 255-285.

Lilis, R.; Eisinger, J.; Blumberg, W.; Fishbein, A.; Selikoff, I. J. (1978) Hemoglobin, serum iron, and zinc protoporphyrin in lead-exposed workers. Environ. Health Perspect. 25: 97-102.

Lilis, R.; Valciukas, J.; Fischbein, A.; Andrews, G.; Selikoff, I. J.; Blumberg, W. (1979) Renal function impairment in secondary lead smelter workers: correlations with zinc protoporphyrin and blood lead levels. J. Environ. Pathol. Toxicol. 2: 1447-1474.

Lilis, R.; Fischbein, A.; Valciukas, J. A.; Blumberg, W.; Selikoff, I. J. (1980) Kidney function and lead: relationships in several occupational groups with different levels of exposure. Am. J. Ind. Med. 1: 405-412.

Lin-Fu, J. S. (1972) Undue absorption of lead among children: a new look at an old problem. N. Engl. J. Med. 286: 702-710.

Lin-Fu, J. S. (1973) Vulnerability of children to lead exposure and toxicity: parts one and two. N. Engl. J. Med. 289: 1229-1233, 1289-1293.

Lindblad, B.; Lindstedt, S.; Steen, G. (1977) On the enzymatic defects in hereditary tyrosinemia. Proc. Natl. Acad. Sci. U.S.A.: 74: 4641-4645.

Linnane, J. W.; Burry, A. F.; Emmerson, B. T. (1981) Urate deposits in the renal medulla. Nephron 29: 216-222.

Loch, R. K.; Rafales, L. S.; Michaelson, I. A.; Bornschein, R. L. (1978) The role of undernutrition in animal models of hyperactivity. Life Sci. 22: 1963-1970.

Lockard, R. B. (1968) The albino rat: a defensible choice or a bad habit? Am. Psychol. 23: 734-742.

Lockard, R. B. (1971) Reflections on the fall of comparative psychology: is there a message for us all? Am. Psychol. 26: 168-179.

Lorimer, G. (1886) Saturnine gout, and its distinguishing marks. Br. Med. J. 2(1334): 163.

Lucchi, L.; Memo, M.; Airaghi, M. L.; Spano, P. F.; Trabucchi, M. (1981) Chronic lead treatment induces in rat a specific and differential effect on dopamine receptors in different brain areas. Brain Res. 213: 397-404.

Ludwig, G. D. (1957) Saturnine gout: a secondary type of gout. Arch. Int. Med. 100: 802-812.

Luster, M. I.; Faith, R. E.; Kimmel, C. A. (1978) Depression of humoral immunity in rats following chronic developmental lead exposure. J. Environ. Pathol. Toxicol. 1: 397-402.

MacGee, J.; Roda, S. M. B.; Elias, S. V.; Lington, E. A.; Tabor, M. W.; Hammond, P. B. (1977) Determination of δ-aminolevulinic acid in blood plasma and urine by gas-liquid chromatography. Biochem. Med. 17: 31-44.

Mahaffey-Six, K.; Goyer, R. A. (1972) The influence of iron deficiency on tissue content and toxicity of ingested lead in the rat. J. Lab. Clin. Med. 79: 129-136.

Mahaffey, K. R. (1981) Nutritional factors in lead poisoning. Nutr. Rev. 39: 353-362.

Mahaffey, K. R. (1983) Biotoxicity: influence of various factors. Fed. Proc. Fed. Am. Soc. Exp. Biol. 42: 1730-1733.

Mahaffey, K. R.; Michaelson, I. A. (1980) The interaction between lead and nutrition. In: Needleman, H. L., ed. Low level lead exposure: the clinical implications of current research. New York, NY: Raven Press; pp. 159-200.

Mahaffey, K. R.; Capar, S. G.; Gladen, B. C.; Fowler, B. A. (1981) Concurrent exposure to lead, cadmium, and arsenic. J. Lab. Clin. Med. 98: 463-481.

Mahaffey, K. R.; Rosen, J. F.; Chesney, R. W.; Peeler, J. T.; Smith, C. M.; De Luca, H. F. (1982) Association between age, blood lead concentration, and serum 1,25-dihydroxycholecalciferol levels in children. Am. J. Clin. Nutr. 35: 1327-1331.

Maines, M. D.; Kappas, A. (1976) The induction of heme oxidation in various tissues by trace metals: evidence for the catabolism of endogenous heme by hepatic heme oxygenase. Ann. Clin. Res. (Suppl. 17)8: 39-46.

Maisin, J. R.; Jade, J. M.; Lambiet-Collier, M. (1975) Progress report on morphological studies of the toxic effects of lead on the reproductive organs and the embryos. Economic Community of Europe Contract No. 080-74-7, Env. B. Brussels, Belgium: ECE.

Maisin, J. R.; Lambiet-Collier, M.; De Saint-Georges, L. (1978) Toxicité du plomb pour les embryons de la souris. [Lead toxicity for mouse embryos.] C. R. Seances Soc. Biol. Ses. Fil. 172: 1041-1043.

Makasev, K. K.; Krivdina, L. V. (1972) Status of the interstitial tissues of vascular walls and their penetration under lead poisoning. Tr. Nauchno-Issled. Inst. Kraev. Pathol. (Alma-Ata) 23: 11-13.

Maker, H. S.; Lehrer, G. M.; Silides, D. J. (1975) The effect of lead on mouse brain development. Environ. Res. 10: 76-91.

Makotchenko, V. M. (1965) The functional condition of the adrenal cortex in chronic poisoning with heavy metals (lead, mercury). Tr. Ukr. Nauch. Issled Inst. Eksp. Endokrinol. 20: 162-170.

Malcolm, D. (1971) Prevention of long-term sequelae following the absorption of lead. Arch. Environ. Health 23: 292-298.

Malpass, C. A.; Asokan, S. K.; Ulmer, F. T. (1971) Experimental lead cardiomyopathy. Circulation 44 (Suppl. 2): 101-104.

Mambeeva, A. A.; Kobkova, I. D. (1969) Soderzhanie katerkholaminov v tkanyakh serdechno-sosudistoi sistemy pri eksperimentalnoi svintsovoi intoksikatsii. [The concentration of catecholamines in tissues of the cardiovascular system in experimental lead intoxication.] Izv. Akad. Nauk Kaz. SSR. Ser. Biol. 1: 77-82.

Manalis, R. S.; Cooper, G. P. (1973) Presynaptic and postsynaptic effects of lead at the frog neuromuscular junction. Nature (London) 243: 354-356.

Mantere, P.; Hänninen, H.; Hernberg, S. (1982) Subclinical neurotoxic lead effects: two-year follow-up studies with psychological test methods. Neurobehav. Toxicol. Teratol. 4: 725-727.

Mao, P.; Molnar, J. J. (1967) The fine structure and histochemistry of lead-induced renal tumors in rats. Am. J. Pathol. 50: 571-603.

Marecek, J.; Shapiro, I. M.; Burke, A.; Katz, S. H.; Hediger, M. L. (1983) Low level lead exposure in childhood influences neuropsychological performance. Arch. Environ. Health.

Marlowe, M.; Errera, J. (1982) Low lead levels and behavior problems in children. Behav. Disorders 7: 163-172.

Marlowe, M.; Folio, R.; Hall, D.; Errera, J. (1982) Increased lead burdens and trace-mineral status in mentally retarded children. J. Spec. Ed. 16: 87-99.

Marlowe, M.; Errera, J.; Jacobs, J. (1983) Increased lead and cadmium burdens among mentally retarded children and children with borderline intelligence. Am. J. Ment. Defic. 87: 477-483.

Maxwell, J. D.; Meyer, U. A. (1976) Effect of lead on hepatic δ-aminolaevulinic acid synthetase activity in the rat: a model for drug sensitivity in intermittent acute porphyria. Eur. J. Clin. Invest. 6: 373-379.

Mayers, M. R. (1947) Industrial exposure to lead. Occup. Med. 3: 77-83.

Mayne, J. G. (1955) Pathological study of the renal lesions found in 27 patients with gout. In: American Rheumatism Association: proceedings of the second interim scientific session; December; Bethesda, MD. Ann. Rheum. Dis. 15: 61-62.

McAllister, R. G., Jr.; Michelakis, A. M.; Sandstead, H. H. (1971) Plasma renin activity in chronic plumbism: effect of treatment. Arch. Intern. Med. 127: 919-923.

McBride, W. G.; Black, B. P.; English, B. J. (1982) Blood lead levels and behaviour of 400 preschool children. Med. J. Aust. 2: 26-29.

McCall, R. B.; Hogarty, P. S.; Hurlburt, N. (1972) Transitions in infant sensorimotor development and the prediction of childhood IQ. Am. Psychologist 27: 728-748.

McCauley, P. T.; Bull, R. J. (1978) Lead-induced delays in synaptogenesis in the rat cerebral cortex. Fed. Proc. Fed. Am. Soc. Exp. Biol. 37: 740.

McCauley, P. T.; Bull, R. J.; Lutkenhoff, S. D. (1979) Association of alterations in energy metabolism with lead-induced delays in rat cerebral cortical development. Neuropharmacology 18: 93-101.

McClain, R. M.; Becker, B. A. (1972) Effect of organolead compounds on rat embryonic and fetal development. Toxicol. Appl. Pharmacol. 21: 265-274.

McClain, R. M.; Becker, B. A. (1975) Teratogenicity, fetal toxicity, and placental transfer of lead nitrate in rats. Toxicol. Appl. Pharmacol. 31: 72-82.

McKay, R.; Druyan, R.; Getz, G. S.; Rabinowitz, M. (1969) Intramitochondrial localization of δ-aminolaevulate synthetase and ferrochelatase in rat liver. Biochem. J. 114: 455-461.

McKhann, C. F.; Vogt, E. C. (1926) Lead poisoning in children: with notes on therapy. Am. J. Dis. Child. 32: 386-392.

McLean, K.; Parker, G. H.; Persinger, M. A. (1982) Lead in the water supply alters swimming-maze behavior in adult mice. Percept. Mot. Skills 55: 507-512.

McLellan, J. S.; VonSmolinski, A. W.; Bederka, J. P., Jr.; Boulos, B. M. (1974) Developmental toxicology of lead in the mouse. Fed. Proc. Fed. Am. Soc. Exp. Biol. 33: 288.

McMichael, A. J.; Johnson, H. M. (1982) Long-term mortality profile of heavily-exposed lead smelter workers. J. Occup. Med. 24: 375-378.

McNeil, J. L.; Ptasnik, J. A. (1975) Evaluation of long-term effects of elevated blood lead concentrations in asymptomatic children. In: Recent advances in the assessment of the health effects of environmental pollution: proceedings, international symposium, vol. 2; June 1974; Paris, France. Luxembourg: Commission of the European Communities; pp. 571-590.

McQueen, E. G. (1951) The syndrome of gout. Med. J. Aust. 1: 644-650.

Melberg, P. E.; Alhenius, S.; Engel, J.; Lundborg, P. (1976) Ontogenetic development of locomotor activity and rate of tyrosine hydroxylation. Psychopharmacology 49: 119-123.

Melgaard, B.; Clausen, J.; Rastogi, S. C. (1976) Electromyographic changes in automechanics with increased heavy metal levels. Acta Neurol. Scand. 54: 227-240.

Mellins, R. B.; Jenkins, C. D. (1955) Epidemiological and psychological study of lead poisoning in children. J. Am. Med. Assoc. 158: 15-20.

Memo, M.; Lucchi, L.; Spano, P. F.; Trabucchi, M. (1980a) Lack of correlation between the neurochemical and behavioural effects induced by d-amphetamine in chronically lead-treated rats. Neuropharmacology 19: 795-799.

Memo, M.; Lucchi, L.; Spano, P. F.; Trabucchi, M. (1980b) Effect of chronic lead treatment on gaba-ergic receptor function in rat brain. Toxicol. Lett. 6: 427-432.

Memo, M.; Lucchi, L.; Spano, P. F.; Trabucchi, M. (1981) Dose-dependent and reversible effects of lead on rat dopaminergic system. Life Sci. 28: 795-799.

Meredith, P. A.; Moore, M. R. (1979) The influence of lead on haem biosynthesis and biodegradation in the rat. Biochem. Soc. Trans. 7: 637-639.

Meredith, P. A.; Moore, M. R. (1980) The in vivo effects of zinc on erythrocyte delta-aminolaevulinic acid dehydratase in man. Int. Arch. Occup. Environ. Health 45: 163-168.

Meredith, P. A.; Campbell, B. C.; Moore, M. R.; Goldberg, A. (1977) The effects of industrial lead poisoning on cytochrome P450 mediated phenazone (antipyrine) hydroxylation. Eur. J. Clin. Pharmacol. 12: 235-239.

Meredith, P. A.; Moore, M. R.; Campbell, B. C.; Thompson, G. G.; Goldberg, A. (1978) Delta-aminolaevulinic acid metabolism in normal and lead-exposed humans. Toxicology 9: 1-9.

Michaelson, I. A. (1973) Effects of inorganic lead on RNA, DNA and protein content in the developing neonatal rat brain. Toxicol. Appl. Pharmacol. 26: 539-548.

Michaelson, I. A. (1980) An appraisal of rodent studies on the behavioral toxicity of lead the role of nutritional status. In: Singhal, P. L.; Thomas, J. A., eds. Lead toxicity. Baltimore, MD: Urban and Schwarzenberg, Inc.; pp. 301-366.

Michaelson, I. A.; Sauerhoff, M. W. (1973) The effects of chronically ingested lead on brain levels of Fe, Zn, Cu, and Mn of 25 day old rat. Life Sci. 13: 417-428.

Milar, C. R.; Schroeder, S. R.; Mushak, P.; Dolcourt, J. L.; Grant, L. D. (1980) Contributions of the caregiving environment to increased lead burden of children. Am. J. Ment. Defic. 84: 339-344.

Milar, C. R.; Schroeder, S. R.; Mushak, P.; Boone, L. (1981a) Failure to find hyperactivity in preschool children with moderately elevated lead burden. J. Pediatr. Psychol. 6: 85-95.

Milar, K. S.; Krigman, M. R.; Grant, L. D. (1981b) Effects of neonatal lead exposure on memory in rats. Neurobehav. Toxicol. Teratol. 3: 369-373.

Milburn, H.; Mitran, E.; Crockford, G. W. (1976) An investigation of lead workers for subclinical effects of lead using three performance tests. Ann. Occup. Hyg. 19: 239-249.

Millar, J. A.; Cummings, R. L. C.; Battistini, V.; Carswell, F.; Goldberg, A. (1970) Lead and δ-aminolaevulinic acid dehydratase levels in mentally retarded children and lead-poisoned suckling rats. Lancet 2(7675): 695-698.

Miller, C. D.; Buck, W. B.; Hembrough, F. B.; Cunningham, W. L. (1982) Fetal rat development as influenced by maternal lead exposure. Vet. Hum. Toxicol. 24: 163-166.

Minsker, D. H.; Moskalski, N.; Peter, C. P.; Robertson, R. T.; Bokelman, D. L. (1982) Exposure of rats to lead nitrate in utero or postpartum: effects on morphology and behavior. Biol. Neonate 41: 193-203.

Mistry, P.; Lucier, G. W.; Fowler, B. A. (1982) Characterization studies on the 63,000 dalton 203Pb binding component of rat kidney cytosol. In: 66th annual meeting of the Federation of American Societies for Experimental Biology; April; New Orleans, LA. Fed. Proc. Fed. Am. Soc. Exp. Biol. 41: 527.

Mitchell, P. R.; Martin, I. L. (1978) Is GABA release modulated by presynaptic receptors [Letter]? Nature (London) 274: 904-905.

Mitchell, R. A.; Drake, J. E.; Wittlin, L. A.; Rejent, T. A. (1977) Erythrocyte porphobilinogen synthase (delta-aminolaevulinate dehydratase) activity: a reliable and quantitative indicator of lead exposure in humans. Clin. Chem. (Winston Salem N.C.) 23: 105-111.

Modak, A. T.; Purdy, R. H.; Stavinoha, W. B. (1978) Changes in acetylcholine concentration in mouse brain following ingestion of lead acetate in drinking water. Drug Chem. Toxicol. 1: 373-389.

Monaenkova, A. M. (1957) K voprosu o funktsionalnom sostoyanii shchitovidnoi zhelezy pri chronicheskikh intoksikatsiyakh nokotorymi promyshlennymi yadami (svinets rtut). [Functional state of the thyroid in chronic intoxication with some industrial poisons.] Gig. Tr. Prof. Zabol. 1: 44-48.

Moore, J. F.; Goyer, R. A.; Wilson, M. (1973) Lead-induced inclusion bodies: solubility, amino acid content, and relationship to residual acidic nuclear proteins. Lab. Invest. 29: 488-494.

Moore, J. F.; Mushak, P.; Krigman, M. R. (1975a) Distribution of lead in subcellular fractions of cerebrums of guinea pigs. Environ. Health Perspect. 10: 266-267.

Moore, M. R.; Meredith, P. A. (1976) The association of delta-aminolevulinic acid with the neurological and behavioural effects of lead exposure. In: Hemphill, D. D., ed. Trace substances in environmental health - X: [proceedings of University of Missouri's 10th annual conference on trace substances in environmental health]; June; Columbia, MO. Columbia, MO: University of Missouri-Columbia; pp. 363-371.

Moore, M. R.; Meredith, P. A. (1979) The effect of carbon monoxide upon erythrocyte δ-aminolevulinic acid dehydratase activity. Arch. Environ. Health 34: 158-161.

Moore, M. R.; Beattie, A. D.; Thompson, G. G.; Goldberg, A. (1971) Depression of δ-aminolaevulinic acid dehydratase activity by ethanol in man and rat. Clin. Sci. 40: 81-88.

Moore, M. R.; Meredith, P. A.; Goldberg, A.; Carr, K. E.; Toner, P. G.; Lawrie, T. D. V. (1975b) Cardiac effects of lead in drinking water of rats. Clin. Sci. Mod. Med. 49: 337-341.

Moore, M. R.; Meredith, P. A.; Goldberg, A. (1977) A retrospective analysis of blood-lead in mentally retarded children. Lancet 1(8014): 717-719.

Moore, M. R.; Meredith, P. A.; Goldberg, A. (1980) Lead and heme biosynthesis. In: Singhal, P. L.; Thomas, J. A., eds. Lead toxicity. Baltimore, MD: Urban and Schwarzenberg, Inc.; pp. 79-118.

Moreau, T.; Orssaud, G.; Juquet, B.; Busquet, G. (1982) Plombémie et pression artérielle: premiers résultats d'une enquête transversale de 431 sujets de sexe mascilin. [Blood lead levels and arterial pressure: initial results of a cross sectional study of 431 male subjects.] [Letter]. Rev. Epidemol. Sante Publique. 30: 395-397.

Morgan, B. B., Jr.; Repko, J. D. (1974) Evaluation of behavioral functions in workers exposed to lead. In: Xintaras, C.; Johnson, B. L.; DeGroot, I., eds. Behavioral toxicology: early detection of occupational hazards. Washington, DC: U.S. Department of Health, Education and Welfare; pp. 248-266.

Morgan, J. M. (1968) The consequences of chronic lead exposure. Ala. J. Med. Sci. 5: 454-457.

Morgan, J. M. (1975) Chelation therapy in lead nephropathy. South. Med. J. 68: 1001-1006.

Morgan, J. M. (1976) Hyperkalemia and acidosis in lead nephropathy. South. Med. J. 69: 881-886.

Morgan, J. M.; Burch, H. B. (1972) Comparative tests for diagnosis of lead poisoning. Arch. Intern. Med. 130: 335-340.

Morgan, J. M.; Hartley, M. W.; Miller, R. E. (1966) Nephropathy in chronic lead poisoning. Arch. Intern. Med. 118: 17-29.

Morris, H. P.; Laug, E. P.; Morris, H. J.; Grant, R. L. (1938) The growth and reproduction of rats fed diets containing lead acetate and arsenic trioxide and the lead and arsenic content of newborn and suckling rats. J. Pharmacol. Exp. Ther. 64: 420-445.

Mouw, D. R.; Vander, A. J.; Cox, J.; Fleischer, N. (1978) Acute effects of lead on renal electrolyte excretion and plasma renin activity. Toxicol. Appl. Pharmacol. 46: 435-447.

Mukherji, S.; Maitra, P. (1976) Toxic effects of lead on growth and metabolism of germinating rice (Oryza sativa L.) seeds and mitosis of onion (Allium cepa L.) root tip cells. Indian J. Exp. Biol. 14: 519-521.

Mullenix, P. (1980) Effect of lead on spontaneous behavior. In: Needleman, H. L., ed. Low level lead exposure: the clinical implications of current research. New York, NY: Raven Press; pp. 211-220.

Müller, S.; Gillert, K-E.; Krause, Ch.; Gross, U.; L'Age-Shehr, J.; Diamantstein, T. (1977) Suppression of delayed type hypersensitivity of mice by lead. Experientia 33: 667-668.

Murakami, M.; Hirosawa, K. (1973) Electron microscope autoradiography of kidney after administration of ^{210}Pb in mice. Nature (London) 245: 153-154.

Murashov, B. F. (1966) Functional state of the adrenal cortex in chronic poisoning with tetraethyl lead. Gig. Tr. Prof. Zabol. 10:46-47.

Muro, L. A.; Goyer, R. A. (1969) Chromosome change in experimental lead poisoning. Arch. Pathol. 87: 660-663.

Murray, H. M.; Guruk, M.; Zenick, H. (1978) Effects of lead exposure on the developing rat parietal cortex. In: Proceedings of the 17th annual Hanford biology symposium; October 1977; Richland, WA; pp. 520-535. U.S. Department of Energy. (Symposium series v. 47.) Available from: NTIS, Springfield, VA; CONF 771017.

Murray, R. E. (1939) Plumbism and chronic nephritis in young people in Queensland. In: Service publication (School of Public Health and Tropical Medicine): number 2. Glebe, New South Wales, Australia: Australasian Medical Publishing Company, Limited; pp. 1-81.

Myerson, R. M.; Eisenhauer, J. H. (1963) Atrioventricular conduction defects in lead poisoning. Am. J. Cardiol. 11: 409-412.

Mykkänen, H. M.; Dickerson, J. W. T.; Lancaster, M. (1980) Strain differences in lead intoxication in rats. Toxicol. Appl. Pharmacol. 52: 414-421.

Nation, J. R.; Clark, D. E.; Bourgeois, A. E.; Rogers, J. K. (1982) Conditioned suppression in the adult rat following chronic exposure to lead. Toxicol. Lett. 14: 63-67.

National Academy of Sciences. (1972) Lead: airborne lead in perspective. Washington, DC: National Academy of Sciences. (Biologic effects of atmospheric pollutants.)

National Academy of Sciences, Committee on Lead in the Human Environment. (1980) Lead in the human environment. Washington, DC: National Academy of Sciences.

Neal, P. A.; Dreessen, W-C.; Edwards, T. I.; Reinhart, W. H.; Webster, S. H.; Castberg, H. T.; Fairhall, L. T. (1941) A study of the effect of lead arsenate exposure on orchardists and consumers of sprayed fruit. Washington, DC: Government Printing Office; U.S. Public Health Bulletin no. 267.

Neal, R. A. (1980) Metabolism of toxic substances. In: Doull, J.; Klaassen, C. D.; Amdur, M. O., eds. Toxicology: the basic science of poisons; 2nd ed. New York, NY: Macmillian Publishing Co., Inc.; pp. 56-69.

Nechay, B. R.; Saunders, J. P. (1978a) Inhibitory characteristics of lead chloride in sodium- and potassium-dependent adenosinetriphosphatase preparations derived from kidney, brain, and heart of several species. J. Toxicol. Environ. Health 4: 147-159.

Nechay, B. R.; Saunders, J. P. (1978b) Inhibition by lead and cadmium of human $Na^+ + K^+$ - ATPase activity. Toxicol. Appl. Pharmacol. 45: 349.

Nechay, B. R.; Saunders, J. P. (1978c) Inhibitory characteristics of cadmium, lead, and mercury in human sodium and potassium dependent adenosinetriphosphatase preparations. J. Environ. Pathol. Toxicol. 2: 283-290.

Nechay, B. R.; Williams, B. J. (1977) Characteristics of sodium and potassium dependent adenosine triphosphatase ($Na^+ + K^+$ ATPase) inhibition by lead. In: Brown, S. S., ed. Clinical chemistry and chemical toxicology of metals. New York, NY: Elsevier/North-Holland Biomedical Press; pp. 69-70.

Needleman, H. L. (1980a) Lead and neuropsychological deficit: finding a threshold. In: Needleman, H. L., ed. Low level lead exposure: the clinical implications of current research. New York, NY: Raven Press; pp. 43-51.

Needleman, H. L. (1980b) Human lead exposure difficulties and strategies in the assessment of neuropsychological impact. In: Singhal, P. L.; Thomas, J. A., eds. Lead toxicity. Baltimore, MD: Urban and Schwarzenberg, Inc.; pp. 1-18.

Needleman, H. L.; Bellinger, D.(1982) The epidemiology of low-level lead exposure in childhood. J. Am. Acad. Child Psych. 20: 496-512.

Needleman, H. L.; Landrigan, P. J. (1981) The health effects of low level exposure to lead. Ann. Rev. Public Health 2: 277-298.

Needleman, H. L.; Gunnoe, C.; Leviton, A.; Reed, R.; Peresie, H.; Maher, C.; Barrett, P. (1979) Deficits in psychological and classroom performance of children with elevated dentine lead levels. N. Engl. J. Med. 300: 689-695.

Neilan, B. A.; Taddeini, C.; McJilton, C. E.; Handwerger, B. S. (1980) Decreased T cell function in mice exposed to chronic, low levels of lead. Clin. Exp. Immunol. 39: 746-749.

Nelson, D. J.; Kiremidjian-Schumacher, L.; Stotzky, G. (1982) Effects of cadmium, lead, and zinc on macrophage-mediated cytotoxicity toward tumor cells. Environ. Res. 28: 154-163.

Nestmann, E. R.; Matula, T. I.; Douglas, G. R.; Bora, K. C.; Kowbel, D. J. (1979) Detection of the mutagenic activity of lead chromate using a battery of microbial tests. Mutat. Res. 66: 357-365.

Nicholls, D. G. (1978) Calcium transport and proton electrochemical potential gradient in mitochondria from guinea-pig cerebral cortex and rat heart. Biochem. J. 170: 511-522.

Nicoll, R. A. (1976) The interaction of porphyrin precursors with GABA receptors in the isolated frog spinal cord. Life Sci. 19: 521-525.

Nieburg, P. I.; Weiner, L. S.; Oski, B. F.; Oski, F. A. (1974) Red blood cell δ-aminolevulinic acid dehydratase activity. Am. J. Dis. Child. 127: 348-350.

Niklowitz, W. J. (1975) Neurofibrillary changes after acute experimental lead poisoning. Neurology 25: 927-934.

Niklowitz, W. J.; Mandybur, T. I. (1975) Neurofibrillary changes following childhood lead encephalopathy: case report. J. Neuropathol. Exp. Neurol. 34: 445-455.

Nishioka, H. (1975) Mutagenic activities of metal compounds in bacteria. Mutat. Res. 31: 185-189.

Nogaki, K. (1957) [On the action of lead on the body of lead refinery workers: particularly on conception, pregnancy and parturition in the case of females and on the vitality of their newborn.] Igaku Kenkyu 27: 1314-1338.

Nordenson, I.; Beckman, G.; Beckman, L.; Nordström, S. (1978) Occupational and environmental risks in and around a smelter in northern Sweden. IV: Chromosomal aberrations in workers exposed to lead. Hereditas 88: 263-267.

Nordström, S.; Beckman, L.; Nordenson, I. (1978a) Occupational and environmental risks in and around a smelter in northern Sweden. I: Variations in birth weight. Hereditas 88: 43-46.

Nordström, S.; Beckman, L.; Nordenson, I. (1978b) Occupational and environmental risks in and around a smelter in northern Sweden. III: Frequencies of spontaneous abortion. Hereditas 88: 51-54.

Nordström, S.; Beckman, L.; Nordenson, I. (1979a) Occupational and environmental risks in and around a smelter in northern Sweden. V: Spontaneous abortion among female employees and decreased birth weight in their offspring. Hereditas 90: 291-296.

Nordström, S.; Beckman, L.; Nordenson, I. (1979b) Occupational and environmental risks in and around a smelter in northern Sweden. VI: Congenital malformations. Hereditas 90: 297-302.

Norton, S.; Culver, B. (1977) A Golgi analysis of caudate neurons in rats exposed to carbon monoxide. Brain Res. 132: 455-465.

Nye, L. J. J. (1933) Chronic nephritis and lead poisoning. Sydney, Australia: Angus and Robertson, Ltd.

Odenbro, A.; Kihlström, J. E. (1977) Frequency of pregnancy and ova implantation in triethyl lead-treated mice. Toxicol. Appl. Pharmacol. 39: 359-363.

Odenbro, A.; Örberg, J.; Lundqvist, E. (1982) Progesterone and oestrogen concentration in plasma during blastocyst implanation in mice exposed to triethyl lead. Acta Pharmacol. Toxicol. 50: 241-245.

Odenbro, A.; Greenberg, N.; Vroegh, K.; Bederka, J.; Kihlstrom, J-E. (1983) Functional disturbances in lead -exposed children. Ambio 12: 40-44.

Odone, P.; Castoldi, M. R.; Guercilena, S.; Alessio, L. (1979) Erythrocyte zinc protoporphyrin as an indicator of the biological effect of lead in adults and children. In: International conference: management and control of heavy metals in the environment; September; London, United Kingdom. Edinburgh, United Kingdom: CEP Consultants, Ltd.; pp 66-69.

Ogilvie, D. M.; Martin, A. H. (1982) Aggression and open-field activity of lead-exposed mice. Arch. Environ. Contam. Toxicol. 11: 249-252.

Ogryzlo, M. A.; Urowitz, M. B.; Weber, H. M.; Houpt, J. B. (1966) Effects of allopurinol on gouty and non-gouty uric acid nephropathy. Ann. Rheum. Dis. 25: 673-680.

Ohnishi, A.; Dyck, P. J. (1981) Retardation of Schwann cell division and axonal regrowth following nerve crush in experimental lead neuropathy. Ann. Neurol. 10: 469-477.

Oliver, T. (1885) A clinical lecture on lead-poisoning. Br. Med. J. 2(1294): 731-735.

Oliver, T. (1891) Lead poisoning in its acute and chronic forms. London, United Kingdom: Yong J. Pentland.

Oliver, T. (1911) Lead poisoning and the race. Br. Med. J. 1(2628): 1096-1098.

Ollivier, A. (1863) De l'albuminurie saturnine. [On lead albuminuria.] Arch. Génér Med. 2: 530-546, 708-724.

Olson, L.; Björklund, H.; Hoffer, B.; Freedman, R.; Marwaha, J.; Palmer, M.; Seiger, A. (1981) Silent purkinje neurons: an effect of early chronic lead treatment on cerebellar grafts. In: Meeting on chemical indices and mechanisms of organ-directed toxicity; March; Barcelona, Spain. Gen. Pharmacol. 12: A6.

O'Riordan, M. L.; Evans, H. J. (1974) Absence of significant chromosome damage in males occupationally exposed to lead. Nature (London) 247: 50-53.

Osheroff, M. R.; Uno, H.; Bowman, R. E. (1982) Lead inclusion bodies in the anterior horn cells and neurons of the substantia nigra in the adult rhesus monkey. Toxicol. Appl. Pharmacol. 64: 570-576.

Oskarsson, A.; Squibb, K. S.; Fowler, B. A. (1982) Intracellular binding of lead in the kidney: the partial isolation and characterization of postmitochondrial lead binding components. Biochem. Biophys. Res. Commun. 104: 290-298.

Östberg, Y. (1968) Renal urate deposits in chronic renal insufficiency. Acta Med. Scand. 183: 197-201.

Otto, D. A.; Benignus, V. A.; Muller, K. E.; Barton, C. N. (1981) Effects of age and body lead burden on CNS function in young children. I: Slow cortical potentials. Electroencephalogr. Clin. Neurophysiol. 52: 229-239.

Otto, D.; Benignus, V.; Muller, K.; Barton, C.; Seiple, K.; Prah, J.; Schroeder, S. (1982) Effects of low to moderate lead exposure on slow cortical potentials in young children: two year follow-up study. Neurobehav. Toxicol. Teratol. 4: 733-737.

Otto, D.; Benignus, V.; Muller, K.; Barton, C. (1983) Electrophysiological evidence of changes in CNS function at low-to-moderate blood lead levels in children. In: Rutter, M.; Russell Jones, R., eds. Lead versus health: sources and effects of low level lead exposure. New York, NY: John Wiley & Sons; pp. 319-331.

Overmann, S. R. (1977) Behavioral effects of asymptomatic lead exposure during neonatal development in rats. Toxicol. Appl. Pharmacol. 41: 459-471.

Overmann, S. R.; Fox, D. A.; Woolley, D. E. (1979) Neurobehavioral ontogeny of neonatally lead-exposed rats. I: Reflex development and somatic indices. Neurotoxicology 1: 125-147.

Overmann, S. R.; Zimmer, L.; Woolley, D. E. (1981) Motor development, tissue weights and seizure susceptibility in perinatally lead-exposed rats. Neurotoxicology 2: 725-742.

Owen, G.; Lippman, G. (1977) Nutritional status of infants and young children: U.S.A. Pediatr. Clin. North Am. 24: 211-227.

Oyasu, R.; Battifora, H. A.; Clasen, R. A.; McDonald, J. H.; Hass, G. M. (1970) Induction of cerebral gliomas in rats with dietary lead subacetate and 2-acetylaminofluorene. Cancer Res. 30: 1248-1261.

Padich, R.; Zenick, H. (1977) The effects of developmental and/or direct lead exposure on FR behavior in the rat. Pharmacol. Biochem. Behav. 6: 371-375.

Paglia, D. E.; Valentine, W. N. (1975) Characteristics of a pyrimidine-specific 5'-nucleotidase in human erythrocytes. J. Biol. Chem. 250: 7973-7979.

Paglia, D. E.; Valentine, W. N.; Dahlgren, J. G. (1975) Effects of low-level lead exposure on pyrimidine 5'-nucleotidase and other erythrocyte enzymes: possible role of pyrimidine 5'-nucleotidase in the pathogenesis of lead-induced anemia. J. Clin. Invest. 56: 1164-1169.

Paglia, D. E.; Valentine, W. N.; Fink, K. (1977) Lead poisoning: further observations on erythrocyte pyrimidine-nucleotidase deficiency and intracellular accumulation of pyrimidine nucleotides. J. Clin. Invest. 60: 1362-1366.

Palmer, M. R.; Björklund, H.; Freedman, R.; Taylor, D. A.; Marwaha, J.; Olson, L.; Seiger, A.; Hoffer, B. J. (1981) Permanent impairment of spontaneous Purkinje cell discharge in cerebellar grafts caused by chronic lead exposure. Toxicol. Appl. Pharmacol. 60: 431-440.

Panova, A. (1972) Early changes in the ovarian function of women in occupational contact with inorganic lead. In: Works of the United Research Institute of Hygiene and Industrial Safety. Sofia, Bulgaria; pp. 161-166.

Pardo, V.; Perez-Stable, E.; Fisher, E. R. (1968) Ultrastructural studies in hypertension. III: Gouty nephropathy. Lab. Invest. 18: 143-150.

Parr, D. R.; Harris, E. J. (1976) The effect of lead on the calcium-handling capacity of rat heart mitochondria. Biochem. J. 158: 289-294.

Paul, C. (1860) Etude sur l'intoxication lente par les preparations de plomb; deson influence par le produit de la conception. Arch. Gen. Med. 15: 513-533.

Paulev, P-E.; Gry, C.; Døssing, M. (1979) Motor nerve conduction velocity in asymptomatic lead workers. Int. Arch. Occup. Environ. Health 43: 37-43.

Pearl, M.; Boxt, L. M. (1980) Radiographic findings in congenital lead poisoning. Radiology 136: 83-84.

Pejić, S. (1928) The nature of the primary renal lesion produced by lead. Ann. Intern. Med. 1: 577-604.

Pentschew, A. (1965) Morphology and morphogenesis of lead encephalopathy. Acta Neuropathol. 5: 133-160.

Pentschew, A.; Garro, F. (1966) Lead encephalo-myelopathy of the suckling rat and its implications on the porphyrinopathic nervous diseases: with special reference to the permeability disorders of the nervous system's capillaries. Acta Neuropathol. 6: 266-278.

Penzien, D. B.; Scott, D. R. C.; Motiff, J. P. (1982) The effects of lead toxication on learning in rats. Arch. Environ. Health 37: 85-87.

Perino, J.; Ernhart, C. B. (1974) The relation of subclinical lead level to cognitive and sensorimotor impairment in black preschoolers. J. Learn. Dis. 7: 616-620.

Perlstein, M. A.; Attala, R. (1966) Neurologic sequelae of plumbism in children. Clin. Pediatr. (Philadelphia) 5: 292-298.

Perry, H. M., Jr.; Erlanger, M. W. (1978) Pressor effects of chronically feeding cadmium and lead together. In: Hemphill, D. D., ed. Trace substances in environmental health-XII: [proceedings of University of Missouri's 12th annual conference on trace substances in environmental health]; June; Columbia, MO. Columbia MO: University of Missouri-Columbia; pp. 268-275.

Petit, T. L.; Alfano, D. P. (1979) Differential experience following developmental lead exposure: effects on brain and behavior. Pharmacol. Biochem. Behav. 11: 165-171.

Petit, T. L.; LeBoutillier, J. C. (1979) Effects of lead exposure during development on neocortical dendritic and synaptic structure. Exp. Neurol. 64: 482-492.

Petrusz, P.; Weaver, C. M.; Grant, L. D.; Mushak, P.; Krigman, M. R. (1979) Lead poisoning and reproduction: effects on pituitary and serum gonadotropins in neonatal rats. Environ. Res. 19: 383-391.

Pienta, R. J. (1980) A transformation bioassay employing cryopreserved hamster embryo cells. In: Mishra, N.; Dunkel, V.; Mehlman, M., eds. Advances in modern environmental toxicology. Vol. 1: Mammalian cell transformation by chemical carcinogens. Princeton Junction, NJ: Senate Press, Inc.; pp. 47-83.

Piepho, R. W.; Ryan, C. F.; Lacz, J. P. (1976) The effects of chronic lead intoxication on the γ-aminobutyric acid content of the rat CNS. Pharmacologist 18: 125.

Pihl, R. O; Parkes, M. (1977) Hair element content in learning disabled children. Science (Washington D.C.) 198: 204-206.

Pines, A. G. (1965) Sostoyanie pokazatelei obshchei reaktivnosti pri svintsovykh intoksikatsiyakh. [Indexes of general reactivity in saturnine toxicity.] Vroch. Delo. 3: 93-96.

Piomelli, S.; Graziano, J. (1980) Laboratory diagnosis of lead poisoning. Pediatr. Clin. North Am. 27: 843-853.

Piomelli, S.; Davidow, B.; Guinee, V. F.; Young, P.; Gay, G. (1973) The FEP (free erythrocyte porphyrins) test: a screening micromethod for lead poisoning. Pediatrics 51: 254-259.

Piomelli, S.; Seaman, C.; Zullow, D.; Curran, A.; Davidow, B. (1977) Metabolic evidence of lead toxicity in "normal" urban children. Clin. Res. 25: 459A.

Piomelli, S.; Seaman, C.; Zullow, D.; Curran, A.; Davidow, B. (1982) Threshold for lead damage to heme synthesis in urban children. Proc. Natl. Acad. Sci. U.S.A. 79: 3335-3339.

Piper, W. N.; Tephly, T. R. (1974) Differential inhibition of erythrocyte and hepatic uroporphyrinogen I synthetase activity by lead. Life Sci. 14: 873-876.

Piper, W. N.; van Lier, R. B. L. (1977) Pteridine regulation of inhibition of hepatic uroporphyrinogen I synthetase activity by lead chloride. Mol. Pharmacol. 13: 1126-1135.

Plechaty, M. M.; Noll, B.; Sunderman, F. W., Jr. (1977) Lead concentrations in semen of healthy men without occupational exposure to lead. Ann. Clin. Lab. Sci. 7: 515-518.

Popoff, N.; Weinberg, S.; Feigin, I. (1963) Pathologic observations in lead encephalopathy: with special reference to the vascular changes. Neurology 13: 101-112.

Porritt, N. (1931) Cumulative effects of infinitesimal doses of lead. Br. Med. J. 2(3680): 92-94.

Pounds, J. G.; Wright, R.; Morrison, D.; Casciano, D. A. (1982a) Effect of lead on calcium homeostasis in the isolated rat hepatocyte. Toxicol. Appl. Pharmacol. 63: 389-401.

Pounds, J. G.; Wright, R.; Kodell, R. L. (1982b) Cellular metabolism of lead: a kinetic analysis in the isolated rat hepatocyte. Toxicol. Appl. Pharmacol. 66: 88-101.

Prendergast, W. D. (1910) The classification of the symptoms of lead poisoning. Br. Med. J. 1(2576): 1164-1166.

Prerovská, I. (1973) Einfluss von Blei auf biochemische Veränderungen im Serum und Veränderungen in der Aderwand im Hinblick auf Atherosklerose. [Influence of lead on biochemical changes in serum and changes in the arteries with regard to arteriosclerosis.] In: Barth, D.; Berlin, A.; Engel, R.; Recht, P.; Smeets, J., eds. Environmental health aspects of lead: proceedings, international symposium; October 1972; Amsterdam, The Netherlands. Luxembourg: Commission of the European Communities, Centre for Information and Documentation; pp. 551-558.

Press, M. F. (1977) Lead encephalopathy in neo-natal Long-Evans rats: morphological studies. J. Neuropathol. Exp. Neurol. 34: 169-193.

Prigge, E.; Greve, J. (1977) Effekte einer Bleiinhalation allein und in Kombination mit Kohlenmonoxid bei bucgttragebdeb und tragenden Ratten und deren Feten. II. Effekte auf die Aktivitat der δ-Aminolavulinsaure-Dehydratase, den Hamatokrit und das Korpergewicht. [Effects of lead inhalation exposures alone and in combination with carbon monoxide in nonpregnant and pregnant rats and fetuses. II: Effects on δ-aminolevulinic acid dehydratase activity, hematocrit and body weight.] Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1: Orig. Reihe B 165: 294-304.

Pueschel, S. M.; Kopito, L.; Schwachman, H. (1972) Children with an increased lead burden: a screening and follow-up study. J. Am. Med. Assoc. 222: 462-466.

Puhac, I.; Hrgović, N.; Stankoviç M.; Popović, S. (1963) [Laboratory investigations of the possibility of application of lead nitrates compounds as a raticide means by decreasing reproductive capability of rats.] Acta Vet. (Belgrade) 13: 3-9.

Purdy, S. E.; Blair, J. A.; Leeming, R. J.; Hilburn, M. E. (1981) Effect of lead on tetrahydrobiopterin synthesis and salvage: a cause of neurological dysfunction. Int. J. Environ. Stud. 17: 141-145.

Qazi, Q. H.; Madahar, C.; Yuceoglu, A. M. (1980) Temporary increase in chromosome breakage in an infant prenatally exposed to lead. Hum. Genet. 53: 201-203.

Qazi, Q. H.; Go, S. C.; Smithwick, E. M.; Madahar, D. P. (1972) Osmotic resistance of abnormal red cells exposed to lead in vitro. Br. J. Hematol. 23: 631-633.

Radosević, Z.; Sarić, M.; Beritić, T.; Knezević, J. (1961) The kidney in lead poisoning. Br. J. Ind. Med. 18: 222-230.

Radulescu, I. C.; Dinischiotu, G. T.; Maugsch, C.; Ionescu, C.; Teodorescu-Exarcu, I. (1957) Recherches sur l'atteinte du rein dans le saturnisme industriel par l'étude du clearance de la créatinine et de l'urée. [Research on damage to the kidney in industrial lead poisoning through the study of creatinine and urine clearance.] Arch. Mal. Prof. 18: 125-137.

Rafales, L. S.; Bornschein, R. L.; Michaelson, I. A.; Loch, R. K.; Barker, G. F. (1979) Drug induced activity in lead-exposed mice. Pharmacol. Biochem. Behav. 10: 95-104.

Raghavan, S. R. V.; Culver, B. D.; Gonick, H. C. (1981) Erythrocyte lead-binding protein after occupational exposure. II: Influence on lead inhibition of membrane Na^+, K^+ - adenosinetriphosphatase. J. Toxicol. Environ. Health 7: 561-568.

Ramel, C. (1973) The effect of metal compounds on chromosome segregation. Mutat. Res. 21: 45-46.

Ramirez-Cervantes, B.; Embree, J. W.; Hine, C. H.; Nelson, K. M.; Varner, M. O.; Putnam, R. D. (1978) Health assessment of employees with different body burdens of lead. J. Occup. Med. 20: 610-617.

Ramsay, L. E. (1979) Hyperuricaemia in hypertension: role of alcohol. Br. Med. J. 1(6164): 653-654.

Ratcliffe, J. M. (1977) Developmental and behavioural functions in young children with elevated blood lead levels. Br. J. Prev. Soc. Med. 31: 258-264.

Reddy, T. P.; Vaidyanath, K. (1978) Synergistic interaction of gamma rays and some metallic salts in the induction of chlorophyll mutations in rice. Mutat. Res. 52: 361-365.

Reif, M. C.; Constantiner, A.; Levitt, M. F. (1981) Chronic gouty nephropathy: a vanishing syndrome? N. Engl. J. Med. 304: 535-536.

Reigart, J. R.; Graber, C. D. (1976) Evaluation of the humoral immune response of children with low level lead exposure. Bull. Environ. Contam. Toxicol. 16: 112-117.

Reiter, L. W. (1982) Age-related effects of chemicals on the nervous system. In: Hunt, V. R.; Smith, M. K.; Worth, D., eds. Banbury report 11: environmental effects on maturation; November 1981; Long Island, NY. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory; pp. 245-267.

Reiter, L. W.; MacPhail, R. C. (1982) Factors influencing motor activity measurements in neurotoxicology. In: Mitchell, C. L., ed. Nervous system toxicology. New York, NY: Raven Press; pp. 45-65. (Dixon, R. L., ed. Target organ toxicology series).

Reiter, L. W.; Anderson, G. E.; Laskey, J. W.; Cahill, D. F. (1975) Developmental and behavioral changes in the rat during chronic exposure to lead. Environ. Health Perspect. 12: 119-123.

Remmer, H.; Schenkman, J.; Estabrook, R. W.; Sasame, H.; Gillette, J.; Narasimhulu, S.; Cooper, D. Y.; Rosenthal, O. (1966) Drug interaction with hepatic microsomal cytochrome. Mol. Pharmacol. 2: 187.

Repko, J. D.; Corum, C. R. (1979) Critical review and evaluation of the neurobiological and behavorial sequelae of inorganic lead absorption. CRC Crit. Rev. Toxicol. 6: 135-187.

Reyes, A.; Mercado, E.; Goicoechea, B.; Rosado, A. (1976) Participation of membrane sulfhydryl groups in the epididymal maturation of human and rabbit spermotozoa. Fertil. Steril. 27: 1452-1458.

Reyners, H.; Gianfelici de Reyners, E.; Maisin, J. R. (1979) An ultrastructural study of the effects of lead in the central nervous system of the rat. In: International conference: management and control of heavy metals in the environment; September; London, United Kingdom. Edinburgh, United Kingdom: CEP Consultants, Ltd.; pp. 58-61.

Rice, D. C.; Willes, R. F. (1979) Neonatal low-level lead exposure in monkeys (Macaca fascicularis): effect on two-choice non-spatial form discrimination. J. Environ. Pathol. Toxicol. 2: 1195-1203.

Rice, D. C.; Gilbert, S. G.; Willes, R. F. (1979) Neonatal low-level lead exposure in monkeys: locomotor activity, schedule-controlled behavior, and the effects of amphetamine. Toxicol. Appl. Pharmacol. 51: 503-513.

Richet, G.; Albahary, C.; Ardaillou, R.; Sultan, C.; Morel-Maroqer, A. (1964) Le rein du saturnisme chronique. [The kidney in chronic lead poisoning.] Rev. Fr. Etud. Clin. Biol. 9: 188-196.

Richet, G.; Mignon, F.; Ardaillou, R. (1965) Goutte secondaire des néphropathies chroniques. [Gout secondary to chronic nephropathy.] Presse Med. 73: 633-638.

Richet, G.; Albahary, C.; Morel-Maroqer, L.; Guillaume, P.; Galle, P. (1966) Les altérations rénales dans 23 cas de saturnisme professionnel. [Renal changes in 23 cases of occupational lead poisoning.] Soc. Med. Hop. Paris 117: 441-466.

Rippe, D. F.; Berry, L. J. (1973) Metabolic manifestations of lead acetate sensitization to endotoxin in mice. J. Reticuloendothel. Soc. 13: 527-535.

Robinson, N. M.; Robinson, H. B. (1976) The mentally retarded child: a psychological approach. 2nd ed. New York, NY: McGraw-Hill; pp. 241-318.

Robinson, R. O. (1978) Tetraethyl lead poisoning from gasoline sniffing. J. Am. Med. Assoc. 240: 1373-1374.

Robinson, T. R. (1976) The health of long service tetraethyl lead workers. J. Occup. Med. 18: 31-40.

Roels, H. A.; Lauwerys, R. R.; Buchet, J-P.; Urelust, M. T. (1975a) Response of free erythrocyte protoporphyrin and urinary-d-aminolevulinic acid in men and women moderately exposed to lead. Int. Arch. Arbeitsmed. 34: 97-108.

Roels, H. A.; Buchet, J-P.; Lauwerys, R. R.; Sonnet, J. (1975b) Comparison of in vivo effect of inorganic lead and cadmium on glutathione reductase system and δ-aminolevulinate dehydratase in human erythrocytes. Br. J. Ind. Med. 32: 181-192.

Roels, H.; Buchet, J-P.; Lauwerys, R.; Hubermont, G.; Bruaux, P.; Claeys-Thoreau, F.; Lafontaine, A.; Van Overschelde, J. (1976) Impact of air pollution by lead on the heme biosynthetic pathway in school-age children. Arch. Environ. Health 31: 310-316.

Roels, H.; Lauwerys, R.; Buchet, J-P.; Hubermont, G. (1977) Effects of lead on lactating rats and their sucklings. Toxicology 8: 107-113.

Roels, H. A.; Buchet, J-P.; Bernard, A.; Hubermont, G.; Lauwerys, R. R.; Masson, P. (1978a) Investigations of factors influencing exposure and response to lead, mercury, and cadmium in man and in animals. Environ. Health Perspect. 25: 91-96.

Roels, H.; Hubermont, G.; Buchet, J-P.; Lauwerys, R. (1978b) Placental transfer of lead, mercury, cadmium, and carbon monoxide in women. III: Factors influencing the accumulation of heavy metals in the placenta and the relationship between metal concentration in the placenta and in maternal and cord blood. Environ. Res. 16: 236-247.

Roels, H. A.; Balis-Jacques, M. N.; Buchet, J-P.; Lauwerys, R. R. (1979) The influence of sex and of chelation therapy on erythrocyte protoporphyrin and urinary δ-aminolevulinic acid in lead-exposed workers. J. Occup. Med. 21: 527-539.

Rom, W. N. (1976) Effects of lead on the female and reproduction: a review. Mt. Sinai J. Med. 43: 542-552.

Rosen, J. F.; Zarate-Salvador, C.; Trinidad, E. E. (1974) Plasma lead levels in normal and lead-intoxicated children. J. Pediatr. (St. Louis) 84: 45-48.

Rosen, J. F.; Chesney, R. W.; Hamstra, A.; DeLuca, H. F.; Mahaffey, K. R. (1980) Reduction in 1,25-dihydroxyvitamin D in children with increased lead absorption. N. Engl. J. Med. 302: 1128-1131.

Rosen, J. F.; Chesney, R. W.; Hamstra, A.; DeLuca, H. F.; Mahaffey, K. R. (1981) Reduction in 1,25-dihydroxyvitamin D in children with increased lead absorption. In: Brown, S. S.; Davis, D. S., eds. Organ-directed toxicity: chemical indices and mechanisms. New York, NY: Peragmon Press; pp. 91-95.

Rosenfeld, J. B. (1974) Effect of long-term allopurinol administration on serial GFR in normotensive and hypertensive hyperuricemic subjects. Adv. Exp. Med. Biol. 41B: 581-596.

Rosenkranz, H. S.; Poirier, L. A. (1979) Evaluation of the mutagenicity and DNA-modifying activity of carcinogens and noncarcinogens in microbial systems. J. Natl. Cancer Inst. (U.S.) 62: 873-892.

Roth, H-P.; Kirchgessner, M. (1981) Zur alimentären Zinkabhangigkeit der δ-Aminolavulinsaure-Dehydratase. [Dependence of delta-aminolaevulinic acid dehydratase upon alimentary zinc.] Z. Tierphysiol. Tiernaehr. Futtermittelkd. 46: 59-63.

Rummo, J. H.; Routh, D. K.; Rummo, N. J.; Brown, J. F. (1979) Behavioral and neurological effects of symptomatic and asymptomatic lead exposure in children. Arch. Environ. Health 34: 120-124.

Rutter, M. (1980) Raised lead levels and impaired cognitive/behavioral functioning. Dev. Med. Child Neurol. (Suppl.) 42: 1-36.

Rutter, M. (1983) Low level lead exposure: sources, effects and implications. In: Rutter, M.; Russell Jones, R., eds. Lead versus health: sources and effects of low level lead exposure. New York, NY: John Wiley & Sons; pp. 333-370.

Sabbioni, E.; Marafante, E. (1976) Identification of lead-binding components in rat liver: in vivo study. Chem. Biol. Interact. 15: 1-20.

Sachs, H. K. (1978) Intercurrent infection in lead poisoning. Am. J. Dis. Child. 132: 315-316.

Sachs, H. K.; Krall, V.; McCaughran, D. A.; Rozenfeld, I. H.; Youngsmith, N.; Growe, G.; Lazar, B. S.; Novar, L.; O'Connell, L.; Rayson, B. (1978) IQ following treatment of lead poisoning: a patient-sibling comparison. J. Pediatr. (St. Louis) 93: 428-431.

Sachs, H. K.; McCaughran, D. A.; Krall, V.; Rozenfeld, I. H.; Youngsmith, N. (1979) Lead poisoning without encephalopathy: effect of early diagnosis on neurologic and psychologic salvage. Am. J. Dis. Child. 133: 786-790.

Sachs, H. K.; Krall, V.; Drayton, M. A. (1982) Neuropsychological assessment after lead poisoning without encephalopathy. Percept. Motor Skills 54: 1283-1288.

Saenger, P.; Forster, E.; Kream, J. (1981) 6β-Hydroxycortisol: a noninvasive indicator of enzyme induction. J. Clin. Endocrinol. Metab. 52: 381-384.

Saenger, P.; Markowitz, M. E.; Rosen, J. F. (1982a) 6β-hydroxycortisol (6 OHF) as index of microsomal inhibition in lead burdened children. Dev. Pharmacol. 16: 130A.

Saenger, P.; Rosen, J. F.; Markowitz, M. E. (1982b) Diagnostic significance of edetate disodium calcium testing in children with increased lead absorption. Am. J. Dis. Child. 136: 312-315.

Salaki, J.; Louria, D. B.; Thind, I. S. (1975) Influence of lead intoxication on experimental infections. Clin. Res. 23: 417A.

Sameroff, A. J.; Chandler, M. J. (1975) Reproductive risk and the continuum of caretaker casualty. In: Horowitz, F., ed. Review of research in child development: vol. 4. Chicago, IL: University of Chicago Press; pp. 187-244.

Sandberg, A. A., ed. (1982) Sister chromatid exchange. New York, NY: Alan R. Liss. (Sandberg, A. A., ed. Progress and topics in cytogenetics: v. 2.)

Sanstead, H. H.; Galloway, R. (1967) Effect of chronic lead intoxication on in vivo I^{131} uptake by the rat thyroid. (31656) Proc. Fed. Soc. Exp. Biol. Med. 124: 18-20.

Sandstead, H. H.; Stant, E. G.; Brill, A. B.; Arias, L. I.; Terry, R. T. (1969) Lead intoxication and the thyroid. Arch. Int. Med. 123: 632-635.

Sandstead, H. H.; Michelakis, A. M.; Temple, T. E. (1970a) Lead intoxication: its effects on the renin-aldosterone response to sodium deprivation. Arch. Environ. Health 20: 356-363.

Sandstead, H. H.; Orth, D. N.; Abe, K.; Stiel, J. (1970b) Lead intoxication: effect on pituitary and adrenal function in man. Clin. Res. 18: 76.

Sarto, F.; Stella, M.; Acqua, A. (1978) Studio citogenetico su un gruppo di lavoratori con indici di aumentato assorbimento di piombo. [Cytogenetic studies in 20 workers occupationally exposed to lead]. Med. Lav. 69: 172-180.

Sassa, S.; Granick, J. L.; Granick, S.; Kappas, A.; Levere, R. D. (1973) Studies in lead poisoning. I: Microanalysis of erythrocyte protoporphyrin levels by spectrofluorometry in the detection of chronic lead intoxication in the subclinical range. Biochem. Med. 8: 135-148.

Sassa, S.; Whetsell, W. J., Jr.; Kappas, A. (1979) Studies on porphyrin-heme biosynthesis in organotypic cultures of chick dorsal root ganglia. II: The effect of lead. Environ. Res. 19: 415-426.

Sauerhoff, M. W.; Michaelson, I. A. (1973) Hyperactivity and brain catecholamines in lead-exposed developing rats. Science (Washington D.C.) 182: 1022-1024.

Schlick, E.; Friedberg, K. D. (1981) The influences of low lead doses on the reticuloendothelial system and leucocytes of mice. Arch. Toxicol. 47: 197-207.

Schlipköter, H-W.; Frieler, L. (1979) Der Einfluss kurzzeitiger Bleiexposition auf die Bakterienclearance der Lunge. [The influence of short-term lead exposure on the bacterial clearance of the lung.] Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1: Orig. Reihe B 168: 256-265.

Schlipköter, H-W.; Winneke, G. (1980) Behavioral studies on the effects of ingested lead on the developing central nervous system of rats. In: Commission of the European Communities, Luxembourg. Environment and quality of life: 2nd environmental research programme, 1976-80; pp. 127-134.

Schmid, E.; Bauchinger, M.; Pietruck, S.; Hall, G. (1972) Die cytogenetische Wirkung von Blei in menschlichen peripheren Lymphocyten in vitro und in vivo. [The cytogenetic effect of lead in human peripheral lymphocytes in vitro and in vivo.] Mutat. Res. 16: 401-406.

Schnitker, M. A.; Richter, A. B. (1936) Nephritis in gout. Am. J. Med. Sci. 192: 241-252.

Schrauzer, G. N.; Kuehn, K.; Hamm, D. (1981) Effects of dietary selenium and of lead on the genesis of spontaneous mammary tumors in mice. Biol. Trace Elem. Res. 3: 185-196.

Schroeder, H. A.; Mitchener, M. (1971) Toxic effects of trace elements on the reproduction of mice and rats. Arch. Environ. Health 23: 102-106.

Schroeder, H. A.; Mitchener, M.; Nason, A. P. (1970) Zirconium, niobium, antimony, vanadium and lead in rats: life term studies. J. Nutr. 100: 59-68.

Schumann, G. B.; Lerner, S. I.; Weiss, M. A.; Gawronski, L.; Lohiya, G. K. (1980) Inclusion-bearing cells in industrial workers exposed to lead. Am. J. Clin. Pathol. 74: 192-196.

Schumer, W.; Erve, P. R. (1973) Endotoxin sensitivity of adrenalectomized rats treated with lead acetate. J. Reticuloendothel. Soc. 13: 122-125.

Schwanitz, G.; Lehnert, G.; Gebhart, E. (1970) Chromosomenschäden bei beruflicher Bleibelastung. [Chromosome damage in occupational exposure to lead.] Dtsch. Med. Wochenschr. 95: 1636-1641.

Schwanitz, G.; Gebhart, E.; Rott, H-D., Schaller, K-H.; Essing, H-G.; Lauer, O.; Prestele, H. (1975) Chromosomenunterschungen bei Personen mit beruflicher Bleiexposition. [Chromosome investigations in subjects with occupational lead exposure.] Dtsch. Med. Wochenschr. 100: 1007-1011.

Scoppa, P.; Raumengous, M.; Penning, W. (1973) Hepatic drug metabolizing activity lead-poisoned rats. Expereentia 29: 970-972.

Scott, K. M.; Hwang, K. M.; Jurkowitz, M.; Brierley, G. P. (1971) Ion transport by heart mitochondria. XXIII: The effects of lead on mitochondrial reactions. Arch. Biochem. Biophys. 147: 557-567.

Scroczynski, J.; Zajusz, K.; Kossman, S.; Wegiel, A. (1967) Effect of experimental lead poisoning on the development of arteriosclerosis. Pol. Arch. Med. Wewn. 38: 641-664.

Seawright, A. A.; Brown, A. W.; Aldridge, W. N.; Verschoyle, R. D.; Street, B. W. (1980) Neuropathological changes caused by trialkyllead compounds in the rat. Dev. Toxicol. Environ. Sci. 8: 71-74.

Secchi, G. C.; Rezzonico, A.; Alessio, L. (1968) Variazioni dell'activita' nelle diverse fasi dellintossicazione saturnina. Med. Lav. 22: 191-196.

Secchi, G.; Alessio, L.; Cambiaghi, G. (1973) Na^+/K^+-ATPase activity of erythrocyte membranes: in urban populations not occupationally exposed to lead. Arch. Environ. Health 27: 399-400.

Secchi, G. C.; Erba, L.; Cambiaghi, G. (1974) Delta-aminolevulinic acid dehydratase activity of erythrocytes and liver tissue in man: relationship to lead exposure. Arch. Environ. Health 28: 130-132.

Selander, S.; Cramér, K. (1970) Interrelationships between lead in blood, lead in urine, and ALA in urine during lead work. Br. J. Ind. Med. 27: 28-39.

Selye, H.; Tuchweber, B.; Bertok, L. (1966) Effect of lead acetate on the susceptibility of rats to bacterial endotoxins. J. Bacteriol. 91: 884-890.

Seppäläinen, A. M. (1977) Diagnostic utility of neuroelectric measures in environmental and occupational medicine. In: Otto, D. A., ed. Multidisciplinary perspectives in event-related brain potential research. Proceedings of the fourth international congress on event-related slow potentials of the brain (EPIC IV); April 1976; Hendersonville, NC. Research Triangle Park, NC: U.S. Environmental Protection Agency, Health Effects Research Laboratory, pp. 448-452; EPA report no. EPA-600/9-77-043. Available from: NTIS, Springfield, VA; PB 297137.

Seppäläinen, A. M.; Hernberg, S. (1972) Sensitive technique for detecting subclinical lead neuropathy. Br. J. Ind. Med. 29: 443-449.

Seppäläinen, A. M.; Hernberg, S. (1980) Subclinical lead neuropathy. Am. J. Ind. Med. 1: 413-420.

Seppäläinen, A. M.; Hernberg, S. (1982) A follow-up study of nerve conduction velocities in lead exposed workers. Neurobehav. Toxicol. Teratol. 4: 721-723.

Seppäläinen, A. M.; Tola, S.; Hernberg, S.; Kock, B. (1975) Subclinical neuropathy at "safe" levels of lead exposure. Arch. Environ. Health 30: 180-183.

Seppäläinen, A. M.; Hernberg, S.; Kock, B. (1979) Relationship between blood lead levels and nerve conduction velocities. Neurotoxicology 1: 313-332.

Sessa, T.; Ferrari, E.; D'Amato, C. C. (1965) Velocitadi conduzione nervosa nei saturnini. [Velocity of nerve conduction in saturnism.] Folia Med. 48: 658-668.

Seto, D. S. Y.; Freeman, J. M. (1964) Lead neuropathy in childhood. Am. J. Dis. Child. 107: 337-342.

Shannon, H. S.; Williams, M. K.; King, E. (1976) Sickness absence of lead workers and controls. Br. J. Ind. Med. 33: 236-242.

Shapiro, I. M.; Marecek, J. (1983) Environmental lead exposure and neuropsychological impairment in inner-city children. Biol. Trace Elem. Res. (in press)

Shapiro, I. M.; Dobkin, B.; Tuncay, O. C.; Needleman, H. L. (1973) Lead levels in dentine and circumpulpal dentine of deciduous teeth of normal and lead poisoned children. Clin. Chim. Acta 46: 119-123.

Sharma, R. M.; Buck, W. B. (1976) Effects of chronic lead exposure on pregnant sheep and their progeny. Vet. Toxicol. 18: 186-188.

Shelton, K. R.; Egle, P. M. (1982) The proteins of lead-induced intranuclear inclusion bodies. J. Biol. Chem. 257: 11802-11807.

Shen-Ong, G. L. C.; Keath, E. J.; Piccoli, S. P.; Cole, M. D. (1982) Novel myc oncogene RNA from abortive immunoglobulin-gene recombination in mouse plasmacytomas. Cell 31 (Part 1): 443-452.

Shenker, B. J.; Matarazzo, W. J.; Hirsch, R. L.; Gray, I. (1977) Trace metal modification of immunocompetence. 1: Effect of trace metals in the cultures on in vitro transformation of lymphocytes. Cell. Immunol. 34: 19-24.

Shigeta, S.; Misawa, T.; Aikawa, H. (1977) Effect of lead on operant behavior in rats. Tokai J. Exp. Clin. Med. 2: 153-161.

Shigeta, S.; Misawa, T.; Aikawa, H.; Hirase, F.; Nagata, M. (1979) Effects of lead on Sidman avoidance behavior by lever pressing in rats. Nippon Eiseigaku Zasshi 34: 677-682.

Silbergeld, E. K.; Adler, H. S. (1978) Subcellular mechanisms of lead neurotoxicity. Brain Res. 148: 451-467.

Silbergeld, E. K.; Goldberg, A. M. (1973) A lead-induced behavioral disorder. Life Sci. 13: 1275-1283.

Silbergeld, E. K.; Goldberg, A. M. (1974a) Hyperactivity: a lead-induced behavior disorder. Environ. Health Perspect. 7: 227-232.

Silbergeld, E. K.; Goldberg, A. M. (1974b) Lead-induced behavioral dysfunction: an animal model of hyperactivity. Exp. Neurol. 42: 146-157.

Silbergeld, E. K.; Lamon, J. M. (1980) Role of altered heme synthesis in lead neurotoxicity. J. Occup. Med. 22: 680-684.

Silbergeld, E. K.; Adler, H. S.; Costa, J. L. (1977) Subcellular localization of lead in synaptosomes. Res. Commun. Chem. Pathol. Pharmacol. 17: 715-725.

Silbergeld, E. K.; Miller, L. P.; Kennedy, S.; Eng, N. (1979) Lead, GABA, and seizures: effects of subencephalopathic lead exposure on seizure sensitivity and GABAergic function. Environ. Res. 19: 371-382.

Silbergeld, E. K.; Hruska, R. E.; Miller, L. P.; Eng, N. (1980a) Effects of lead in vivo and in vitro on GABAergic neurochemistry. J. Neurochem. 34: 1712-1718.

Silbergeld, E. K.; Wolinski, J. S.; Goldstein, G. W. (1980b) Electron probe microanalysis of isolated brain capillaries poisoned with lead. Brain Res. 189: 369-376.

Silbergeld, E. K.; Hruska, R. E.; Bradley, D. Lamon, J. M.; Frykholm, B. C. (1982) Neurotoxic aspects of porphyrinopathies: lead and succinylacetone. Environ. Res. 29: 459-471.

Silver, W.; Rodriguez-Torres, R. (1968) Electrocardiographic studies in children with lead poisoning. Pediatrics 41: 1124-1127.

Simmon, V. F. (1979) In vitro mutagenicity assays of chemical carcinogens and related compounds with Salmonella typhimurium. J. Natl. Cancer Inst. (U.S.) 62: 893-899.

Simmon, V. F.; Rosenkranz, H. S.; Zeiger, E.; Poirier, L. A. (1979) Mutagenic activity of chemical carcinogens and related compounds in the intraperitoneal host-mediated assay. J. Natl. Cancer Inst. (U.S.) 62: 911-918.

Singh, N.; Donovan, C. M.; Hanshaw, J. B. (1978) Neonatal lead intoxication in a prenatally exposed infant. J. Pediatr. (St. Louis) 93: 1019-1021.

Sirover, M. A.; Loeb, L. A. (1976) Infidelity of DNA synthesis in vitro: screening for potential metal mutagens or carcinogens. Science (Washington D.C.) 194: 1434-1436.

Smith, C. M.; DeLuca, H. F.; Tanaka, Y.; Mahaffey, K. R. (1981) Effect of lead ingestion on functions of vitamin D and its metabolites. J. Nutr. 111: 1321-1329.

Smith, F. L., 2nd; Rathmell, T. K.; Marcil, G. E. (1938) The early diagnosis of acute and latent plumbism. Am. J. Clin. Pathol. 8: 471-508.

Smith, M.; Delves, T.; Lansdown, R.; Clayton, B.; Graham, P. (1983) The effects of lead exposure on urban children: the Institute of Child Health/Southampton study. London, United Kingdom: Department of the Environment.

Snodgrass, S. R. (1978) Use of ^{3}H-muscimol for GABA receptor studies. Nature (London) 273: 392-394.

Snowdon, C. T. (1973) Learning deficits in lead-injected rats. Pharmacol. Biochem. Behav. 1: 599-603.

Sobotka, T. J.; Cook, M. P. (1974) Postnatal lead acetate exposure in rats: possible relationship to minimal brain dysfunction. Am. J. Ment. Defic. 79: 5-9.

Sobotka, T. J.; Brodie, R. E.; Cook, M. P. (1975) Psychophysiologic effects of early lead exposure. Toxicology 5: 175-191.

Sommers, S. C.; Churg, J. (1982) Kidney pathology in hyperuricemia and gout. In: Yü, T. F.; Berger, L., eds. The kidney in gout and hyperuricemia. Mt. Kisco, New York: Futura Publishing Company; pp. 95-174.

Sontag, J. M.; Page, N. P.; Saffiotti, U. (1976) Guidelines for carcinogen bioassay in small rodents. Bethesda, MD: U.S. Department of Health, Education and Welfare, National Cancer Institute; report no. NCI-CG-TR-1. DHEW publication no. (NIH) 76-801.

Sotelo, C.; Palay, S. L. (1971) Altered axons and axon terminals in the lateral vestibular nucleus of the rat: possible example of axonal remodeling. Lab. Invest. 25: 653-671.

Soyka, L. F.; Joffe, J. M. (1980) Male medicated drug effects on offspring. In: Schwarz, R. H.; Yaffe, S. J., eds. Drug and chemical risks to the fetus and newborn. Proceedings of a symposium; May 1979; New York, NY. New York, NY: Alan R. Liss; pp. 49-66.

Specter, M. J.; Grinee, V. F.; Davidow, B. (1971) The unsuitability of random urinary delta aminolevulinic acid samples as a screening test for lead poisoning. J. Pediatr. (St. Louis) 79: 799-804.

Spit, B. J.; Wibowo, A. A. E.; Feron, V. J.; Zielhuis, R. L. (1981) Ultrasonic changes in the kidneys of rabbits treated with lead acetate. Arch. Toxicol. 49: 85-91.

Spivey, G. H.; Brown, C. P.; Baloh, R. W.; Campion, D. S.; Valentine, J. L.; Massey, F. J., Jr.; Browdy, B. L.; Culver, B. D. (1979) Subclinical effects of chronic increased lead absorption--a prospective study. I: Study design and analysis of symptoms. J. Occup. Med. 21: 423-429.

Stanković, V.; Jugo, S. (1976) Supressive effect of lead on antibody response of rats to S. typhimurium. Period. Biol. 78 (Suppl. 1): 64-65.

Stephens, M. C. C.; Gerber, G. B. (1981) Development of glycolipids and gangliosides in lead treated neonatal rats. Toxicol. Lett. 7: 373-378.

Stevenson, A. J.; Kacew, S.; Singhal, R. L. (1977) Reappraisal of the use of a single dose of lead for the study of cell proliferation in kidney, liver, and lung. J. Toxicol. Environ. Health 2: 1125-1134.

Stewart, D. D. (1895) Lead convulsions: a study of sixteen cases. Am. J. Med. Sci. 109: 288-306.

Stewart, W. E. (1979) The interferon system. New York, NY: Springer-Verlag.

Stöfen, D. (1974) Blei als Umweltgift; die verdeckte Bleivergiftung-ein Massenphanomen? [Lead as an environmental toxin; hidden lead poisoning - a mass phenomenon?] Eshwege: G. E. Schroeder-Verlag.

Stoner, G. D.; Shimkin, M. B.; Troxell, M. C.; Thompson, T. L.; Terry, L. S. (1976) Test for carcinogenicity of metallic compounds by the pulmonary tumor response in strain A mice. Cancer Res. 36: 1744-1747.

Stowe, H. D.; Goyer, R. A. (1971) The reproductive ability and progeny of F_1 lead-toxic rats. Fertil. Steril. 22: 755-760.

Stowe, H. D.; Goyer, R. A.; Krigman, M. M.; Wilson, M.; Cates, M. (1973) Experimental oral lead toxicity in young dogs: clinical and morphologic effects. Arch. Pathol. 95: 106-116.

Studnitz, W. von; Haeger-Aronsen, B. (1962) Urinary excretion of amino acids in lead-poisoned rabbits. Acta Pharmacol. Toxicol. 19: 36-42.

Stuik, E. J. (1974) Biological response of male and female volunteers to inorganic lead. Int. Arch. Arbeitsmed. 33: 83-97.

Stumpf, W. E.; Sar, M.; Grant, L. D. (1980) Autoradiographic localization of ^{210}Pb and its decay products in rat forebrain. Neurotoxicology 1: 593-606.

Suga, R. S.; Fischinger, A. J.; Knoch, F. W. (1981) Establishment of normal values in adults for zinc protoporphyrin (ZPP) using hematofluorometer: correlation with normal blood lead values. Am. Ind. Hyg. Assoc. J. 42: 637-642.

Suketa, Y.; Hasegawa, A.; Yamamoto, T. (1979) Changes in sodium and potassium in urine and serum of lead-intoxicated rats. Toxicol. Appl. Pharmacol. 47: 203-207.

Suketa, Y.; Ban, K.; Yamamoto, T. (1981) Effects of ethanol and lead ingestion on urinary sodium excretion and related enzyme activity in rat kidney. Biochem. Pharmacol. 30: 2293-2297.

Swanson, M. S.; Angle, C. R.; Stohs, S. J. (1982) Effect of lead chelation therapy with EDTA in children on erythrocyte pyrimidine 5'-nucleotidase and cytidine triphospate levels. Int. J. Clin. Pharmacol. Ther. Toxicol. 20: 497-500.

Szold, P. D. (1974) Plumbism and iron deficiency [Letter]. N. Engl. J. Med. 290: 520.

Takaku, F.; Aoki, Y.; Urata, G. (1973) Delta-aminolevulinic acid synthetase activity in erythroblasts of patients with various hematological disorders. Jpn. J. Clin. Haematol. 14: 1303-1310.

Talbott, J. H. (1949) Diagnosis and treatment of gouty arthritis. Postgrad. Med. 5: 386-393.

Talbott, J. H.; Terplan, K. L. (1960) The kidney in gout. Medicine (Baltimore) 39: 405-467.

Tanis, A. L. (1955) Lead poisoning in children. Am. J. Dis. Child. 89: 325-331.

Tanqueral Des Plances. (1839) Traite des maladies de Plumb on Saturnines. Paris, Terra, Libraire Editeur, Vol. 2.

Tapp, J. T. (1969) Activity, reactivity, and the behavior-directing properties of stimuli. In: Tapp, J. T., ed. Reinforcement and behavior. New York, NY: Academic Press; pp. 146-177.

Tara, S.; Francon, F. (1975) Deux cas de goutte saturnine a modalité mineure. [Two cases of saturnine gout of minor modality.] Rhumatologic (Paris) 5: 238-243.

Taussig, F. J. (1936) Abortion spontaneous and induced: medical and social aspects. St. Louis, MO: The C. V. Mosby Company; pp. 111, 354-355.

Taylor, D.; Nathanson, J.; Hoffer, B.; Olson L.; Seiger, A. (1978) Lead blockade of norepinephrine-induced inhibition of cerebellar Purkinje neurons. J. Pharmacol. Exp. Ther. 206: 371-381.

Taylor, D. H.; Noland, E. A.; Brubaker, C. M.; Crofton, K. M.; Bull, R. J. (1982) Low level lead (Pb) exposure produces learning deficits in young rat pups. Neurobehav. Toxicol. Teratol. 4: 311-314.

Teisinger, J.; Stýblová, V. (1961) Neurologický obraz chronické otravy olovem. [Neurological picture of chronic lead poisoning.] Acta Univ. Carol. Med. Suppl. 14: 199-206.

Tennekoon, G.; Aitchison, C. S.; Frangia, J.; Price, D. L.; Goldberg, A. M. (1979) Chronic lead intoxication: effects on developing optic nerve. Ann. Neurol. 5: 558-564.

Tepper, L. B. (1963) Renal function subsequent to childhood plumbism. Arch. Environ. Health 7: 76-85.

Thatcher, R. W.; Lester, M. L.; McAlaster, R.; Horst, R. (1982a) Effects of low levels of cadmium and lead on cognitive functioning in children. Arch. Environ. Health 37: 159-166.

Thatcher, R. W.; Lester, M. L.; McAlaster, R.; Horst, R.; Ignasisa, S. W. (1982b) Intelligence and lead toxins in rural children. J. Learning Disabilities (in press)

Thind, I. S.; Khan, M. Y. (1978) Potentiation of the neurovirulence of Langat virus infection by lead intoxication in mice. Exp. Mol. Pathol. 29: 342-347.

Thind, I. S.; Singh, N. P. (1977) Potentiation of Langat virus infection by lead intoxication: influence on host defenses. Acta Virol. (Engl. Ed.) 21: 317-325.

Tilson, H. A.; Harry, G. J. (1982) Behavioral principles for use in behavioral toxicology and pharmacology. In: Mitchell, C. L., ed. Nervous system toxicology. New York, NY: Raven Press; pp. 1-27. (Dixon, R. L., ed. Target organ toxicology series.)

Timm, F.; Schulz, G. (1966) Hoden und Schwermetalle. [Testicles and heavy metals.] Histochemistry 7: 15-21.

Timpo, A. E.; Amin, J. S.; Casalino, M. B.; Yuceoglu, A. M. (1979) Congenital lead intoxication. J. Pediatr. (St. Louis) 94: 765-767.

Toews, A. D.; Kolber, A.; Hayward, J.; Krigman M. R.; Morell, P. (1978) Experimental lead encephalopathy in the suckling rat: concentration of lead in cellular fractions enriched in brain capillaries. Brain Res. 147: 131-138.

Tola, S.; Nordman, C. H. (1977) Smoking and blood lead concentrations in lead-exposed workers and an unexposed population. Environ. Res. 13: 250-255.

Tola, S.; Hernberg, S.; Asp, S.; Nikkanen, J. (1973) Parameters indicative of absorption and biological effect in new lead exposure: a prospective study. Br. J. Ind. Med. 30: 134-141.

Toriumi, H.; Kawai, M. (1981) Free erythrocyte protoporphyrin (FEP) in a general population, workers exposed to low-level lead, and organic-solvent workers. Environ. Res. 25: 310-316.

Trejo, R. A.; Di Luzio, N. R. (1971) Impaired detoxification as a mechanism of lead acetate-induced hypersensitivity to endotoxin (35388). Proc. Soc. Exp. Biol. Med. 136: 889-893.

Trejo, R. A.; Di Luzio, N. R.; Loose, L. D.; Hoffman, E. (1972) Reticuloendothelial and hepatic functional alterations following lead acetate administration. Exp. Mol. Pathol. 17: 145-158.

Truscott, R. B. (1970) Endotoxin studies in chicks: effect of lead acetate. Can. J. Comp. Med. 34: 134-137.

Tschudy, D. P.; Ebert, P. S.; Hess, R. A.; Frykholm, B. C.; Weinbach, E. C. (1980) Effect of heme depletion on growth, protein synthesis and respiration of murine erythroleukemia cells. Biochem. Pharmacol. 29: 1825-1831.

Tschudy, D. P.; Hess, R. A.; Frykholm, B. C. (1983) Inhibition of d-aminolevulinic acid dehydrase by 4,5-dioxoheptanoic acid. J. Biol. Chem.: (in press)

Tsuchiya, K.; Sugita, M.; Sakurai, H. (1978) Dose-response relationships at different exposure levels: re-examination of establishing no-effect levels. Sangyo Igaku 20: 247-253.

U.S. Centers for Disease Control. (1978) Preventing lead poisoning in young children. J. Pediatr. (St. Louis) 93: 709-720.

U.S. Environmental Protection Agency, Health Effects Research Lab. (1977) Air quality criteria for lead. Research Triangle Park, NC: U.S. Environmental Protection Agency, Criteria and Special Studies Office; EPA report no. EPA-600/8-77-017. Available from: NTIS, Springfield, VA; PB 280411.

U.S. Health Care Financing Administration. (1982) 1981 End-stage renal disease annual report to Congress. Washington, DC: U.S. Department of Health and Human Services, Health Care Financing Administration; HCFA report no. 82-02144.

Ulmer, D. D.; Vallee, B. L. (1969) Effects of lead on biochemical systems. In: Hemphill, D. D., ed. Trace substances in environmental health - II: [proceedings of the University of Missouri's 2nd annual conference on trace substances in environmental health]; July 1968; Columbia, MO. Columbia, MO: University of Missouri-Columbia; pp. 7-27.

Valciukas, J. A.; Lilis, R. (1980) Psychometric techniques in environmental research. Environ. Res. 21: 275-297.

Valciukas, J. A.; Lilis, R.; Eisinger, J.; Blumberg, W. E.; Fischbein, A.; Selikoff, I. J. (1978) Behavioral indicators of lead neurotoxicity: results of a clinical field survey. Int. Arch. Occup. Environ. Health 41: 217-236.

Valentine, J. L.; Baloh, R. W.; Browdy, B. L.; Gonick, H. C.; Brown, C. P.; Spivey, G. H.; Culver, B. D. (1982) Subclinical effects of chronic increased lead absorption--a prospective study. J. Occup. Med. 24: 120-125.

Valentine, W. N.; Paglia, D. E. (1980) Erythrocyte disorders of purine and pyrimidine metabolism. Hemoglobin 4: 669-681.

Valentine, W. N.; Paglia, D. E.; Fink, K.; Madokoro, G. (1976) Lead poisoning: association with hemolytic anemia, basophilic stippling, erythrocyte pyrimidine 5'-nucleotidase deficiency, and intraerythrocytic accumulation of pyrimidines. J. Clin. Invest. 58: 926-932.

Vallee, B. L.; Ulmer, D. D. (1972) Biochemical effects of mercury, cadmium, and lead. Annu. Rev. Biochem. 41: 91-128.

Van Assen, F. J. J. (1958) Een geval van loodvergiftiging als oorzaak van aangeboren afwijkingen bij het nageslacht. [A case of lead poisoning as cause of congenital anomalies in the offspring?] Ned. Tijdschr. Verloskd. Gynaecol. 58: 258-263.

Van den Bergh, A. A. H.; Grotepass, W. (1933) Porphyrinämie ohne Porphyrinurie. [Porphyrinemia without porphyrinuria.] Klin. Wochenschr. 12: 586-589.

Van Esch, G. J.; Kroes, R. (1969) The induction of renal tumours by feeding basic lead acetate to mice and hamsters. Br. J. Cancer 23: 765.

Van Esch, G. J.; Van Genderen, H.; Vink, H. H. (1962) The induction of renal tumors by feeding of basic lead acetate to rats. Br. J. Cancer 16: 289-297.

Vander, A. J.; Johnson, B. (1981) Accumulation of lead by renal slices in the presence of organic anions. Proc. Soc. Exp. Biol. Med. 166: 583-586.

Vander, A. J.; Taylor, D. L.; Kalitis, K.; Mouw, D. R.; Victery, W. (1977) Renal handling of lead in dogs: clearance studies. Am. J. Physiol. 2: F532-F538.

Vander, A. J.; Mouw, D. R.; Cox, J.; Johnson, B. (1979) Lead transport by renal slices and its inhibition by tin. Am. J. Physiol. 236: F373-F378.

Varma, M. M.; Joshi, S. R.; Adeyemi, A. O. (1974) Mutagenicity and infertility following administration of lead sub-acetate to Swiss male mice. Experientia 30: 486-487.

Vasilescu, C. (1973) Motor nerve conduction velocity and electromyogram in chronic lead poisoning. Rev. Roum. Neurol. 10: 221-226.

Vengris, V. E.; Mare, C. J. (1974) Lead poisoning in chickens and the effect of lead on interferon and antibody production. Can. J. Comp. Med. 38: 328-335.

Verger, D.; Leroux-Robert, C.; Ganter, P.; Richet, G. (1967) Les tophus goutteux de la médullaire rénale des urémiques chroniques. [Gouty tophus of the renal medulary in chronic uremics.] Nephron 4: 356-370.

Verlangieri, A. J. (1979) Prenatal and postnatal chronic lead intoxication and running wheel activity in the rat. Pharmacol. Biochem. Behav. 11: 95-98.

Vermande-Van Eck, G. J.; Meigs, J. W. (1960) Changes in the ovary of the Rhesus monkey after chronic lead intoxication. Fertil. Steril. 11: 223-234.

Victery, W.; Vander, A. J.; Mouw, D. R. (1979a) Renal handling of lead in dogs: stop-flow analysis. Am. J. Physiol. 237: F408-F414.

Victery, W.; Vander, A. J.; Mouw, D. R. (1979b) Effect of acid-base status on renal excretion and accumulation of lead in dogs and rats. Am. J. Physiol. 237: F398-F407.

Victery, W.; Soifer, N. E.; Weiss, J. S.; Vander, A. J. (1981) Acute effects of lead on the renal handling of zinc in dogs. Toxicol. Appl. Pharmacol. 61: 358-367.

Victery, W.; Vander, A. J.; Markel, H.; Katzman, L.; Shulak, J. M.; Germain, C. (1982a) Lead exposure, begun in in utero, decreases renin and angiotensin II in adult rats (41398). Proc. Soc. Exp. Biol. Med. 170: 63-67.

Victery, W.; Vander, A. J.; Shulak, J. M.; Schoeps, P.; Juluis, S. (1982b) Lead, hypertension, and the renin-angiotensin system in rats. J. Lab. Clin. Med. 99: 354-362.

Victery, W.; Vander, A. J.; Schoeps, P.; Germain, C. (1983) Plasma renin is increased in young rats exposed to lead in utero and during nursing (41517). Proc. Soc. Exp. Biol. Med. 172: 1-7.

Vigdortchik, N. A. (1935) Lead intoxication in the etiology of hypertonia. J. Ind. Hyg. 17: 1-6.

Vitale, L. F.; Joselow, M. M.; Wedeen, R. P.; Pawlow, M. (1975) Blood lead--an inadequate measure of occupational exposure. J. Occup. Med. 17: 155-156.

Wada, O.; Yano, Y.; Toyokawa, K.; Suzuki, T.; Suzuki, S.; Katsunuma, H. (1972) Human responses to lead: in special reference to porphyrin metabolism in bone marrow erythroid cells, and clinical and laboratory study. Ind. Health 10: 84-92.

Wada, O; Yano, Y.; Ono, T.; Toyokawa, K. (1973) The diagnosis of different degrees of lead absorption: in special references to choice and evaluation of various parameters indicative of an increased lead absorption. Ind. Health 11: 55-67.

Wada, O.; Takeo, K.; Yano, Y.; Ono, T.; Nagahashi, M.; Seki, H. (1976) δ-Aminolevulinic acid dehydratase in low level lead exposure. Arch. Environ. Health 31: 211-214.

Waldron, H. A. (1964) The effect of lead on the fragility of the red cell incubated in vitro. J. Clin. Pathol. 17: 405-406.

Waldron, H. A. (1966) The anaemia of lead poisoning: a review. Br. J. Ind. Med. 23: 83-100.

Walton, J. R. (1973) Granules containing lead in isolated mitochondria. Nature (London) 243: 100-101.

Walton, J.; Buckley, I. K. (1977) The lead-poisoned cell: a fine structural study using cultured kidney cells. Exp. Mol. Pathol. 27: 167-182.

Wapnir, R. A.; Moak, S. A.; Lifshitz, F.; Teichberg, S. (1979) Alterations of intestinal and renal functions in rats after intraperitoneal injections of lead acetate. J. Lab. Clin. Med. 94: 144-151.

Wardell, R. E.; Seegmiller, R. E.; Bradshaw, W. S. (1982) Induction of prenatal toxicity in the rat by diethylstilbestrol, zeranol, 3,4,3',4',-tetrachlorobiphenyl, cadmium, and lead. Teratology 26: 229-237.

Watson, R. J.; Decker, E.; Lichman, H. C. (1958) Hematologic studies of children with lead poisoning. Pediatrics 21: 40-46.

Watson, W. S.; Hume, R.; Moore, M. R. (1980) Oral absorption of lead and iron. Lancet 2(8188): 236-238.

Webb, R. C.; Winquist, R. J.; Victery, W.; Vander, A. J. (1981) In vivo and in vitro effects of lead on vascular reactivity in rats. Am. J. Physiol. 24: H211-H216.

Wedeen, R. P. (1981) Punch cures the gout. J. Med. Soc. N.J. 78: 201-206.

Wedeen, R. P. (1982) Lead nephrotoxicity. In: Porter, G., ed. Nephrotoxic mechanisms of drugs and environmental toxins. New York, NY: Plenum Publishing Corp.; pp. 255-265.

Wedeen, R. P.; Maesaka, J. K.; Weiner, B.; Lipat, G. A.; Lyons, M. M.; Vitale, L. F.; Joselow, M. M. (1975) Occupational lead nephropathy. Am. J. Med. 59: 630-641.

Wedeen, R. P.; Mallik, D. K.; Batuman, V.; Bogden, J. D. (1978) Geophagic lead nephropathy: case report. Environ. Res. 17: 409-415.

Wedeen, R. P.; Mallik, D. K.; Batuman, V. (1979) Detection and treatment of occupational lead nephropathy. Arch. Intern. Med. 139: 53-57.

Wedeen, R. P.; Batuman, V.; Landy, E. (1983) The safety of the EDTA lead-mobilization test. Environ. Res. 30: 58-62.

Weinreich, K.; Stelte, W.; Bitsch, I. (1977) Effect of lead acetate on the spontaneous activity of young rats. Nutr. Metab. 21 Suppl.: 201-203.

Weir, P. A.; Hine, C. H. (1970) Effects of various metals on behavior of conditioned goldfish. Arch. Environ. Health 20: 45-51.

Weisburger, J. H.; Williams, G. M. (1980) Chemical carcinogens. In: Doull, J.; Klaassen, C. D.; Amdur, M. O., eds. Toxicology: the basic science of poisons. 2nd ed. New York, NY: Macmillan Publishing Co., Inc.; pp. 84-138.

Weiss, B. (1980) Conceptual issues in the assessment of lead toxicity. In: Needleman, H. L., ed. Low level lead exposure: the clinical implications of current research. New York, NY: Raven Press; pp. 127-134.

Weller, C. V. (1915) The blastophthoric effect of chronic lead poisoning. J. Med. Res. 33: 271-293.

Whetsell, W. O., Jr.; Sassa, S.; Bickers, D.; Kappas, A. (1978) Studies on prophyrin-heme biosynthesis in organotypic cultures of chick dorsal root ganglion. I: Observations on neuronal and non-neuronal elements. J. Neuropathol. Exp. Neurol. 37: 497-507.

White, D. J. (1977) Histochemical and histological effects of lead on the liver and kidney of the dog. Br. J. Exp. Pathol. 58: 101-112.

White, J. M.; Harvey, D. R. (1972) Defective synthesis of α and β globin chains in lead poisoning. Nature (London) 236: 71-73.

Wibberley, D. G.; Khera, A. K.; Edwards, J. H.; Rushton, D. I. (1977) Lead levels in human placentae from normal and malformed births. J. Med. Genet. 14: 339-345.

Wide, M. (1978) Effect of inorganic lead on the mouse blastocyst in vitro. Teratology 17: 165-169.

Wide, M. (1980) Interference of lead with implantation in the mouse: effect of exogenous oestradiol and progesterone. Teratology 21: 187-191.

Wide, M.; Nilsson, O. (1977) Differential susceptibility of the embryo to inorganic lead during periimplantation in the mouse. Teratology 16: 273-276.

Wide, M.; Nilsson, B. O. (1979) Interference of lead with implantation in the mouse: a study of the surface ultrastructure of blastocysts and endometrium. Teratology 20: 101-113.

Wide, M.; Wide, L. (1980) Estradiol receptor activity in uteri of pregnant mice given lead before implantation. Fertil. Steril. 34: 503-508.

Wildt, K.; Eliasson, R.; Berlin, M. (1983) Effects of occupational exposure to lead on sperm and semen. Presented at: A joint meeting of the Rochester conference and scientific committee (PCIAOH) on the toxicology of metals: reproductive and developmental toxicity of metals; May; Rochester, NY.

Willems, M. I.; de Schepper, G. G.; Wibowo, A. A. E.; Immel H. R.; Dietrich, A. J. J.; Zielhuis, R. L. (1982) Absence of an effect of lead acetate on sperm morphology, sister chromatid exchanges or on micronuclei formation in rabbits. Arch. Toxicol. 50: 149-157.

Williams, B. J.; Griffith, W. H.; Albrecht, C. M.; Pirch, J. H.; Hejtmancik, M. R., Jr.; Nechay, B. R. (1977a) Cardiac effect of chronic lead poisoning. In: Brown, S. S., ed. Clinical chemistry and chemical toxicology of metals. New York, NY: Elsevier/North-Holland Biomedical Press; pp. 127-130.

Williams, B. J.; Griffith, W. H., III.; Albrecht, C. M.; Pirch, J. H.; Hejtmancik, M. R., Jr. (1977b) Effects of chronic lead treatment on some cardiovascular responses to norepinephrine in the rat. Toxicol. Appl. Pharmacol. 40: 407-413.

Williamson, C. S. (1920) Gout: a clinical study of one hundred and sixteen cases. J. Am. Med. Assoc. 74: 1625-1629.

Wilson, J. D.; Simmonds, H. A.; North, J. D. K. (1967) Allopurinol in the treatment of uraemic patients with gout. Ann. Rheum. Dis. 26: 136-142.

Wilson, V. K.; Thompson, M. L; Dent, C. E. (1953) Amino-aciduria in lead poisoning: a case in childhood. Lancet 2(6776): 66-68.

Wince, L. C.; Donovan, C. A.; Azzaro, A. J. (1980) Alterations in the biochemical properties of central dopamine synapses following chronic postnatal $PbCO_3$ exposure. J. Pharmacol. Exp. Ther. 214: 642-650.

Windebank, A. J.; Dyck, P. J. (1981) Kinetics of ^{210}Pb entry into the endoneurium. Brain Res. 225: 67-73.

Windebank, A. J.; McCall, J. T.; Hunder, H. G.; Dyck, P. J. (1980) The endoneurial content of lead related to the onset and severity of segmental demyelination. J. Neuropathol. Exp. Neurol. 39: 692-699.

Winneke, G. (1980) Non-recovery of lead-induced changes of visual evoked potentials in rats. Toxicol. Lett. Spec. Iss. 1: 77.

Winneke, G.; Brockhaus, A.; Baltissen, R. (1977) Neurobehavioral and systemic effects of long-term blood lead-elevation in rats. I: Discrimination learning and open field-behavior. Arch. Toxicol. 37: 247-263.

Winneke, G.; Hrdina, K-G.; Brockhaus, A. (1982a) Neuropsychological studies in children with elevated tooth-lead concentrations. Part I: Pilot study. Int. Arch. Occup. Environ. Health 51: 169-183.

Winneke, G.; Krämer, U.; Brockhaus, A.; Ewers, U.; Kujanek, G.; Lechner, H.; Janke, W. (1982b) Neuropsychological studies in children with elevated tooth lead concentrations. Part II: Extended study. Int. Arch. Occup. Environ. Health 51: 231-252.

Winneke, G.; Lilienthal, H.; Werner, W. (1982c) Task dependent neurobehavioral effects of lead in rats. Arch. Toxicol. Suppl. 5: 84-93.

Woods, J. S.; Fowler, B. A. (1982) Selective inhibition of δ-aminolevulinic acid dehydratase by indium chloride in rat kidney: biochemical and ultrastructural studies. Exp. Mol. Pathol. 36: 306-315.

World Health Organization/United Nations Environmental Programme. (1977) Lead. Geneva, Switzerland: World Health Organization. (Environmental health criteria 3.)

Wyngaarden, J. B. (1958) The role of the kidney in the pathogenesis and treatment of gout. J. Am. Rheum. Assoc. 1: 191-203.

Wyrobek, A. J.; Bruce, W. R. (1978) The induction of sperm-shape abnormalities in mice and humans. In: Hollaender, A.; de Serres, F. J., eds. Chemical mutagens: principles and methods for their detection: vol. 5. New York, NY: Plenum Press; pp. 257-285.

Yip, R.; Norris, T. N.; Anderson, A. S. (1981) Iron status of children with elevated blood lead concentrations. J. Pediatr. (St. Louis) 98: 922-925.

Yü, T-F. (1982) Clinicial aspects of nephropathy in gout and hyperuricemic states. In: Yü, T-F.;
Berger, L., eds. The kidney and gout in hyperuricemia. Mt. Kisco, NY: Futura Publishing Co.; pp. 261-292.

Yü, T. A.; Berger, L. (1982) Impaired renal function in gout: its association with hypertensive vascular disease and intrinsic renal disease. Am. J. Med. 72: 95-100.

Yule, W.; Lansdown, R. (1983) Lead and children's development: recent findings. To be presented at: International conference: management and control of heavy metals in the environment; September; Heidelberg, West Germany. Edinburgh, United Kingdom: CEP Consultants, Ltd.

Yule, W.; Lansdown, R.; Millar, I. B.; Urbanowicz, M-A. (1981) The relationship between blood lead concentrations, intelligence and attainment in a school population: a pilot study. Dev. Med. Child Neurol. 23: 567-576.

Yule, W.; Lansdown, R.; Urbanowicz, M-A. (1982) Predicting educational attainment from WISC-R in a primary school sample. Br. J. Clin. Psychol. 21: 43-46.

Yule, W.; Urbanowicz, M. A.; Lansdown, R.; Millar, I. (1983) Teachers' ratings of children's behaviour in relation to blood lead levels. Br. J. Dev. Psychol.: (in press)

Zawirska, B.; Medraś, K. (1968) Tumoren und Storungen des Porphyrinstoffwechsels bei Ratten mit chronischer experimenteller Bleiintoxication. I: Morphologische Studien. [Tumors and porphyrin metabolism disturbances in rats with chronic experimental lead intoxication. I: Morphological studies.] Zentralbl. Allg. Pathol. Pathol. Anat. 3: 1-12.

Zawirska, B.; Medraś, K. (1972) The role of the kidneys in disorders of porphyrin metabolism during carcinogenesis induced with lead acetate. Arch. Immunol. Ther. Exp. 20: 257-272.

Zegarska, Z.; Kilkowska, K.; Romankiewicz-Woźniczko, G. (1974) Developmental defects in white rats caused by acute lead poisoning. Folia Morphol. (Warsaw) 33: 23-28.

Zeigler, H. P. (1973) The problem of comparison in comparative psychology. Ann. N.Y. Acad. Sci. 23: 126-134.

Zel'tser, M. E. (1962) K voprosu o funktsional'nom sostoyanii shchitovidnoi zhelezy pri caturnizme (predvarital'noe soobshchenie). [The functional state of the thyroid gland in lead poisoning.] Tr. Inst. Kraev. Patol. Akad. Nauk Kaz. SSR 10: 116-120.

Zenick, H.; Padich, R.; Tokarek, T.; Aragon, P. (1978) Influence of prenatal and postnatal lead exposure on discrimination learning in rats. Pharmacol. Biochem. Behav. 8: 347-350.

Zenick, H.; Pecorraro, F.; Price, D.; Saez, K.; Ward, J. (1979) Maternal behavior during chronic lead exposure and measures of offspring development. Neurobehav. Toxicol. 1: 65-71.

Zielhuis, R. L.; Wibowo, A. A. E. (1976) Review paper: susceptibility of adult females to lead: effects on reproductive function in females and males. Presented at: 2nd international workshop: permissible limits for occupational exposure to lead; September; Amsterdam, The Netherlands.

Zielhuis, R. L.; del Castilho, P.; Herber, R. F. M; Wibowo, A. A. E. (1978) Levels of lead and other metals in human blood: suggestive relationships, determining factors. Environ. Health Perspect. 25: 103-109.

Zimering, R. T.; Burright, R. G.; Donovick, P. J. (1982) Effects of pre-natal and continued lead exposure on activity levels in the mouse. Neurobehav. Toxicol. Teratol. 4: 9-14.

Zollinger, H. U. (1953) Durch chronische Bleivergiftung erzeugte Nierenadenome und -carcinome bei Ratten und ihre Beziehungen zu den entsprechenden Neubildungen des Menschen. [Kidney adenomas and carcinomas in rats caused by chronic lead poisoning and their relationship to corresponding human neoplasms.] Virchows Arch. Pathol. Anat. Physiol. 323: 694-710.

APPENDIX 12-A
ASSESSMENT OF STUDIES REPORTING THE POTENTIAL ESSENTIALITY OF LEAD

Available information concerning the potential essentiality of lead is quite limited, due in part to the inherent difficulties surrounding such investigations. The presence of lead as a ubiquitous contaminant requires that studies of the effects of lead deficiency use synthetic or semi-synthetic diets prepared from components extremely low in lead or use chemical agents to reduce the level of background lead in the diet components. Such procedures, particularly the use of chelating agents to remove lead, can entail risk in terms of their potential effect on the nutritional integrity of the particular diet used.

Schwarz (1975) used synthetic diets prepared from low-lead constituents with or without lead supplementation to determine the effect of low lead on the growth rate of adult rats. It was reported that lead supplementation, usually over the range of 0.5 to 2.5 ppm lead, was associated with measurable enhancement in growth rate compared to low-lead animals. In a critique of the Schwarz results, Nielsen (1980) pointed out that all of the animals in the Schwarz study, both low-lead and supplementation groups, showed sub-optimal growth, which could be ascribed to riboflavin deficiency (Morgan and Schwarz, 1978); hence, the question remains as to what the effect of lead supplementation would be in animals not riboflavin-deficient and growing optimally. Nielsen (1980) has also questioned the statistical methods used in the Schwarz studies and pointed out that addition of lead to the diet was of no apparent benefit to deficient controls in subsequent studies. Problems associated with lead deprivation studies are exemplified by the inability of Schwarz to duplicate his growth rate data over time. He attributed this to the inadvertent use of a dietary component with an elevated lead content for diets of the low-lead animals.

In a series of recent reports, Reichlmayr-Lais and Kirchgessner have described results showing that rats maintained on a semi-synthetic diet low in lead (to levels of either 18 or 45 ppb) over several generations showed reduced growth rate (Reichlmayr-Lais and Kirchgessner, 1981a), disturbances in hematological indices, tissue iron and iron absorption (Reichlmayr-Lais and Kirchgessner, 1981b,c,d; Kirchgessner and Reichlmayr-Lais, 1981a,b), and changes in certain enzyme activities and metabolite levels (Reichlmayr-Lais and Kirchgessner, 1981e; Kirchgessner and Reichlmayr-Lais, 1982). Diets containing 18 ppb were associated with the most pronounced effects on iron metabolism and growth as well as on enzyme activities and metabolite levels. Animals maintained on a 45 ppb lead diet showed moderate changes in some hematological indices in the F_1-group. In these studies, controls were maintained on the same dietary matrix to which 1.0 ppm lead was added.

In the above reports, EDTA was used to remove lead (and other elements) from casein, and the chelating agent ammonium pyrrolidinodithiocarbamate (APDC) was employed to remove lead from the starch and cellulose components to achieve the final diet level of 18 ppb. For the 45 ppb diet experiment, only the starch and cellulose components were treated with APDC (Schnegg, 1975). Although the report of Reichlmayr-Lais and Kirchgessner (1981b) indicated that the cellulose and starch extraction treatment was done on all of the material, a communication in this regard (Kirchgessner, 1982) noted that only a portion of the starch and cellulose for the 45 ppb study was extracted with APDC. After chelant treatment, the components were washed with solvents to remove the complexed metals originally present. Washing was also assumed to remove the chelants.

Caution must be exercised in interpreting these studies as they currently stand, owing to the use of the chelating agents EDTA and/or APDC. Retention of free chelating agent(s) in the diets could potentially affect the bioavailability of certain metals. In the report of Davis et al. (1962), it was noted that diets containing soybean protein that had been extracted with EDTA to lower iron content, followed by supplementation with iron and copper, were associated with iron deficiency in chicks maintained on these diets when compared to chicks fed the same level of iron and copper in untreated diets. Clearly, EDTA treatment of the soybean protein affected iron bioavailability in this study. Subsequently, the authors (Davis et al., 1964) attempted to determine the presence of EDTA in the diets by simulating those used earlier. The crude methodology employed made accurate quantification difficult, but the amounts of EDTA measured ranged up to 67 μg/g diet. Other investigations through the years have documented that EDTA will affect iron absorption/retention and utilization in various species (e.g., Larsen et al., 1960; Brise and Hallberg, 1962; Saltman and Helbock, 1965; Günther, 1969; Cook and Monson, 1976).

In this connection, retention of EDTA by proteins appears to be a general problem, based on information available for casein (Hegenauer et al., 1979), transferrin (Price and Gibson, 1972), the enzyme alkaline phosphatase (Csopak and Szajn, 1973), photoprotein aequonin (Shimomura and Shimomura, 1982), and human fibrinogen (Nieuwenhuizen et al., 1981). Furthermore, complete removal of EDTA from these rather diverse proteins is reported to involve forcing conditions, and the washing procedure used by the authors of the studies in question gives no assurance of being adequate for chelant removal.

Available information also suggests that diets retaining free EDTA and/or APDC, even at quite low levels, may pose problems by affecting the bioavailability of the essential metal, nickel. The studies of Schnegg and Kirchgessner (see review of Kirchgessner and Schnegg, 1980) have shown that nickel deficiency in rats followed over several generations is associated with reduced growth rate, disturbed hematological indices, lowered tissue iron, reduced

iron absorption, and disturbances in enzyme activities and metabolite levels. According to Nielsen (1980), nickel plays a role in the intestinal absorption of trivalent iron. In the nickel deficiency studies of Schnegg and Kirchgessner, low-nickel diets contained 15 ppb nickel, while control groups were maintained on the same basal diet supplemented with 20 ppm nickel. In the lead deficiency studies under discussion, nickel was added back to the treated diets at a level of 1.0 ppm (Reichlmayr-Lais and Kirchgessner, 1981b; Kirchgessner and Reichlmayr-Lais, 1981b).

The interaction of nickel with the chelants EDTA and/or APDC in the context of bioavailability has been documented. Dithiocarbamates such as APDC are effective chelation therapy agents in protecting against nickel toxicity (see review of Sunderman, 1981), while the report of Solomons et al. (1982) described the significant effect of EDTA on nickel bioavailability in human subjects. In the latter study, human volunteers ingested a single dose of 5 mg of nickel, and the resulting effect on plasma nickel was monitored. When nickel was co-ingested with EDTA (40 mg of $Na_2EDTA \cdot H_2O$, a 1.3:1 ratio of EDTA to Ni), not only was the rise in plasma seen with just nickel abolished, but the plasma nickel level was reduced below the fasting background level.

It is not possible to draw a close comparison of the data of Schnegg and Kirchgessner for nickel deficiency with the potential effects of impaired nickel bioavailability in the lead deficiency studies since 1) the actual level of bioavailable nickel in the studies cannot be defined and 2) the age points for most of the effects seen in nickel deficiency are different from those in the lead studies. Interestingly, one can calculate that the decrements in body weight of animals of the F_1-generation in both groups of studies at various common time points, e.g., 20, 22, 30, 38 days, are virtually identical.

Any mechanism by which lead supplementation at 1.0 ppm in the lead deficiency studies would operate in a situtation of altered bioavailability of nickel or iron in the diets can only be inferred, given the absence of any further experiemental data which would more fully elucidate an essential vs. an artifactive role for lead.

In terms of any simple competitive binding mechanism involving lead, chelating agents, and nickel or iron, the presence of lead at a level of 1.0 ppm would be seen to most immediately affect nickel bound up with EDTA (as the common 1:1 complex) or APDC (as the common 1:2 complex). Nickel was added back to the diets at a level of 1.0 ppm. Since the binding constants for lead and nickel with EDTA are roughly comparable (Shapiro and Papa, 1959; Pribl, 1972), while complexes of lead with dithiocarbamates are vastly greater in stability than the corresponding nickel complexes (Sastri et al., 1969), lead at 1.0 ppm can displace up to its molar equivalent of nickel from complexation, which calculates to be 0.3 ppm nickel. This amount of liberated nickel, 0.3 ppm, appears to be nutritionally adequate, since the minimal

nutritional requirement is noted to be around 50 ppb (Kirchgessner and Schnegg, 1980; Nielson, 1980). The corresponding amounts of APDC and EDTA required to bind up 1.0 ppm nickel calculate to be 5.4 μg/g (1:2 complex, NiAPDC) and slightly under 5 μg/g (1:1 complex, Ni-EDTA). Hence, mere traces of free chelants could be a potential problem.

Similar direct competitive binding involving lead and iron cannot be invoked as likely, given the relative amounts of iron and lead in lead-supplemented diets, although lead forms more stable complexes than divalent iron with EDTA or APDC (Pribl, 1972; Sastri et al., 1969). A cyclic mechanism would have to be invoked whereby Pb-EDTA is formed by exchange of ligand from Fe-EDTA, is then dissociated _in vivo_, and the displacement process repeated.

Nickel supplementation at 20 ppm in the Schnegg and Kirchgessner studies, where a similar APDC procedure was used to purify starch and cellulose components, as well as in the study of Nielsen et al. (1979), where APDC at 10 ppm was employed to assess the role of nickel in iron metabolism, do not permit comparison with the studies in question because of the 20-fold disparity in the level of supplementation.

Given the above concerns, it would appear that: 1) further experiments, using methodology such as scintillography and labeled chelants, are necessary to conclusively determine that diet preparation in the Reichlmayr-Lais and Kirchgessner studies did not involve retention of free chelating agents, 2) determination of levels of nickel and lead in tissues, blood, and excreta would greatly help to elucidate the true role of lead, and 3) replication of the results in the authors' or another laboratory, preferably with minimal chelant treatment of components, should be done. It appears that the various reports describe basically single experiments over several generations, one at a diet level of 18 ppb lead, and one at 45 ppb lead.

12A REFERENCES

Brise, H.; Hallberg, L. (1962) Iron absorption studies: a method for comparative studies on iron absorption in man using 2 radio-iron isotopes. Acta Med. Scand. 171 (Suppl.): 23-27.

Cook, J. D.; Monsen, E. R. (1976) Food iron absorption by man. II: The effect of EDTA on absorption of dietary non-heme iron. Am. J. Clin. Nutr. 29: 614-620.

Csopak, H.; Szajn, H. (1973) Factors affecting the zinc content of E. coli alkaline phosphatase. Arch. Biochem. Biophys. 157: 374-379.

Davis, P. N.; Norris, L. C.; Kratzer, F. H. (1962) Iron deficiency studies in chicks using treated isolated soybean protein diets. J. Nutr. 78: 445-453.

Davis, P. N.; Norris, L. C.; Kratzer, F. H. (1964) Iron deficiency studies in chicks. J. Nutr. 84: 93-94.

Günther, R. (1969) Der Einfluss von chelatbildnern auf die Verteilung und Ausscheidung von Radioeisen bei der Ratte. [Distribution and excretion of radioiron in the rat as influenced by chelating agents.] Naunyn Schmiedebergs Arch. Pharmakol. Exp. Pathol. 262: 405-418.

Hegenauer, J.; Saltman, P.; Nace, G. (1979) Iron (III)-phosphoprotein chelates: stoichiometric equilibrium constants for interaction of iron and phosphoryl serine residues of phosvitin and casein. Am. J. Clin. Nutr. 32: 809-816.

Kirchgessner, M. (1982) [Letter to D. Weil]. October 6. Available for inspection at: U.S. Environmental Protection Agency, Environmental Criteria and Assessment Office, Research Triangle Park, NC.

Kirchgessner, M.; Reichlmayr-Lais, A. M. (1982) Konzentrationen verschiedener Stoffwechselmetaboliten im experimentellen Bleimangel. [Concentrations of various metabolites with experimental lead deficiency.] Ann. Nutr. Metab. 26: 50-55.

Kirchgessner, M.; Schnegg, A. (1980) Biochemical and physiological effects of nickel deficiency. In: Nriagu, J. O., ed. Nickel in the environment. New York, NY: John Wiley & Sons; pp. 635-652.

Kirchgessner, M.; Reichlmayr-Lais, A. M. (1981a) Changes of iron concentration and iron-binding capacity in serum resulting from alimentary lead deficiency. Biol. Trace Elem. Res. 3: 279-285.

Kirchgessner, M.; Reichlmayr-Lais, A. M. (1981b) Retention, Absorbierbarkeit und intermeditäre Verfügbarkeit von Eisen bei alimentärem Bleimangel. [Retention, absorbability and intermediate availability of iron with alimentary lead deficiency.] Int. J. Vitam. Nutr. Res. 51: 421-424.

Larsen, B. A.; Bidwell, R. G. S.; Hawkins, W. W. (1960) The effect of ingestion of disodium ethylenediaminetetraacetate on the absorption and metabolism of radioactive iron by the rat. Can. J. Biochem. Physiol. 38: 51-55.

Morgan, J. K.; Schwarz, K. (1978) Light sensitivity of riboflavin in amino acid diets. Fed. Proc. Fed. Am. Soc. Exp. Biol. 37:671.

Nielsen, F. H. (1980) Effect of form of iron on the interaction between nickel and iron in rats: growth and blood parameters. J. Nutr. 110: 965-973.

Nielsen, F. H.; Zimmerman, T. J.; Collings, M. E.; Myron, D. R. (1979) Nickel deprivation in rats: nickel-iron interactions. J. Nutr. 109: 1623-1632.

Nieuwenhuizen, W.; Vermond, A.; Hermans, T. (1981) Human fibrinogen binds EDTA and citrate. Thromb. Res. 22: 659-663.

Pribl, R. (1972) Analytical applications of EDTA and Related Compounds. New York, NY: Pergamon Press; p. 27. (International series of monographs in analytical chemistry, v. 52.)

Price, E. M.; Gibson, J. F. (1972) A re-interpretation of bicarbonate-free ferric transferrin E.P.R. spectra. Biochem. Biophys. Res. Commun. 46: 646-651.

Reichlmayr-Lais, A. M.; Kirchgessner, M. (1981a) Zur Essentialität von Blei für das tierische Wachstum. [Why lead is essential for animal growth.] Z. Tierphysiol. Tierernaehr. Futtermittelkd. 46: 1-8.

Reichlmayr-Lais, A. M.; Kirchgessner, M. (1981b) Depletionsstudien zur Essentialität von Blei an wachsenden Ratten. [Depletion studies on the essentiality of lead in growing rats.] Arch. Tierenaehr. 31: 731-737.

Reichlmayr-Lais, A. M.; Kirchgessner, M. (1981c) Hämatologische Veränderungen bei alimentärem Bleimangel. [Hematological changes with alimentary lead deficiency.] Ann. Nutr. Metab. 25: 281-288.

Reichlmayr-Lais, A. M.; Kirchgessner, M. (1981d) Eisen-,Kupfer- und Zinkgehalte in Neugeborenen sowie in Leber und Milz wachsender ratten bei alimentärem Blei-Mangel. [Iron, copper and zinc contents in newborns as well as in the liver and spleen of growing rats in the case of alimentary lead deficiency.] Z. Tierphysiol. Tierernaehr. Futtermittelkd. 46: 8-14.

Reichlmayr-Lais, A. M.; Kirchgessner, M. (1981e) Aktivitäts-veränderungen verschiedener Enzyme im alimentären Blei-Mangel. [Activity changes of different enzymes in alimentary lead deficiency.] Z. Tierphysiol. Tierernaehr. Futtermittelkd. 46: 145-150.

Saltman, P.; Helbock, H. (1965) The regulation and control of intestinal iron transport. In: Proc. Symp. Radio-isotope Anim. Nutr. Physiol., Prague, Czeckoslovakia; pp. 301-317.

Sastri, V. S.; Aspila, K. I.; Chakrabarti, C. L. (1969) Studies on the solvent extraction of metal dithiocarbamates. Can. J. Chem. 47: 2320-2323.

Schnegg, A. (1975) [Dissertation.] Technische Universität München-Weihenstephan. [Technical University, Munich-Weihenstephan, West Germany.] Available for inspection at: U.S. Environmental Protection Agency, Environmental Criteria and Assessment Office, Research Triangle Park, NC.

Schwarz, K. (1975) Potential essentiality of lead. Arh. Rada Toksikol. 26 (Suppl): 13-28.

Shapiro, S.; Papa, D. (1959) Heavy metal chelates and cesium salts for contrast radiography. Ann. N.Y. Acad. Sci. 78: 756-763.

Shimomura, O.; Shimomura, A. (1982) EDTA-binding and acylation of the Ca(2+)-sensitive photoprotein aequorin. FEBS Lett. 138: 201-204.

Solomns, N. W.; Viteri, F.; Shuler, T. R.; Nielsen, F. H. (1982) Bio-availability of nickel in men: effects of foods and chemically-defined dietary constituents on the absorption of inorganic nickel. J. Nutr. 112: 39-50.

Sunderman, F. W. (1981) Chelation therapy in nickel poisoning. Ann. Clin. Lab. Sci. 11: 1-8.

APPENDIX 12-B

SUMMARY OF PSYCHOMETRIC TESTS USED TO ASSESS COGNITIVE AND BEHAVIORAL DEVELOPMENT IN PEDIATRIC POPULATIONS

TABLE 12B. TESTS COMMONLY USED IN A PSYCHO-EDUCATIONAL BATTERY FOR CHILDREN

	Age range	Norms	Scores	Advantages	Disadvantages
General Intelligence Tests					
Stanford-Binet (Form L-M)	2 yrs - Adult	1972	1. Deviation IQ: Mean = 100 SD = 16 2. Mental Age Equivalent	1. Good reliability & validity 2. Predicts school performance 3. Covers a wide age range	1. Tests mostly verbal skills especially after 6 yrs 2. Does not give a profile of skills
Wechsler Preschool & Primary Scales of Intelligence (WPPSI)	4 - 6½ yrs Best for 5-yr-olds	1967	1. Deviation IQ: Mean = 100 SD = 15 2. Scaled Scores for 10 sub tests: Mean = 10 SD = 3	1. Good reliability & validity 2. Predicts school performance 3. Gives a profile of verbal & non-verbal skills. 4. Useful in early identification of learning disability	1. Narrow age range 2. Mentally retarded children find this a disproportionate difficult test
Wechsler Intelligence Scale for Children-Revised (WISC-R)	6 - 16 yrs	1974	1. Deviation IQ: Mean = 100 SD = 15 2. Scaled Scores for 10 subtests: Mean = 10 SD = 3	1. Good reliability & validity 2. Predicts school performance 3. Gives a profile of verbal and non-verbal skills 4. Useful in identification of learning disability	1. Gives a lower IQ than Stanford-Binet for normal and bright children
McCarthy Scales of Children's Abilities (MSCA)	2½ - 8½ yrs Best for ages 4 - 6	1972	1. General Cognitive Index: Mean = 100 SD = 16 2. Scaled scores for 5 subtests: mean = 50 SD = 10 Age equivalents can be derived.	1. Good reliability & validity 2. Good predictor of school performance 3. Useful in identification of learning disabilities when given with a WISC-R or Stanford-Binet 4. Gives good information for educational programming	1. Children score much lower than on WISC-R or Stanford-Binet 2. Narrow age range
Bayley Scales of Mental Development	2 - 30 mos.	1969	1. Standard scores (M = 100 SD = 16) 2. Mental Development Psychomotor Index	1. Norms are excellent 2. Satisfactory reliability and validity 3. Best measure of infant development	1. Not a good predictor of later functioning in average as in below average children

PRELIMINARY DRAFT

TABLE 12B (continued)

	Age range	Norms	Scores	Advantages	Disadvantages
Slosson Intelligence Test	Infancy - 27 yrs	1963	1. Ratio IQ: is not related to general population	1. Good reliability & validity 2. Quick to administer. A good screening test	1. Many items taken from Stanford Binet 2. Responses require good language skills 3. Measures a narrow range of skills 4. A screening test: not to be used for classification or placement
Peabody Picture Vocabulary Test	2½ - 18 yrs	1959,rev.1981 White, Middle class sample	1. Verbal IQ 2. Age equivalent	1. Easily administered 2. Does not require language or motor skills	1. Fair reliability and validity 2. Tests only receptive vocabulary 3. Lower class children score lower 4. Mentally Retarded children score higher than on other tests 5. Not to be used for classification or placement.
Visual-Motor Tests					
Frostig Developmental Test of Visual Perception	3 - 8 yrs & older learning disabled (L.D.) children	1963 White, middle class sample	1. Perceptual Quotient: Median = 100 Quartile Deviation = 10 2. Perceptual Age Equivalent 3. Scaled Scores for 5 subtests	1. Good reliability for L.D. children	1. Fair reliability for normal children 2. Poor Validity 3. No known relationship to reading or learning 4. Remedial program based on test of questionable value 5. Not useful in identifying children at risk for L.D.
Bender-Gestalt	4 yrs - Adult	1964 Normal and Brain-injured Children	1. Age equivalent	1. Easily administered 2. Long history of research makes it a good research tool	1. Fair reliability 2. Poor predictive and validity 3. Responses influenced by fatigue & variations in administration 4. No known relationship to reading or subtle neurological dysfunction
Beery-Buktenica Developmental Test of Visual Motor Integration (VMI)	2 - 15 yrs	1967	1. Age equivalent	1. Easily administered 2. Good normative sample	1. Moderate reliability and validity 2. Correlates better with mental age than chronological age

PRELIMINARY DRAFT

TABLE 12B (continued)

	Age range	Norms	Scores	Advantages	Disadvantages
Educational Tests					
Wide Range Achievement Test (WRAT)	5 yrs - Adult	1976 Revised	1. Standard Score: mean = 100 SD = 15 2. Grade equivalent	1. Good reliability & validity Reading scores predict grade level 2. Tasks similar to actual work	1. Reading portion tests word recognition only 2. Responses require good organizational skills (could be an advantage)
Peabody Individual Achievement Test (PIAT)	5 - 18 yrs	1969	1. Standard Scores: Mean = 100 SD = 15 2. Grade equivalent 3. Age equivalent	1. Tests word recognition and 2. Breaks down skills into 5 areas	1. Moderate reliability. Low stability for Kindergarten 2. No data on predictive validity 3. A multiple choice test requiring child to recognize correct answer (could be an advantage). 4. Heavily loaded on verbal reasoning. 5. Factor structure changes with age.
Woodcock Reading Mastery Tests	Kgn - 12 grade	1971-72 adjusted for social class	1. Grade equivalent 2. Standard Score 3. Percentile Rank	1. Good reliability 2. Breakdown of reading skills useful diagnostically and in planning remediation 3. Easy to administer and score	1. No data on validity
Spache Diagnostic Reading Scales	1st - 8th grade	1972	1. Instructional level of reading (grade equivalent). 2. Independent level of reading. 3. Potential level of reading	1. Independent level score predicts gains following remediation 2. Good breakdown of reading skills	1. Fairly complex scoring 2. Moderate reliability 3. No good data on validity
Key Math Diagnostic Arithmetic Test	Pre-school - 6th grade	1971	1. Grade equivalent	1. Excellent breakdown of math skills 2. Easy to administer and score	1. Moderate reliability 2. No data on validity

PRELIMINARY DRAFT

TABLE 12B (continued)

	Age range	Norms	Scores	Advantages	Disadvantages
Tests of Adaptive Functioning					
Vineland Social Maturity Scale	Birth - 25 yrs	1983 Revised	1. Social Quotient (Ratio) 2. Social Age Equivalent	1. Easily administered 2. Good reliability for normal and MR chidren	1. Poor norms 2. No data on validity 3. Items are limited past preschool years 4. Scores decrease with age for MR children
AAMD Adaptive Behavior Scale	3 yrs - Adult	1974 Institutionalized Retardates; Public School Children (1982)	1. Percentile Ranks 2. Scaled scores	1. Discriminates between EMR and regular classes 2. Useful for class placement and monitoring progress	1. Moderate reliability for independent living skills scale. Poor reliability for maladaptive behaviour scale. 2. Lengthy administration 3. Items & scoring are not behaviorally objective
Progress Assessment Chart of Social Development (PAC)	Birth - Adult	1976	No Scores	1. Useful for training and assessing progress 2. Gives profile of skills	1. No data on reliability or validity
Developmental Profile	Birth - 12 yrs	1972	1. Age equivalents in 5 5 areas 2. IQ equivalency (IQE)	1. Good reliability and validity. Excellent study of construct validity reported in manual. 2. Gives a profile of skills	1. IQE underestimates IQ of above average children, overestimates IQ of below average children.
Conners Rating Scale	3 yrs - 17 yrs	1978	1. Age equivalents	1. Most widely used measure of attention deficit disorder 2. Four factors: conduct problems; hyperactivity; inattentive-passive; hyperactivity index	1. Parents' ratings don't predict as well as teachers' ratings 2. Works best middle class children
Werry-Weiss-Peters Hyperactivity Scale	1 yr - 9 yrs	1974, 1977	1. Age equivalents	1. Good measure of hyperactivity 2. Seven Factors	1. Limited age range 2. Standardized on middle class children

PRELIMINARY DRAFT

APPENDIX 12-C
WILL BE FORTHCOMING UNDER A SEPARATE COVER.

ABSTRACT OF A REVIEW OF THREE STUDIES ON THE EFFECTS OF LEAD SMELTER EMISSIONS IN EL PASO, TEXAS

Presented by Warren R. Muir
Council on Environmental Quality
Washington, D.C.
At the International Conference on Heavy Metals in the Environment
Toronto, Ontario, Canada
October 1975

The committee reviewed two independent studies conducted in 1973 by Dr. Landrigan (CDC) and Dr. McNeil (ILZRO) to determine the effects of community lead exposures near the ASARCO smelter in El Paso, Texas. The CDC study used a random sample approach to group participating children, and in the ILZRO study match paired groups were selected on the basis of residence. In both studies the criteria for subclassification with regard to lead exposure were blood lead levels. Neuropsychological dysfunction was evaluated by several tests including WISC, WPPSI, and McCarthy scales. Statistical differences in test results could not be directly correlated to blood lead levels.

The opinion of the committee was that no firm conclusions could be drawn from the studies as to whether or not there are subclinical effects of lead on children in El Paso and that the reports and data made available have not clearly demonstrated any psychologic or neurologic effects in the children under study. It noted the absence of major chronic clinical effects, and concluded that these studies therefore do not bear upon the conclusions of other investigations under different conditions and those in which clinical effects have been confirmed. However, because of inherent problems of study design and the limitations in the tests used, this finding should not lead to a conclusion that low levels of lead have no effects on neuropsychological performance. Ellen Silbergeld, Ph.D., NIH, Eileen Higham, Ph.D., and Mr. Russell Jobaris, Johns Hopkins University, Department of Medical Psychology, served as special consultants.

The committee decided to limit its focus to a review of the three studies, and to attempt to account for and interpret the differences between the studies. Thus, aspects not related to differences were not emphasized.

The committee limited its consideration to the following materials: (1) reports of the three studies under consideration; (2) other materials provided by the authors of the studies; (3) background information and documents collected by Dr. Muir in El Paso. This presentation today consists of excerpts from a draft committee report.

D.1 HISTORY

El Paso is situated on the Mexican border in the western part of Texas. A lead smelter owned by American Smelting and Refining Company (ASARCO) has been located on the southwestern border of the city, on the Rio Grande River, since 1887. The area most conspicuously involved in the studies, Smeltertown, was a 2 x 6 block area located between the plant and the river. Smeltertown is no longer in existence, having been destroyed in December 1972. About 2 km south of Smeltertown is Old Fort Bliss, a considerably smaller community, whose inhabitants were considered in some, but not all, of the studies.

The ASARCO smelter produces lead, zinc, copper, and cadmium. Particulate matter is removed from airborne wastes in a series of baghouses; remaining emissions contain approximately 40 lb of lead per day.

The El Paso City County Health Department began an investigation of the ASARCO smelter in early 1970, in preparation for an air pollution suit filed by the city in April 1970. As part of this investigation, Dr.

Bertram Carnow was hired by the city as a consultant. At his suggestion, the city began to sample the blood lead levels of El Paso children to determine whether any had been over-exposed to lead. This included a large number of Smeltertown children. Based upon early results in 1971, Dr. Carnow visited El Paso, and saw a selected group of children with high blood lead levels. He interviewed the children, and reviewed their medical records. The information contained in the medical histories, and Dr. Carnow's interviews, constitute the observations reported by Dr. Carnow in the paper presented to the American Pollution Control Association (APCA). The clinical observations were in a paragraph of a paper otherwise devoted to a consideration of the effects of the smelter on the environment as a whole, and the extent of its emissions. This report contains no details on the age, exposures, individual signs and symptoms, or diagnostic criteria used in the ten cases reported. Our committee focused its attention, therefore, upon the two full-scale follow-up epidemiological studies conducted by Dr. Landrigan (CDC) and Dr. McNeil (ILZRO).

In 1973 ASARCO began a separate investigation of the population of Smeltertown, and asked Dr. James McNeil of the International Lead Zinc Research Organization (ILZRO) for his assistance in the examination and possible treatment of children with elevated blood levels greater than 60 mg/100 ml.

As a result of public concern over widespread lead poisoning throughout the city of El Paso, the mayor requested aid from the Federal Government. A separate protocol for a Center for Disease Control (CDC) study was submitted to and approved by the Public Health Board in 1973 with the understanding that the two studies would proceed independently, with those children in the ILZRO sponsored study being excluded from the CDC study.

In the summer of 1973, CDC and ILZRO proceeded independently to collect data for their respective studies. CDC's examinations were done in two weeks in June 1973, while McNeil's were carried out over the course of the summer with the aid of the El Paso public school system.

The CDC group supplied to the Committee data in detail, which were sufficient to allow the committee to conduct statistical tests and analyze characteristics of groups. For the ILZRO study, this committee requested data sufficient to carry out similar in-depth analyses. All of the requested data were supplied; however, they were not in such a form as to allow recalculation of most of the statistical findings of the study or to allow comparison with the CDC findings.

D.2 STUDY DESIGN

The environmental sampling that was performed was common for both of these studies. In the selection of study and control populations, the Landrigan CDC study used a classical approach of a random sample survey to determine the prevalence of abnormal blood lead values. The 13 census tracts most adjacent to the smelter were divided into three areas. The sampling frame was designed to obtain about 100 study subjects from each area for various age groups. Of 833 occupied residences, interviews were obtained from 758 study subjects in the 1-19 age group. The participating children were divided into a lead-absorption group (40-80 μg/100 ml) of 46 and a control group ($<$ 40 μg/100 ml) of 78. There is no detailed description as to how the children were chosen.

CDC used these same children as the basis for the later study of neuropsychological dysfunction. All but 3 children chosen for study came from the 1972 prevalence survey; 5 children with known preexisting defects such as with a history of symptoms compatible with acute lead poisoning or acute lead encephalopathy and those who had received chelation therapy were excluded.

While it is understood that a number of Smeltertown children with blood lead levels over 40 μg/100 ml were eventually involved in litigation, most of them took part in the studies. However, on the recommendation of the lawyers representing the children, at least one group of 18 did not participate in the ILZRO study. In the absence of identification by names of the individuals in the three studies, it has been impossible to evaluate the effects of non-participation.

The ILZRO study was very different; 138 children from Smeltertown agreed to participate in a study. Residence, not blood lead, was the selection criterion. Two control groups were chosen, and were reported to have been matched on age, sex, ethnic background, and income, with one set chosen from El Paso and another set for those 8 years of age or under from a rural area about 12 miles from the smelter. This classification had the effect of grouping together children who, under the CDC criteria, would have been in "lead" and "control" groups.

The criteria used for subclassification of children with regard to lead exposure were based in both studies on the blood lead level. Whereas the CDC study utilized blood lead values obtained at only two points in time, ILZRO, which was faced with the problem that many children had repeated blood lead measurements with marked variations over a period of 18 months (the levels being generally lower after exposure was discontinued), classified children on the basis of the average of the "two highest" recorded values.

This criterion results in a substantial increase in the number of children in the apparently higher blood lead category and a corresponding decrease in the number of those in the apparently lower blood lead level category.

Although it is understandable that this type of selection was used to avoid underestimating the problem of lead intoxication in the population examined, it ultimately resulted in muddling of the separation between groups (and possibly obscuring eventual differences). For example, the selection for analysis of children from the same geographical area, subclassified according to blood lead level, in the ILZRO study, may give the impression that the effects of lead itself are being studied in a homogeneous population. However, since exposure was geographically the same, other factors inherent to each individual child may be responsible for the difference in blood lead level observed.

An additional method of classification could have been the use of free erythrocytic protoporphyrin measurements (FEP) which have been shown to provide an indication of metabolic effects of lead absorption on metabolism, particularly useful in blood lead level ranges (40-60 μg/100 ml) where analytical and biological fluctuation may result in uncertain classification. (The ILZRO study included this test but did not include it as a basis for data analysis.) Absence of elevation of free erythrocytic protoporphyrin may indicate those instances where high blood lead levels were spurious.

The following psychometric tests were employed by the two studies:

1. Wechsler Intelligence Scale for Children, WISC (CDC, ILZRO)
2. McCarthy Scales of Children's Abilities (ILZRO)
3. Wechsler Preschool and Primary Scale of Intelligence, WPPSI (CDC)
4. Lincoln-Oseretsky Motor Development Scale (ILZRO)
5. California Test of Personality Adjustment (ILZRO)
6. Frosting Perceptual Quotient (ILZRO)
7. Bender Visual-Motor Gestalt Test (CDC, ILZRO)
8. Peabody
9. WRAT
10. Wepman
11. Draw-a-person

All of the tests selected by both studies were appropriate for the ages of the children to whom they were administered. Since the common ground for these studies is the WISC test, with the WPPSI used by CDC and the McCarthy Scales by ILZRO for the younger children in their studies, the Committee concentrated on these three tests and the results obtained for them.

D.3 RESULTS

The study by CDC reports results for 27 children given the WPPSI (12 with blood lead levels 40-80 μg/100 ml and 15 with blood lead levels less than 40 μg/100 ml) and for 97 children tested with the WISC (34 in the "lead group" and 63 in the "control group"). Statistical analyses were performed on grouped data with one-tailed tests. Significant differences between lead and control groups are reported in this study for the performance IQ's of the WICS and WPPSI. In subtest scores, significant differences were found in Coding on the WISC and Geometric Design on the WPPSI. When data from both tests are combined, a significant difference between lead and control groups on performance IQ is found. No differences were found between groups in verbal IQ's or full-scale IW's of the WISC or WPPSI.

The ILZRO study based on match pairing solely by residences reports no significant differences in scores on the WISC or McCarthy scales between groups with increased lead absorption and pair-matched controls. Statistical analysis was by means of two-way analysis of variance by age and blood lead levels.

The two studies base much of their conclusions upon psychometric and neurological testing of children from El Paso and Smeltertown. The reported significant differences and psychometric and neuromotor functions in the CDC study were clouded by potentially important methodological difficulties. These included

age differences between case and control groups, limited statistical treatment of the psychometric data collected, and, in the ILZRO study, the use of an average of the two highest blood lead levels to categorize lead exposure.

In addition, both the studies shared the following inherent problems:

1. Non-random exclusion of large groups of children
2. Uncertainties as to the selection of control groups
3. Reliance upon blood lead as the indicator of lead exposure and intoxication in analyses of data
4. Measurement of a limited aspect of psychological behavior
5. Lack of consideration of the potentially disruptive influences on test taking of the razing of Smeltertown, closing of its school, resettlement, litigation, and public controversy
6. Inability to rule out possible preexisting conditions

The Committee stressed the last issue, noting the likelihood that any behavioral or genetic factors that predispose an individual child to ingest or absorb more lead than another child equally exposed may itself be correlated to he result of psychometric testing. In other words an increased blood lead level may reflect, rather than cause, a preexisting difference in intelligence or behavior, an issue inherent in virtually all retrospective studies of the effects of low level blood lead.

The opinion of the committee was that no firm conclusions could be drawn from the studies as to whether or not there are subclinical effects of lead on children in El Paso and that the reports and data made available have not clearly demonstrated any psychologic or neurologic effects in the children under study. It noted the absence of major chronic clinical effects, and concluded that these studies therefore do not bear upon the conclusions of other investigations under different conditions and those in which clinical effects have been confirmed. However, because of inherent problems of study design and the limitations in the tests used, this finding should not lead to a conclusion that low levels of lead have no effects on neuropsychological performance.

13. EVALUATION OF HUMAN HEALTH RISKS ASSOCIATED WITH EXPOSURE TO LEAD AND ITS COMPOUNDS

13.1 INTRODUCTION

This chapter attempts to integrate, concisely, key information and conclusions discussed in preceding chapters into a coherent framework by which interpretation and judgments can be made concerning the risk to human health posed by present levels of lead contamination in the United States.

Towards this end, the chapter is organized into seven sections, each of which discusses one or more of the following major components of the overall health risk evaluation: (1) external and internal exposure aspects of lead; (2) lead metabolism, which determines the relationship of external lead exposure to associated health effects of lead; (3) qualitative and quantitative characterization of key health effects of lead; and (4) identification of population groups at special risk for health effects associated with lead exposure.

The various aspects of lead exposure discussed include: (1) an historical perspective on the input of lead into the environment as well as the nature and magnitude of current lead input; (2) the cycling of lead through the various environmental compartments; and (3) levels of lead in those media most relevant to lead exposure of various segments of the U.S. population. These various aspects of lead exposure are summarized in Section 13.2.

With respect to lead metabolism, some of the relevant issues addressed include: (1) the major quantitative characteristics of lead absorption, distribution, retention, and excretion in humans and how these differ between adults and children; (2) the toxicokinetic bases for external/internal lead exposure relationships with respect to both internal indicators and target tissue lead burdens; and (3) the relationships between internal and external indices of lead exposure, i.e., blood-lead levels in relation to lead concentrations in air, food, water, dust/soil. Section 13.3 summarizes the most salient features of lead metabolism, whereas Section 13.4 addresses experimental and epidemiological data concerning various blood lead-environmental media lead relationships.

In regard to various health effects of lead, the main emphasis here is on the identification of those effects most relevant to various segments of the general U.S. population and the placement of such effects in a dose-effect/dose-response framework. In regard to the latter, a crucial issue has to do with relative response of various segments of the population in terms of effect thresholds as indexed by some exposure indicator. Furthermore, it is of interest to assess the extent to which available information supports the notion of a continuum of effects as one proceeds across the spectrum of exposure levels. Finally, it is of interest to ascertain the availability of data on the relative number or percentage of members

(i.e., "responders") of specific population groups that can be expected to experience a particular effect at various lead exposure levels in order to permit delineation of dose-response curves for the relevant effects in different segments of the population. These matters are discussed in Sections 13.5 and 13.6.

Melding of information from the sections on lead exposure, metabolism, and biological effects permits the identification of population segments at special risk in terms of physiological and other host characteristics, as well as heightened vulnerability to a given effect; and these risk groups are discussed in Section 13.7. With demographic identification of individuals at risk, one may then draw upon population data from other sources to obtain a numerical picture of the magnitude of population groups at potential risk. This is also discussed in Section 13.7.

13.2 EXPOSURE ASPECTS

13.2.1 Sources of Lead Emission in the United States

The important issues to be raised concerning the sources of lead in the human environment are: What additional pathways to human consumption have been added in the course of civilization? What are the relative contributions of natural and anthropogenic lead? From the available data, what trends can be expected in the potential consumption of lead by humans? What is the impact of normal lead cycling processes on total human exposure? And finally, are there population segments particularly at risk due to a higher potential exposure?

Figure 13-1 is a composite of similar figures appearing in Chapters 7 and 11. This figure shows that four of the five sources of lead in the human environment are of anthropogenic origin. The only significant natural source is from the geochemical weathering of parent rock material as an input to soils. Of the four anthropogenic pathways, two are closely associated with atmospheric emissions and two (pigments and solder) are more directly related to the use of metallurgical compounds in products consumed by humans.

It is clear that natural sources contribute only a very small fraction to total lead in the biosphere. Levels of lead in the atmosphere, the main conduit for lead movement from sources into various environmental compartments are 10,000 to 20,000-fold higher in some urban areas than in the most remote regions of the earth. Chronological records assembled using reliable lead analysis techniques which show that atmospheric lead levels were at least 2,000-fold lower than at present before abrupt anthropological inputs accelerated with the industrial revolution and more recently, with the introduction of leaded gasoline. For actual comparison, estimates indicate a general background air lead level of 0.0005 μg Pb/m^3 versus

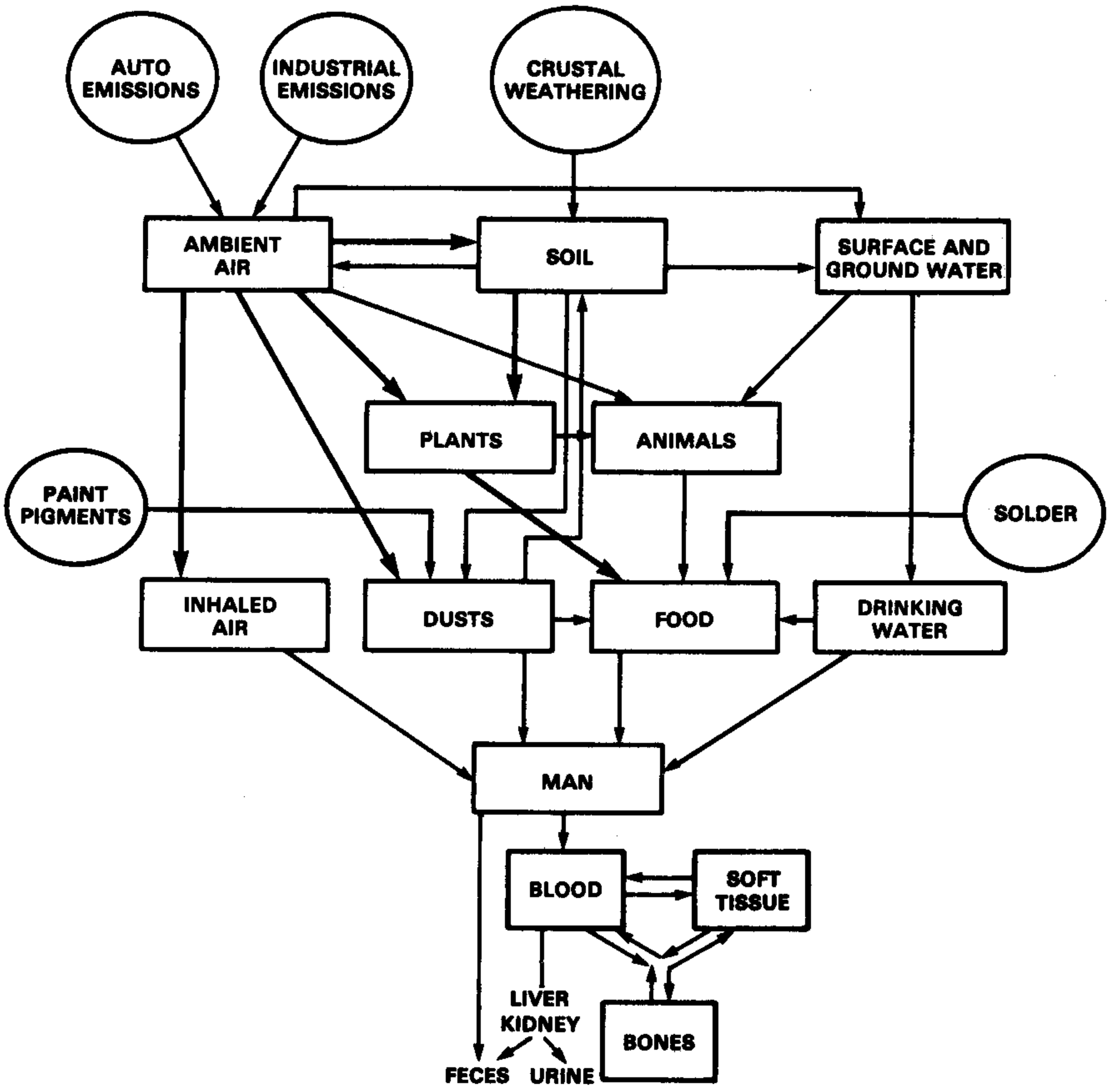

Figure 13-1. Pathways of lead from the environment to man.

urban air lead concentrations frequently approaching 1.0 μg Pb/m^3. A recent measurement of 0.000076 μg Pb/m^3 at the South Pole, using highly reliable lead analysis, suggests an anthropogenic enrichment factor of 13,000-fold compared to the same urban air level of 1.0 μg Pb/m^3.

Lead occupies an important niche in the U.S. economy, with consumption averaging 1.36 x 10^6 metric tons/year over the period 1971-1980. Of the various categories of lead consumption, those of pigments, gasoline additives, ammunition, foil, solder and steel products are widely dispersed and therefore unrecoverable. In the United States, about 41,000 tons are emitted to the atmosphere each year, including 35,000 tons as gasoline additives. Lead and its compounds enter the atmosphere at various points during mining, smelting, processing, use, recycling, or disposal. Leaded gasoline combustion in vehicles accounted for 86 percent of the total anthropogenic input into the atmosphere in the U.S. in 1981. Of the remaining 14 percent of total emissions from stationary sources, 7 percent was from the metallurgical industry, 2 percent was from waste oil combustion, and 2 percent from coal combustion. Atmospheric emissions have declined in recent years with the phase-down of lead in gasoline.

The fate of emitted particulate lead depends on particle size. It has been estimated that, of the 75 percent of combusted gasoline lead which immediately departs the vehicle in exhaust, 46 percent is in the form of particles <0.25 μm mass median equivalent diameter (MMED) and 54 percent has an average particle size of >10 μm. The sub-micron fraction is involved in long-range transport, whereas the larger particles settle mainly near the roadway.

13.2.2 Environmental Cycling of Lead

The atmosphere is the main conduit for movement of lead from emission sources to other environmental compartments. Removal of lead from the atmosphere occurs by both wet and dry deposition processes, each mechanism accounting for about one-half of the atmospheric lead removed. The fraction of lead emitted as alkyl lead vapor (1 to 6 percent) undergoes subsequent transformation to other, more stable compounds such as triethyl- or trimethyl lead, as a complex function of sunlight, temperature and ozone level.

Studies of the movement of lead emitted into the atmosphere indicate that air lead levels decrease logarithmically with distance away from the source: (1) away from emission sites, e.g., roadways and smelters; (2) within urban regions away from central business districts; (3) from urban to rural areas; and (4) from developed to remote areas.

By means of wet and dry deposition, atmospheric lead is transferred to terrestrial surfaces and bodies of water. Transfer to water occurs either directly from the atmosphere or through runoff from soil to surface waters. A sizeable fraction of water-borne lead becomes lodged in aquatic sediments. Percolation of water through soil does not transport much lead to ground water because most of the lead is retained at the soil surface.

The fate of lead particles on terrestrial surfaces depends upon such factors as the mechanism of deposition, the chemical form of the particulate lead, the chemical nature of the receiving soil, and the amount of vegetation cover. Lead deposited on soils is apparently immobilized by conversion to the carbonate, by binding to humic or fulvic acids, or by ion exchange on clays and hydrous oxides. In industrial, playground, and household environments, atmospheric particles accumulate as dusts with lead concentrations often greater than 1000 μg/g. It is important to distinguish these dusts from windblown soil dust, which typically has a lead concentration of 10 to 30 μg/g.

It has been estimated that soils adjacent to roadways have been enriched in lead content by as much as 10,000 μg Pb/g soil since 1930, while in urban areas and sites adjacent to smelters as much as 130,000 μg Pb/g has been measured in the upper 2.5 cm layer of soil.

Soil appears to be the major sink for emitted lead, with a residency half-time of decades; but soil as a reservoir for lead cannot be considered as an infinite sink, because lead will continue to pass into the grazing and detrital food chains and sustain elevated lead levels in them until equilibrium is reached. It was estimated in Chapters 7 and 8 that lead in soils not adjacent to major sources such as highways and smelters contain 3 to 5 μg/g of anthropogenic lead and that this lead has caused an increase of lead in soil moisture by a factor of 2 to 4. Thus, movement of lead from soils to other environmental compartments is at least twice the prehistoric rate and will continue to increase for the foreseeable future.

Lead enters the aquatic compartment by direct transfer from the atmosphere via wet and dry precipitation as well as indirectly from the terrestrial compartment via surface runoff. Water-borne lead, in turn, may be retained in some soluble fraction or may undergo sedimentation, depending on such factors as pH, temperature, suspended matter which may entrap lead, etc. Present levels of lead in natural waters represent a 50-fold enrichment over background content, from 0.02 to 1.0 μg Pb/l, due to anthropogenic activity. Surface waters receiving urban effluent represent a 2500-fold and higher enrichment (50 μg Pb/l and higher).

13.2.3 Levels of Lead in Various Media of Relevance to Human Exposure

Human populations in the United States are exposed to lead in air, food, water, and dust. In rural areas, Americans not occupationally exposed to lead consume 50 to 75 μg Pb/day. This level of exposure is referred to as the baseline exposure because it is unavoidable except by drastic change in lifestyle or by regulation of lead in foods or ambient air. There are several environmental circumstances that can increase human exposures above baseline levels. Most of these circumstances involve the accumulation of atmospheric dusts in the work and play environments. A few, such as pica and family home gardening, may involve consumption of lead from chips of exterior or interior house paint.

13.2.3.1 Ambient Air Lead Levels. Monitored ambient air lead concentration values in the U.S. are contained in two principal data bases: (1) EPA's National Air Sampling Network (NASN), recently renamed National Filter Analysis Network (NFAN); and (2) EPA's National Aerometric Data Bank, consistting of measurements by state and local agencies in conjunction with compliance monitoring for the current ambient air lead standard.

NASN data for 1982, the most current year in the annual surveys, indicate that most of the urban sites show reported annual averages below 0.7 μg Pb/m^3, while the majority of the non-urban locations have annual figures below 0.2 μg Pb/m^3. Over the interval 1976-1981, there has been a downward trend in these averages, mainly attributable to decreasing lead content of leaded gasoline and the increasing usage of lead-free gasoline. Furthermore, examination of quarterly averages over this interval shows a typical seasonal variation, characterized by maximum air lead values in winter and minimum values in summer.

With respect to the particle size distribution of ambient air lead, EPA studies using cascade impactors in six U.S. cities have indicated that 60 to 75 percent of such air lead was associated with sub-micron particles. This size distribution is significant in considering the distance particles may be transported and the deposition of particles in the pulmonary compartment of the respiratory tract. The relationship between airborne lead at the monitoring station and the lead inhaled by humans is complicated by such variables as vertical gradients, relative positions of the source, monitor, and the person, and the ratio of indoor to outdoor lead concentrations. To obtain an accurate picture of the amount of lead inhaled during the normal activities of an individual, personal monitors would probably be the most effective. But the information gained would be insignificant, considering that inhaled lead is only a small fraction of the total lead exposure, compared to the lead in food, beverages, and dust. The critical question with respect to airborne lead is how much lead becomes entrained in dust. In this respect, the existing monitoring network may provide an adequate estimate of the air concentration from which the rate of deposition can be determined. The percentage of ambient air lead which represents alkyl forms was noted in one study to range from 0.3 to 2.7 percent, rising up to about 10 percent at service stations.

13.2.3.2 Levels of Lead In Dust. The lead content of dusts can figure prominently in the total lead exposure picture for young children. Lead in aerosol particles deposited on rigid surfaces in urban areas (such as sidewalks, porches, steps, parking lots, etc.) does not undergo dilution compared to lead transferred by deposition onto soils. Dust can approach extremely high concentrations. Dust lead can accumulate in the interiors of dwellings as well as in the outside surroundings, particularly in urban areas.

Measurements of soil lead to a depth of 5 cm in areas of the U.S., using sites near roadways, were shown in one study to range from 150 to 500 μg Pb/g dry weight close to roadways

(i.e., within 8 meters). By contrast, lead in dusts deposited on or near heavily traveled traffic arteries show levels in major U.S. cities ranging up to 8000 μg Pb/g and higher. In residential areas, exterior dust lead levels are 1000 μg/g or less. Levels of lead in house dust can be significantly elevated. A study of house dust samples in Boston and New York City revealed levels of 1000 to 2000 μg Pb/g. Some soils adjacent to houses with exterior lead-based paints may have lead concentrations greater than 10,000 μg/g.

Thirty-four percent of the baseline consumption of lead by children comes from the consumption of 0.1 g of dust per day (Tables 13-1 and 13-2). Ninety percent of this dust lead is of atmospheric origin. Dust also accounts for more than ninety percent of the additive lead attributable to residences in an urban environment or near a smelter (Table 13-3).

13.2.3.3 Levels of Lead in Food. The route by which adults and older children in the baseline population of the U.S. receive the largest proportion of lead intake is through foods, with reported estimates of the dietary lead intake for Americans ranging from 60 to 75 μg/day. The added exposure from living in an urban environment is about 30 μg/day for adults and 100 μg/day for children, all of which can be attributed to atmospheric lead.

Atmospheric lead may be added to food crops in the field or pasture, during transportation to the market, during processing, and during kitchen preparation. Metallic lead, mainly solder, may be added during processing and packaging. Other sources of lead, as yet undetermined, increase the lead content of food between the field and dinner table. American children, adult females, and adult males consume 29, 33 and 46 μg Pb/day, respectively, in milk and nonbeverage foods. Of these amounts, 38 percent is of direct atmospheric origin, 36 percent is of metallic origin and 20 percent is of undetermined origin.

Processing of foods, particularly canning, can significantly add to their background lead content, although it appears that the impact of this is being lessened with the trend away from use of lead-soldered cans. The canning process can increase lead levels 8-to 10-fold higher than for the corresponding uncanned food items. Home food preparation can also be a source of additional lead in cases where food preparation surfaces are exposed to moderate amounts of high-lead household dust.

13.2.3.4 Lead Levels in Drinking Water. Lead in drinking water may result from contamination of the water source or from the use of lead materials in the water distribution system. Lead entry into drinking water from the latter is increased in water supplies which are plumbosolvent, i.e., with a pH below 6.5. Exposure of individuals occurs through direct ingestion of the water or via food preparation in such water.

The interim EPA drinking water standard for lead is 0.05 μg/g (50 μg/l) and several extensive surveys of public water supplies indicate that only a limited number of samples exceeded this standard on a nationwide basis. For example, a survey of interstate carrier water supplies conducted by EPA showed that only 0.3 percent exceeded the standard.

TABLE 13-1. SUMMARY OF BASELINE HUMAN EXPOSURES TO LEAD†

Source	Total Lead Consumed	Percent of Total Consumption	Soil: Natural Lead Consumed	Soil: Indirect Atmospheric Lead*	Direct Atmospheric Lead*	Lead from Solder or Other Metals	Lead of Undetermined Origin
Child 2-yr old							
Inhaled Air	0.5	0.8%	0.001	-	0.5	-	-
Food	28.7	46.7	0.9	0.9	10.9	10.3	17.6
Water & beverages	11.2	18.3	0.01	2.1	1.2	7.8	-
Dust	21.0	34.2	0.6	-	19.0	-	1.4
Total	61.4		1.5	3.0	31.6	18.1	19.0
Percent	100%		2.4%	4.9%	51.5%	29.5%	22.6%
Adult female							
Inhaled Air	1.0	1.8%	0.002	-	1.0	-	-
Food	33.2	58.7	1.0	1.0	12.6	11.9	21.6
Water & beverages	17.9	31.6	0.01	3.4	2.0	12.5	-
Dust	4.5	7.9	0.2	-	2.9	-	1.4
Total	56.6		1.2	4.4	18.5	24.4	23.0
Percent	100%		2.1%	7.8%	32.7%	43.1%	26.8%
Adult male							
Inahaled air	1.0	1.3%	0.002	-	1.0	-	-
Food	45.7	59.9	1.4	1.4	17.4	16.4	31.5
Water & beverages	25.1	32.9	0.1	4.7	2.8	17.5	-
Dust	4	5.9	0.2	-	2.9	-	1.4
Total	76.3		1.7	6.1	24.1	33.9	32.9
Percent	100%		2.2%	8.0%	31.6%	44.4%	27.1%

*Indirect atmospheric lead has been previously incorporated into soil, and will probably remain in the soil for decades or longer. Direct atmospheric lead has been deposited on the surfaces of vegetation and living areas or incorporated during food processing shortly before human consumption. It may be assumed that 85 percent of direct atmospheric lead derives from gasoline additives.

†units are in μg/day.

PRELIMINARY DRAFT

TABLE 13-2. RELATIVE BASELINE HUMAN LEAD EXPOSURES EXPRESSED PER KILOGRAM BODY WEIGHT*

	Total Lead Consumed	Total Lead Consumed Per Kg Body Wt µg/Kg·Day	Atmospheric Lead Per Kg Body Wt µg/Kg·Day
Child (2 yr old)	(µg/day)		
Inhaled air	0.5	0.05	0.05
Food	28.7	2.9	1.1
Water and beverages	11.2	1.1	0.12
Dust	21.0	2.1	1.9
Total	61.4	6.15	3.17
Adult female			
Inhaled air	1.0	0.02	0.02
Food	33.2	0.66	0.25
Water and beverages	17.9	0.34	0.04
Dust	4.5	0.09	0.06
Total	56.6	1.13	0.37
Adult male			
Inhaled air	1.0	0.014	0.014
Food	45.7	0.65	0.25
Water and beverages	25.1	0.36	0.04
Dust	4.5	0.064	0.04
Total	76.3	1.088	0.344

*Body weights: 2 year old child = 10/kg; adult female = 50 kg; adult male = 70 kg.

The major source of lead contamination of drinking water is the distribution system itself, particularly in older urban areas. Highest levels are encountered in "first-draw" samples, i.e., water sitting in the piping system for an extended period of time. In a large community water supply survey of 969 systems carried out in 1969-1970, it was found that the prevalence of samples exceeding 0.05 µg/g was greater where water was plumbo-solvent.

Most drinking water, and the beverages produced from drinking water, contain 0.008 to 0.02 µg Pb/g. The exceptions are canned juices and soda pop, which range from 0.033 to 0.052 µg/g. About 11 percent of the lead consumed in drinking water and beverages is of direct atmospheric origin, 70 percent comes from solder and other metals.

TABLE 13-3. SUMMARY OF POTENTIAL ADDITIVE EXPOSURES TO LEAD

	Total Lead Consumed (μg/day)	Atmospheric Lead Consumed (μg/day)	Other Lead Sources (μg/day)
Baseline exposure:			
Child (2 yr old)			
Inhaled air	0.5	0.5	-
Food, water & beverages	39.9	12.1	27.8
Dust	21.0	19.0	2.0
Total baseline	61.4	31.6	29.8
Additional exposure due to:			
urban atmospheres:[1]			
air inhalation	7	7	0
dust	72	71	1
family gardens[2]	800	200	600
interior lead paint[3]	85	-	85
residence near smelter:[4]			
air inhalation	60	60	-
dust	2250	2250	-
secondary occupational[5]	150	-	-
Baseline exposure:			
Adult Male			
Inhaled air	1.0	1.0	-
Food, water & beverages	70.8	20.2	50.6
Dust	4.5	2.9	1.6
Total baseline	76.3	24.1	52.2
Additional exposure due to:			
urban atmospheres:[1]			
air inhalation	14	14	-
dust	7	7	-
family gardens[2]	2000	500	1500
interior lead paint[3]	17	-	17
residence near smelter:[4]			
air inhalation	120	120	-
dust	250	250	-
occupational[6]	1100	1100	-
secondary occupational[5]	21	-	-
smoking	30	27	3
wine consumption	100	?	?

[1]includes lead from household and street dust (1000 μg/g) and inhaled air (.75 μg/m^3)

[2]assumes soil lead concentration of 2000 μg/g; all fresh leafy and root vegetables, sweet corn of Table 7-15 replaced by produce from garden. Also assumes 25% of soil lead is of atmospheric origin.

[3]assumes household dust rises from 300 to 2000 μg/g. Dust consumption remains the same as baseline. Does not include consumption of paint chips.

[4]assumes household and street dust increases to 25,000 μg/g, inhaled air increases to 6 μg/m^3.

[5]assumes household dust increases to 2400 μg/g.

[6]assumes 8 hr shift at 10 μg Pb/m^3 or 90% efficiency of respirators at 100 μg/ Pb/m^3. and occupational dusts at 100,000 μg/m^3.

13.2.3.5 Lead in Other Media. Flaking lead paint in deteriorated housing stock in urban areas of the Northeast and Midwest has long been recognized as a major source of lead exposure for young children residing in this housing stock, particularly for children with pica. Individuals who are cigarette smokers may inhale significant amounts of lead in tobacco smoke. One study has indicated that the smoking of 30 cigarettes daily results in lead intake equivalent to that of inhaling lead in ambient air at a level of 1.0 μg Pb/m^3.

13.2.3.6 Cumulative Human Lead Intake From Various Sources.

Table 13-1 shows the baseline of human lead exposures as described in detail in Chapter 7. These data show that atmospheric lead accounts for at least 30 percent of the baseline adult consumption and 50 percent of the daily consumption by a 2 yr old child. These percentages are conservative estimates because a part of the lead of undetermined origin may originate from atmospheric lead not yet accounted for.

From Table 13-2, it can be seen that young children have a dietary lead intake rate that is 5-fold greater than for adults, on a body weight basis. To these observations must be added that absorption rates for lead are higher in children than in adults by at least 3-fold. Overall, then, the rate of lead entry into the blood stream of children, on a body weight basis, is estimated to be twice that of adults from the respiratory tract and 6 and 9 times greater from the GI tract. Since children consume more dust than adults, the atmospheric fraction of the baseline exposure is ten-fold higher for children than for adults, on a body weight basis. These differences generally tend to place young children at greater risk, in terms of relative amounts of proportions of atmospheric lead absorbed per kg body weight, than adults under any given lead exposure situation.

13.3 LEAD METABOLISM: KEY ISSUES FOR HUMAN HEALTH RISK EVALUATION

From the detailed discussion of those various quantifiable characteristics of lead toxicokinetics in humans and animals presented in Chapter 10, several clear issues emerge as being important for full evaluation of the human health risk posed by lead:

1) Differences in systemic or internal lead exposure of groups within the general population in terms of such factors as age/development and nutritional status; and

2) The relationship of indices of internal lead exposures to both environmental levels of lead and tissues levels/effects.

Item 1 provides the basis for identifying segments within human populations at increased risk in terms of exposure criteria and is used along with additional information on relative sensitivity to lead health effects for identification of risk populations. The chief concern

with item 2 is the adequacy of current means for assessing internal lead exposure in terms of providing adequate margins of protection from lead exposures producing health effects of concern.

13.3.1 Differential Internal Lead Exposure Within Population Groups

Compared to adults, young children take in more lead through the gastrointestinal and respiratory tracts on a unit body weight basis, absorb a greater fraction of this lead intake, and also retain a greater proportion of the absorbed amount.

Unfortunately, such amplification of these basic toxicokinetic parameters in children vs. adults also occurs at the time when: (1) humans are developmentally more vulnerable to the effects of toxicants such as lead in terms of metabolic activity, and (2) the interactive relationships of lead with such factors as nutritive elements are such as to induce a negative course toward further exposure risk.

Typical of physiological differences in children vs. adults in terms of lead exposure implications is a more metabolically active skeletal system in children. In children, turnover rates of bone elements such as calcium and phosphorus are greater than in adults, with correspondingly greater mobility of bone-sequestered lead. This activity is a factor in the observation that the skeletal system of children is relatively less effective as a depository for lead than in adults.

Metabolic demand for nutrients, particularly calcium, iron, phosphorus, and the trace nutrients, is such that widespread deficiencies of these nutrients exist, particularly among poor children. The interactive relationships of these elements with lead are such that deficiency states both enhance lead absorption/retention and, as in the case of lead-induced reductions in 1,25-dihydroxyvitamin D, establish increasingly adverse interactive cycles.

Quite apart from the physiological differences which enhance internal lead exposure in children is the unique relationship of 2- to 3-year-olds to their exposure setting by way of normal mouthing behavior and the extreme manifestation of this behavior, pica. This behavior occurs in the same age group which studies have consistently identified as having a peak in blood lead. A number of investigations have addressed the quantification of this particular route of lead exposure, and it is by now clear that such exposure will dominate other routes when the child's surroundings, e.g., dust and soil, are significantly contaminated by lead.

Information provided in Chapter 10 also makes it clear that lead traverses the human placental barrier, with lead uptake by the fetus occurring throughout gestation. Such uptake of lead poses a potential threat to the fetus via an impact on the embryological developement of the central nervous and other systems. Hence, the only logical means of protecting the fetus from lead exposure is exposure control during pregnancy.

Within the general population, then, young children and pregnant women qualify as definale risk groups for lead exposure. Occupational exposure to lead, particularly among lead workers, logically defines these individuals as being in a high-risk category; work place contact is augmented by those same routes and levels of lead exposure affecting the rest of the adult population. From a biological point of view, lead workers do not differ from the general adult population with respect to the various toxicokinetic parameters and any differences in exposure control--occupational vs. non-occupational populations--as they exist are based on factors other than toxicokinetics.

13.3.2 Indices of Internal Lead Exposure and Their Relationship To External Lead Levels and Tissue Burdens/Effects

Several points are of importance in this area of lead toxicokinetics. They are: 1) the temporal characteristics of indices of lead exposure; 2) the relationship of the indicators to external lead levels; 3) the validity of indicators of exposure in reflecting target tissue burdens; 4) the interplay between these indicators and lead in body compartments; and 5) those various aspects of the issue with particular reference to children.

At this time, blood lead is widely held to be the most convenient, if imperfect, index of both lead exposure and relative risk for various adverse health effects. In terms of exposure, however, it is generally accepted that blood lead is a temporally variable measure which yields an index of relatively recent exposure because of the rather rapid clearance of absorbed lead from the blood. Such a measure, then, is of limited usefulness in cases where exposure is variable or intermittent over time, as is often the case with pediatric lead exposure.

Mineralizing tissue, specifically deciduous teeth, accumulate lead over time in proportion to the degree of lead exposure, and analysis of this material provides an assessment integrated over a greater time period and of more value in detecting early childhood exposure.

These two methods of assessing internal lead exposure have obvious shortcomings. A blood lead value will say little about any excessive lead intake at early periods, even though such remote exposure may have resulted in significant injury. On the other hand, whole tooth or dentine analysis is retrospective in nature and can only be done after the particularly vulnerable age in children under 4 to 5 years-- has passed. Such a measure, then provides little utility upon which to implement regulatory policy or clinical intervention.

The dilemmas posed by these existing methods may be able to be resolved by *in situ* analysis of teeth and bone lead, such that the intrinsic advantage of mineral tissue as a cumulative index is combined with measurement which is temporally concordant with on-going exposure. Work in several laboratories offers promise for such *in situ* analysis (See Chapters 9 and 10).

A second issue concerning internal indices of exposure and environmental lead is the relationship of changes in lead content of some medium with changes in blood content. Much of Chapter 11 was given over to description of the mathematical relationships of blood lead with lead in some external medium-- air, food, water, etc., without consideration of the biological underpinnings for these relationships.

Over a relatively broad range of lead exposure through some medium, the relationship of lead in the external medium to blood lead is curvilinear, such that relative change in blood lead per unit change in medium level generally becomes increasingly less as exposure increases. This behavior may reflect changes in tissue lead kinetics, reduced lead absorption, or increased excretion. Limited animal data would suggest that changes in excretion or absorption are not factors in this phenomenon. In any event, modest changes in blood levels with exposure at the higher end of this range are in no way to be taken as reflecting concomitantly modest changes in body or tissue lead uptake. Evidence continues to accumulate which suggests that an indicator such as blood lead is an imperfect measure of tissue lead burdens and of changes in such tissue levels in relation to changes in external exposure.

In Chapter 10, it was pointed out that blood lead is logarithmically related to chelatable lead (the latter being a more useful measure of the potentially toxic fraction of body lead), such that a unit change in blood lead is associated with an increasingly larger amount of chelatable lead. One consequence of this relationship is that moderately elevated blood lead values will tend to mask the "margin of safety" in terms of mobile body lead burdens. Such masking is apparent in one study of children where chelatable lead levels in children showing moderate elevations in blood lead overlapped those obtained in subjects showing frank plumbism, i.e. overt lead intoxication.

Related to the above is the question of the source of chelatable lead. It was noted in Chapter 10 that some sizable fraction of chelatable lead is derived from bone and this compels reappraisal of the notion that bone is an "inert sink" for otherwise toxic body lead.

The notion of bone lead as toxicologically inert never did accord with what was known from studies of bone physiology, i.e., that bone is a "living" organ, and the thrust of recent studies of chelatable lead as well as interrelationships of lead and bone metabolism is more to a view of bone lead as actually an insidious source of long-term systemic lead exposure rather than evidence of a protective mechanism permitting significant lead contact in industrialized populations.

The complex interrelationships of lead exposure, blood lead, and lead in body compartments is of particular interest in considering the disposition of lead in young children. Since children take in more lead on a weight basis, and absorb and retain more of this lead than the adult, one might expect that either tissue and blood levels would be significantly elevated or that the child's skeletal system would be more efficient in lead sequestration.

Blood lead levels in young children are either similar to adults (males) or somewhat higher (adult females). Limited autopsy data, furthermore, indicate that soft tissue levels in children are not markedly different from adults, whereas the skeletal system shows an approximate 2-fold increase in lead concentration from infancy to adolescence. Neglected in this observation is the fact that the skeletal system in children grows at an exponential rate, so that skeletal mass increases 40-fold during the interval in childhood when bone lead levels increase 2-fold, resulting in an actual increase of approximately 80-fold in total skeletal lead. If the skeletal growth factor is taken into account, along with growth in soft tissue and the expansion of vascular fluid volumes, the question of lead disposition in children is better understood.

Finally, limited animal data indicate that blood lead alterations with changes in lead exposure are poor indicators of such changes in target tissue. Specifically, it appears that abrupt reduction of lead exposure will be more rapidly reflected in blood lead than in such target tissues as the central nervous system, especially in the developing organism. This discordance may underlie the observation that severe lead neurotoxicity in children is associated with a rather broad range of blood lead values (see Section 12.4).

The above discussion of some of the problems with the use of blood lead in assessing target tissue burdens or the toxicologically active fraction of total body lead is really a summary of the inherent toxicokinetic problems with use of blood lead levels in defining margins of safety for avoiding internal exposure or undue risk of adverse effects.

If, for example, blood lead levels of 40-50 μg/dl in "asymptomatic" children are associated with chelatable lead burdens which overlap those encountered in frank pediatric plumbism, as documented in one series of lead-exposed children, then there is no margin of safety at these blood levels for severe effects which are not at all a matter of controversy. Were it both logistically feasible to do so on a large scale and were the use of chelants free of health risk to the subjects, serial provocative chelation testing would appear to be the better indicator of exposure and risk. Failing this, the only prudent alternative is the use of a large safety factor applied to blood lead which would translate to an "acceptable" chelatable burden. It is likely that this blood lead value would lie well below the currently accepted upper limit of 30 μg/dl, since the safety factor would have to be large enough to protect against frank plumbism as well as more subtle health effects seen with non-overt lead intoxication. This rationale from the standpoint of lead toxicokinetics is in accord also with the growing data base for dose-effect relationships of lead's effects on heme biosynthesis, erythropoiesis, and the nervous system in humans as detailed in Sections 12.3 and 12.4.

The future developement and routine use of in situ mineral tissue testing at time points concordant with on-going exposure and the comparison of such results with simultaneous blood

lead and chelatable lead measurement would be of significant value in further defining what level of blood lead is indeed an acceptable upper limit.

13.4 DEMOGRAPHIC CORRELATES OF HUMAN LEAD EXPOSURE AND RELATIONSHIPS BETWEEN EXTERNAL AND INTERNAL LEAD EXPOSURE INDICES

13.4.1 Demographic Correlates of Lead Exposure

Studies of ancient populations using bone and teeth show that levels of internal exposure of lead today are substantially elevated over past levels. Studies of current populations living in remote areas far from urbanized cultures show blood lead levels in the range of 1 to 5 μg/dl. In contrast to the blood lead levels found in remote populations, data from current U.S. populations have geometric means ranging from 10 to 20 μg/dl depending on age, race, sex and degree of urbanization. These increases of current exposure appear to be associated with industrialization and widespread commercial use of lead, for example gasoline combustion.

Age appears to be one of the single most important demographic covariate of blood lead levels. Blood lead levels in children up to six years are generally higher than those in non-occupationally exposed adults. Children aged two to three years tend to have the highest levels as shown in Figure 13-2. Blood lead levels in non-occupationally exposed adults may increase slightly with age due to skeletal lead accumulation.

Sex has a differential impact on blood lead levels depending on age. No significant differences exist between males and females less than seven years of age. Males above the age of seven generally have higher blood lead levels than females.

Race also plays a role, in that blacks have higher blood lead levels than either whites or Hispanics. Race has yet to be fully disentangled from exposure.

Blood lead levels also seem to increase with degree of urbanization. Data from NHANES II show that blood lead levels in the United States, averaged from 1976 to 1980, increase from a geometric mean of 11.9 μg/dl in rural populations to 12.8 μg/dl in urban populations less than one million, and increase again to 14.0 μg/dl in urban populations of one million or more.

Recent U.S. blood lead levels show a downward trend occurring consistently across race, age and geographic location. The downward pattern commenced in the early part of the 1970's and has continued into 1980. The downward trend has occurred from a shift in the entire distribution and not through a truncation in the high blood lead levels. This consistency suggests a general causative factor, and attempts have been made to identify the causative element. Reduction in lead emitted from the combustion of leaded gasoline is a prime candidate, but at present no causal relationship has been definitively established.

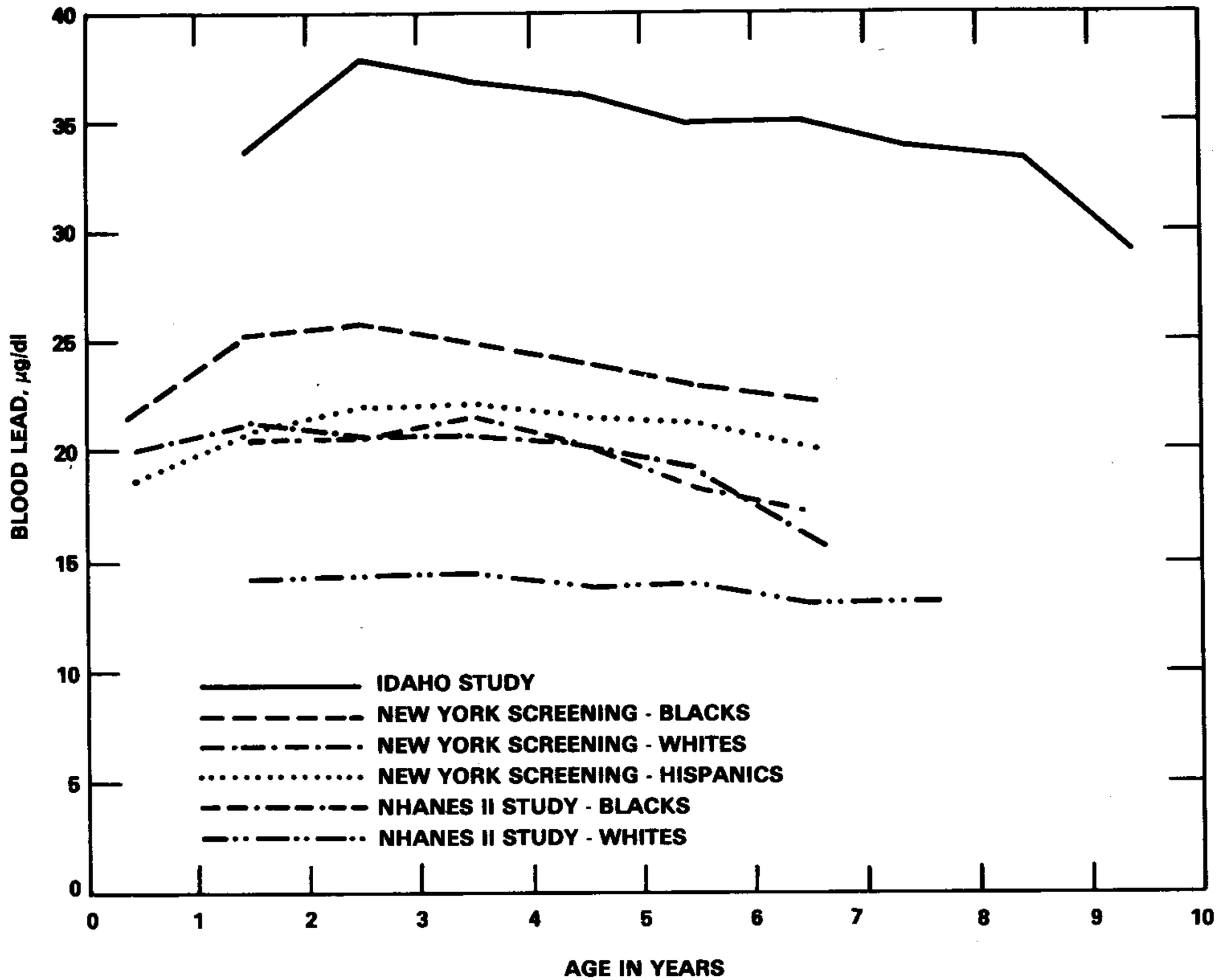

Figure 13-2. Geometric mean blood lead levels by race and age for younger children in the NHANES II study, and the Kellogg/Silver Valley and New York Childhood Screening Studies.

Blood lead levels, examined on a population basis, have similarly skewed distributions. Blood lead levels, from a population thought to be homogenous in terms of demographic and lead exposure characteristics, approximately follow a lognormal distribution. Geometric standard deviations, an estimation of dispersion, from four different studies discussed in Chapter 11, including analytic error, are about 1.4 for children and possibly somewhat smaller for adults. This allows an estimation of the upper tail of the blood lead distribution, which is the population segment in the United States at higher risk.

13.4.2 Relationships Between External and Internal Lead Exposure Indices

Because one main purpose of this chapter is to examine relationships of lead in air and lead in blood under ambient conditions, the results of studies most appropriate to this area have been emphasized. A summary of the most appropriate studies appears in Table 13-4. At air lead exposures of 3.2 μg/m³ or less, there is no statistically significant difference between curvilinear and linear blood lead inhalation relationships. At air lead exposures at 10 μg/m³ or more, either nonlinear or linear relationships can be fitted. Thus, a reasonably consistent picture emerges in which the blood lead air lead relationship by direct inhalation was approximately linear in the range of normal ambient exposures (0.1 - 2.0 μg/m³) as discussed in Chapter 7. Differences among individuals in a given study, and among several studies are large, so that pooled estimates of the blood lead inhalation slope depend upon the the weight given to various studies. Several studies were selected for analysis, based upon factors described earlier. EPA analyses of experimental and clinical studies (Griffin et al., 1975; Rabinowitz et al., 1974, 1976, 1977; Kehoe 1961a,b,c; Gross 1981; Hammond et al., 1981) suggest that blood lead in adults increases by 1.64 ± 0.22 μg/dl from direct inhalation of each additional μg/m³ of air lead. EPA analyses of population studies (Yankel et al., 1977; Roels et al., 1980; Angle and McIntire, 1979) suggest that, for children, the blood lead increase is 1.97 ± 0.39 μg/dl per μg/m³ for air lead. EPA anaylsis of Azar's population study (Azar et al., 1975) yields a slope of 1.32 ± 0.38 for adult males.

These slope estimates are based on the assumption that an equilibrium level of blood lead is achieved within a few months after exposure begins. This is only approximately true, since lead stored in the skeleton may return to blood after some years. Chamberlain et al. (1978) suggest that long term inhalation slopes should be about 30 percent larger than these estimates. Inhalation slopes quoted here are associated with a half-life of blood lead in adults of about 30 days. O'Flaherty et al. (1982) suggest that the blood-lead half-life may increase slightly with duration of exposure, but this has not been confirmed (Kang et al., 1983).

One possible approach would be to regard all inhalation slope studies as equally informative and to calculate an average slope using reciprocal squared standard error estimates as

TABLE 13-4. SUMMARY OF BLOOD INHALATION SLOPES, (β) μg/dl per μg/m³

POPULATION	STUDY	STUDY TYPE	N	(β) SLOPE μg/dl per μg/m³	MODEL SENSITIVITY OF SLOPE*
Children	Angle and McIntire, 1979 Omaha, NE	Population	1074	1.92	(1.40 - 4.40)[1,2,3]
	Roels et al. (1980) Belgium	Population	148	2.46	(1.55 - 2.46)[1,2]
	Yankel et al. (1977); Walter et al. (1980) Idaho	Population	879	1.52	(1.07 - 1.52)[1,2,3]
Adult Males	Azar et al. (1975). Five groups	Population	149	1.32	(1.08 - 2.39)[2,3]
	Griffin et al. (1975), NY prisoners	Experiment	43	1.75	(1.52 - 3.38)[4]
	Gross (1979)	Experiment	6	1.25	(1.25 - 1.55)[2]
	Rabinowitz et al. (1973,1976, 1977)	Experiment	5	2.14	(2.14 - 3.51)[5]

*Selected from among the most plausible statistically equivalent models. For nonlinear models, slope at 1.0 μg/m³.

[1]Sensitive to choice of other correlated predictors such as dust and soil lead.

[2]Sensitive to linear vs. nonlinear at low air lead.

[3]Sensitive to age as a covariate.

[4]Sensitive to baseline changes in controls.

[5]Sensitive to assumed air lead exposure.

weights. This approach has been rejected for two reasons. First, the standard error estimates characterize only the internal precision of an estimated slope, not its representativeness (i.e., bias) or predictive validity. Secondly, experimental and clinical studies obtain more information from a single individual than do population studies. Thus, it may not be appropriate to combine the two types of studies.

Estimates of the inhalation slope for children are only available from population studies. The importance of dust ingestion as a non-inhalation pathway for children is established by many studies. A slope estimate has been derived for air lead inhalation based on those studies (Angle and McIntire 1979; Roels et al., 1980; Yankel et al., 1977) from which the air inhalation and dust ingestion contributions can both be estimated.

While direct inhalation of air lead is stressed, this is not the only air lead contribution that needs to be considered. Smelter studies allow partial assessment of the air lead contributions to soil, dust and finger lead. Conceptual models allow preliminary estimation of the propagation of lead through the total food chain as shown in Chapter 7. Useful mathematical models to quantify the propagation of lead through the food chain need to be developed. The direct inhalation relationship does provide useful information on changes in blood lead as responses to changes in air lead on a time scale of several months. The indirect pathways through dust and soil and through the food chain may thus delay the total blood lead response to changes in air lead, perhaps by one or more years. The Italian ILE study facilitates partial assessment of this delayed response from leaded gasoline as a source.

Dietary absorption of lead varies greatly from one person to another and depends on the physical and chemical form of the carrier, on nutritional status, and on whether lead is ingested with food or between meals. These distinctions are particularly important for consumption by children of leaded paint, dust and soil. Typical values of 10 percent absorption of ingested lead into blood have been assumed for adults and 25 to 50 percent for children.

It is difficult to determine accurate relationships between blood lead levels and lead levels in food or water. Dietary intake must be estimated by duplicate diets or fecal lead determinations. Water lead levels can be determined with some accuracy, but the varying amounts of water consumed by different individuals adds to the uncertainty of the estimated relationships.

Quantitative analyses relating blood lead levels and dietary lead exposures have been reported. Studies on infants provide estimates that are in close agreement. Only one individual study is available for adults (Sherlock et al. 1982); another estimate from a number of pooled studies is also available. These two estimates are in good agreement. Most of the subjects in the Sherlock et al. (1982) and United Kingdom Central Directorate on Environmental Pollution (1982) studies received quite high dietary lead levels (>300 μg/day). The fitted

cube root equations give high slopes at lower dietary lead levels. On the other hand, the linear slope of the United Kingdom Central Directorate on Environmental Pollution (1982) study is probably an underestimate of the slope at lower dietary lead levels. For these reasons, the Ryu et al. (1983) study is the most believable, although it only applies to infants. Estimates for adults should be taken from the experimental studies or calculated from assumed absorbtion and half-life values. Most of the dietary intake supplements were so high that many of the subjects had blood lead concentrations much in excess of 30 $\mu g/m^3$ for a considerable part of the experiment. Blood lead levels thus may not completely reflect lead exposure, due to the previously noted nonlinearity of blood lead response at high exposures. The slope estimates for adult dietary intake are about 0.02 µg/dl increase in blood lead per µg/day intake, but consideration of blood lead kinetics may increase this value to about 0.04. Such values are a bit lower than those estimated from the population studies extrapolated to typical dietary intakes about 0.05 µg/dl per µg/day. The value for infants is much larger.

The relation between blood lead and water lead is not clearly defined and is often described as nonlinear. Water lead intake varies greatly from one person to another. It has been assumed that children can absorb 25 to 50 percent of lead in water. Many authors chose to fit cube root models to their data, although polynomial and logarithmic models were also used. Unfortunately, the form of the model greatly influences the estimated contributions to blood lead levels from relatively low water lead concentration.

Although there is close agreement in the quantitative analyses of the relationship between blood lead level and dietary lead, there is a larger degree of variability in results of the various water lead studies. The relationship is curvilinear, but its exact form is yet to be determined. At typical levels for U.S. populations, the relationship appears linear. The only study that determines the relationship based on lower water lead values (<100 µg/l) is the Pocock et al. (1983) study. The data from this study, as well as the authors themselves, suggest that in this lower range of water lead levels, the relationship is linear. Furthermore, the estimated contributions to blood lead levels from this study are quite consistent with the polynomial models from other studies. For these reasons, the Pocock et al. (1983) slope of 0.06 is considered to represent the best estimate. The possibility still exists, however, that the higher estimates of the other studies may be correct in certain situations, especially at higher water lead levels (>100 µg/l).

Studies relating soil lead to blood lead levels are difficult to compare. The relationship obviously depends on depth of soil lead, age of the children, sampling method, cleanliness of the home, mouthing activities of the children, and possibly many other factors. Various soil sampling methods and sampling depths have been used over time, and as such they may not be directly comparable and may produce a dilution effect of the major lead concentration

contribution from dust which is located primarily in the top 2 cm of the soil. Increases in soil dust lead significantly increase blood lead in children. From several studies (Yankel et al., 1977; Angle and McIntire, 1979) EPA estimates an increase of 0.6 to 6.8 μg/dl in blood lead for each increase of 1000 μg/g in soil lead concentration. The values from the Stark et al. (1982) study of about 2, may represent a reasonable median estimate. The relationship of housedust lead to blood lead is difficult to obtain. Household dust also increases blood lead, children from the cleanest homes in the Silver Valley/Kellogg Study having 6 μg/dl less lead in blood, on average, than those from the households with the most dust.

A number of specific environmental sources of airborne lead have been evaluated for potential direct influence on blood lead levels. Combustion of leaded gasoline appears to be the largest contributor to airborne lead. Two studies used isotope ratios of lead to estimate the relative proportion of lead in the blood coming from airborne lead.

From the Manton study it can be estimated that between 7 to 41 percent of the blood lead in study subjects in Dallas resulted from airborne lead. Additionally, these data provide a means of estimating the indirect contribution of air lead to blood lead. By one estimate, only 10 to 20 percent of the total airborne contribution in Dallas is from direct inhalation.

From the ILE data of Facchetti and Geiss (1982), as shown in Table 13-5, the direct inhalation of air lead may account for 54 percent of the total adult blood lead uptake from leaded gasoline in a large urban center, but inhalation is a much less important pathway in

TABLE 13-5. ESTIMATED CONTRIBUTION OF LEADED GASOLINE TO BLOOD LEAD BY INHALATION AND NON-INHALATION PATHWAYS

Location	Air Lead Fraction From Gasoline[a]	Blood Lead Fraction From Gasoline[b]	Blood Lead From Gasoline In Air[c] (μg/dl)	Blood Lead Not Inhaled From Gasoline[d] (μg/dl)	Estimated Fraction Gas-Lead Inhalation[e]
Turin	0.873	0.237	2.79	2.37	0.54
<25 km	0.587	0.125	0.53	2.60	0.17
>25 km	0.587	0.110	0.28	3.22	0.08

[a]Fraction of air lead in Phase 2 attributable to lead in gasoline.

[b]Mean fraction of blood lead in Phase 2 attributable to lead in gasoline.

[c]Estimated blood lead from gas inhalation = $\beta \times (a) \times (b)$, $\beta = 1.6$.

[d]Estimated blood lead from gas, non-inhalation = (f)-(e)

[e]Fraction of blood lead uptake from gasoline attributable to direct inhalation = (f)/(e)

Source: Facchetti and Geiss (1982), pp. 52-56.

suburban parts of the region (17 percent of the total gasoline lead contribution) and in the rural parts of the region (8 percent of the total gasoline lead contribution). EPA analyses of the preliminary results from the ILE study separated the inhalation and non-inhalation contributions of leaded gasoline to blood lead into the following three parts: (1) An increase of about 1.7 μg/dl in blood lead per μg/m^3 of air lead, attributable to direct inhalation of the combustion products of leaded gasoline; (2) a sex difference of about 2 μg/dl attributable to lower exposure of women to indirect (non-inhalation) pathways for gasoline lead; and (3) a non-inhalation background attributable to indirect gasoline lead pathways, such as ingestion of dust and food, increasing from about 2 μg/dl in Turin to 3 μg/dl in remote rural areas. The non-inhalation background represents only two to three years of environmental accumulation at the new experimental lead isotope ratio. It is not clear how to numerically extrapolate these estimates to U.S. subpopulations; but it is evident that even in rural and suburban parts of a metropolitan area, the indirect (non-inhalation) pathways for exposure to leaded gasoline make a significant contribution to blood lead. This can be seen in Table 13-5. It should also be noted that the blood lead isotope ratio responded fairly rapidly when the lead isotope ratio returned to its pre-experimental value, but it is not yet possible to estimate the long term change in blood lead attributable to persistent exposures to accumulated environmental lead.

Studies of data from blood lead screening programs suggest that the downward trend in blood lead levels noted earlier is due to the reduction in air lead levels, which has been attributed to the reduction of lead in gasoline.

Primary lead smelters, secondary lead smelters and battery plants emit lead directly into the air and ultimately increase soil and dust lead concentrations in their vicinity. Adults, and especially children, have been shown to exhibit elevated blood lead levels when living close to these sources. Blood lead levels in these residents have been shown to be related to air, as well as to soil or dust exposures.

13.4.3 Proportional Contributions of Lead in Various Media to Blood Lead in Human Populations

The various mathematical descriptions of the relationship of blood lead to lead in individual media--air, food, water, dust, soil--were discussed in some detail in Chapter 11 and concisely in the preceding section (13.4.2) of this chapter. Using values for lead intake/content of these media which appear to represent the current exposure picture for human populations in the U.S., these relationships are further employed in this section to estimate proportional inputs to total blood lead levels in U.S. populations. Such an exercise is of help in providing an overall perspective on which routes of exposure are of most significance in terms of contributions to blood lead levels seen in U.S. populations.

Table 13-6 tabulates the relative direct contributions (in percentages) of air lead to blood lead at different air-lead levels for calculated typical background levels of lead from food and water in adults. The blood lead contributions from diet are estimated using the slope 0.02 μg/dl increase in blood lead μg/day intake as discussed in Section 11.4.2.4.

TABLE 13-6. DIRECT CONTRIBUTIONS OF AIR LEAD TO BLOOD LEAD (PbB) IN ADULTS AT FIXED INPUTS OF WATER AND FOOD LEAD

Air Lead ($\mu g/m^3$)	PbB (Air)[a]	PbB (Food)[b]	PbB (Water)[c]	% PbB From Air
0.1	0.2	2.0	0.6	7.1
1.0	2.0	2.0	0.6	43.4
1.5	3.0	2.0	0.6	53.5

[a] $\frac{\Delta \text{ PbB}}{\Delta \text{ Pb Air}} = 2.0$ for 3.2 $\mu g/m^3$ or less.

[b] Assuming 100 μg/day lead from diet and slope 0.02 as discussed in Section 11.4.2.4.

[c] Assuming 10 μg/ℓ water, Pocock et al. (1983).

In Table 13-7 are listed the direct contributions of air lead to blood lead at varying air lead levels for children given calculated typical background levels of blood lead for food and water. Diet contribution is based on the work of Ryu et al. (1983). Table 13-8 shows the relative contributions of dust/soil to blood lead at varying dust/soil levels for children given calculated background levels of blood lead from air, food, and water. Assuming that virtually all soil/dust lead is due to atmospheric fallout of lead particles, the percentage contribution of air directly and indirectly to blood lead becomes significantly greater than when considering just the direct impact of inhaling lead in the ambient air.

TABLE 13-7. DIRECT CONTRIBUTIONS OF AIR LEAD TO BLOOD LEAD IN CHILDREN AT FIXED INPUTS OF FOOD AND WATER LEAD

Air Lead ($\mu g/m^3$)	PbB (Air)[a]	PbB (Food)[b]	PbB (Water)[c]	% PbB From Air
0.1	0.2	16.0	0.6	1.2
0.5	1.0	16.0	0.6	5.7
1.0	2.0	16.0	0.6	10.8
1.5	3.0	16.0	0.6	15.3
2.5	5.0	16.0	0.6	23.1

[a] $\frac{\Delta \text{ PbB}}{\Delta \text{ Pb Air}} = 2.0$ for 3.2 $\mu g/m^3$ or less.

[b] Assuming 100 μg Pb/day based upon Ryu et al. (1983).

[c] Assuming 10 μg Pb/l water, using Pocock et al. (1983).

PRELIMINARY DRAFT

TABLE 13-8. CONTRIBUTIONS OF DUST/SOIL LEAD TO BLOOD LEAD IN CHILDREN AT FIXED INPUTS OF AIR, FOOD, AND WATER LEAD

Dust-Soil (μg/g)	Air Lead μg/m³	PbB (Air)[a]	PbB (Food)[b]	PbB (Water)[c]	PbB (Dust-Soil)[d]	% PbB From Dust/Soil
500	0.5	1.0	16.0	0.6	0.3/3.4	1.7/16.2
1000	0.5	1.0	16.0	0.6	0.6/6.8	3.3/27.8
2000	0.5	1.0	16.0	0.6	1.2/13.6	6.4/43.6

[a] $\frac{\Delta \text{ PbB}}{\Delta \text{ Pb Air}} = 2.0$ for 3.2 $\mu g/m^3$ or less.

[b] Assuming 100 μg Pb/day based on Ryu et al. (1983).

[c] Assuming 10 μg Pb/l water, based on Pocock et al. (1983).

[d] Based on range 0.6 to 6.8 μg/dl for 1000 μg/g (Angle and McIntire, 1979).

PRELIMINARY DRAFT

13.5 BIOLOGICAL EFFECTS OF LEAD RELEVANT TO THE GENERAL HUMAN POPULATION

13.5.1 Introduction

It is clear from the wealth of available literature reviewed in Chapter 12, that there exists a continuum of biological effects associated with lead across a broad range of exposure. At rather low levels of lead exposure, biochemical changes, e.g., disruption of certain enzymatic activities involved in heme biosynthesis and erythropoietic pyrimidine metabolism, are detectable. Heme biosynthesis is a generalized process in mammalian species, including man, with importance for normal physiological functioning of virtually all organ systems. With increasing lead exposure, there are sequentially more intense effects on heme synthesis and a broadening of lead effects to additional biochemical and physiological mechanisms in various tissues, such that increasingly more severe disruption of the normal functioning of many different organ systems becomes apparent. In addition to heme biosynthesis impairment at relatively low levels of lead exposure, disruption of normal functioning of the erythropoietic and the nervous systems are among the earliest effects observed as a function of increasing lead exposure. With increasingly intense exposure, more severe disruption of the erythropoietic and nervous systems occur and additional organ systems are affected so as to result, for example, in the manifestation of renal effects, disruption of reproductive functions, and impairment of immunological functions. At sufficiently high levels of exposure, the damage to the nervous system and other effects can be severe enough to result in death or, in some cases of non-fatal lead poisoning, long-lasting sequelae such as permanent mental retardation.

As discussed in Chapter 12 of this document, numerous new studies, reviews, and critiques concerning Pb-related health effects have been published since the issuance of the earlier EPA lead criteria document in 1977. Of particular importance for present criteria development purposes are those new findings, taken together with information earlier available at the writing of the 1977 Criteria Document, which have bearing on the establishment of quantitative dose-effect or dose-response relationships for biological effects of lead potentially viewed as adverse health effects likely to occur among the general population at or near existing ambient air concentrations of lead in the United States. Key information regarding observed health effects and their implications are discussed below for adults and children.

For the latter group, children, emphasis is placed on the discussion of (1) heme biosynthesis effects, (2) certain other biochemical and hematological effects, and (3) the disruption of nervous system functions. All of these appear to be among those effects of most concern for potential occurrence in association with exposure to existing U.S. ambient air lead levels of the population group (i.e., children ≤6 years old) at greatest risk for lead-induced health effects. Emphasis is also placed on the delineation of internal lead exposure levels, as defined mainly by blood-lead (PbB) levels, likely associated with the occurrence of such

effects. Also discussed are characteristics of the subject effects that are of crucial importance in regard to the determination of which might reasonably be viewed as constituting "adverse health effects" in affected human populations.

Over the years, there has been superimposed on the continuum of lead-induced biological effects various judgments as to which specific effects observed in man constitute "adverse health effects". Such judgments involve not only medical concensus regarding the health significance of particular effects and their clinical management, but also incorporate societal value judgments. Such societal value judgments often vary depending upon the specific overall contexts to which they are applied, e.g., in judging permissible exposure levels for occupational versus general population exposures to lead. For some lead exposure effects, e.g., severe nervous system damage resulting in death or serious medical sequelae consequent to intense lead exposure, there exists little or no disagreement as to these being significant "adverse health effects." For many other effects detectable at sequentially lower levels of lead exposure, however, the demarcation lines as to which effects represent adverse health effects and the lead exposure levels at which they are accepted as occurring are neither sharp nor fixed, having changed markedly during the past several decades. That is, from a historical perspective, levels of lead exposure deemed to be acceptable for either occupationally exposed persons or the general population have been steadily revised downward as more sophisticated biomedical techniques have revealed formerly unrecognized biological effects and concern has increased in regard to the medical and social significance of such effects.

It is difficult to provide a definitive statement of all criteria by which specific biological effects associated with any given agent can be judged to be "adverse health effects". Nevertheless, several criteria are currently well-accepted as helping to define which effects should be viewed as "adverse". These include: (1) impaired normal functioning of a specific tissue or organ system itself; (2) reduced reserve capacity of that tissue or organ system in dealing with stress due to other causative agents; (3) the reversibility/irreversibility of the particular effect(s); and (4) the cumulative or aggregate impact of various effects on individual organ systems on the overall functioning and well-being of the individual.

Examples of possible uses of such criteria in evaluating lead effects can be cited for illustrative purposes. For example, impairment of heme synthesis intensifies with increasing lead exposure until hemeprotein synthesis is inhibited in many organ systems, leading to reductions in such functions as oxygen transport, cellular energetics, and detoxification of xenobiotic agents. The latter effect can also be cited as an example of reduced reserve capacity pertinent to consideration of effects of lead, the reduced capacity of the liver to detoxify certain drugs or other xenobiotic agents resulting from lead effects on hepatic detoxification enzyme systems.

In regard to the issue of reversibility/irreversibility of lead effects, there are really two dimensions to the issue that need to be considered, i.e.: (1) biological reversibility or irreversibility characteristic of the particular effect in a given organism; and (2) the generally less-recognized concept of exposure reversibility or irreversibility. Severe central nervous system damage resulting from intense, high level lead exposure is generally accepted as an irreversible effect of lead exposure; the reversibility/irreversibility of certain more difficult-to-detect neurological effects occurring at lower lead exposure levels, however, remains a matter of some controversy. The concept of exposure reversibility/irreversibility can be illustrated by the case of urban children of low socioecomomic status showing disturbances in heme biosynthesis and erythropoiesis. Biologically, these various effects may be considered reversible; the extent to which actual reversibility occurs, however, is determined by the feasibility of removing these subjects from their particular lead exposure setting. If such removal from exposure is unlikely or does not occur, then such effects will logically persist and, defacto, constitute essentially irreversible effects.

13.5.2 Dose-Effect Relationships for Lead-Induced Health Effects

13.5.2.1 Human Adults

Table 13-9 concisely summarizes the lowest observed effect levels (in terms of blood lead concentrations) thus far credibly associated with particular health effects of concern for human adults in relation to specific organ systems or generalized physiological processes, e.g. heme synthesis.

The most serious effects associated with markedly elevated blood lead levels are severe neurotoxic effects that include irreversible brain damage as indexed by the occurrence of acute or chronic encephalopathic symptoms observed in both humans and experimental animals. For most human adults, such damage typically does not occur until blood lead levels exceed 100-120 μg/dl. Often associated with encephalopathic symptoms at such blood lead levels or higher are severe gastrointestinal symptoms and objective signs of effects on several other organ systems as well. The precise threshold for occurrence of overt neurological and gastrointestinal signs and symptoms of lead intoxication remains to be established but such effects have been observed in adult lead workers at blood lead levels as low as 40-60 μg/dl, notably lower than the 60 or 80 μg/dl levels previously established or discussed as being "safe" for occupational lead exposure.

Other types of health effects occur coincident with the above overt neurological and gastrointestinal symptoms indicative of marked lead intoxication. These range from frank peripheral neuropathies to chronic renal nephropathy and anemia. Toward the lower range of blood lead levels associated with overt lead intoxication or somewhat below, less severe but important signs of impairment in normal physiological functioning in several organ systems are evident, including: (1) slowed nerve conduction velocities indicative of peripheral nerve

TABLE 13-9. SUMMARY OF LOWEST OBSERVED EFFECT LEVELS FOR KEY LEAD-INDUCED HEALTH EFFECTS IN ADULTS

Lowest Observed Effect Level (PbB)	Heme Synthesis and Hematological Effects	Neurological Effects	Renal System Effects	Reproductive Function Effects	Gastrointestinal Effects
100-120 µg/dl		Encephalopathic signs and symptoms	Chronic renal nephropathy		Overt gastrointestinal symptoms (colic, etc.)
80 µg/dl	Frank anemia				
60 µg/dl					
50 µg/dl	Reduced hemoglobin production	Overt subencephalopathic neurological symptoms ?		Altered testicular function	
40 µg/dl	Increased urinary ALA and elevated coproporphyrins				
30 µg/dl		Peripheral nerve dysfunction (slowed nerve conduction)			
25-30 µg/dl	Erythrocyte protoporphyrin (EP) elevation in males	?			
15-20 µg/dl	Erythrocyte protoporphyrin (EP) elevation in females				
<10 µg/dl	ALA-D inhibition				

Abbreviations: PbB = blood lead concentrations.

PRELIMINARY DRAFT

dysfunction (at 30-40 μg/dl, or possibly lower levels); (2) altered testicular function (at 40-50 μg/dl); and (3) reduced hemoglobin production (at approximately 50 μg/dl) and other signs of impaired heme synthesis evident at still lower blood lead levels. All of these effects point toward a generalized impairment of normal physiological functioning across several different organ systems, which becomes abundantly evident as adult blood lead levels approach or exceed 30-40 μg/dl. Evidence for impaired heme synthesis effects in blood cells exists at still lower blood lead levels in human adults and the significance of this and evidence of impairment of other biochemical processes important in cellular energetics are the subject of discussion below in relation to health effects observed in children.

13.5.2.2 Children

Table 13-10 summarizes lowest observed effect levels for a variety of imporatnt health effects observed in children. Again, as for adults, it can be seen that lead impacts many different organ systems and biochemical/physiological processes across a wide range of exposure levels. Also, again, the most serious of these effects is the severe, irreversible central nervous system damage manifested in terms of encephalopathic signs and symptoms. In children, effective blood lead levels for producing encephalopathy or death are lower than for adults, starting at approximately 80-100 μg/dl. Other overt neurological symptoms are evident at somewhat lower blood lead levels associated with lasting neurological sequalae. Colic and other overt gastrointestinal symptoms clearly occur at similar or still lower blood lead levels in children, at least down to 60 μg/dl and, perhaps, below. Renal dysfunction is also manifested along with the above overt signs of lead intoxication in children and has been reported at blood lead levels as low as 40 μg/dl in some pediatric populations. Frank anemia is also evident at 70 μg/dl, representing an extreme manifestation of reduced hemoglobin synthesis observed at blood lead levels as low as 40 μg/dl along with other signs of marked heme synthesis inhibition at that exposure level. Again, all of these effects are reflective of widespread impact of lead on the normal physiological functioning of many different organ systems in children at blood lead levels at least as low as 40 μg/dl.

Among the most important and controversial of the issues discussed in Chapter 12 are the evaluation of neuropsychological or electrophysiological effects associated with low-level lead exposures in non-overtly lead intoxicated children. None of the available studies on the subject, individually, can be said to prove conclusively that significant neurological effects occur in children at blood-Pb levels <30 μg/dl. The collective neurobehavioral studies of CNS (cognitive; IQ) effects, for example, can probably now be most reasonably interpreted as most clearly being indicative of a likely association between neuropsychologic deficits and low-level Pb-exposures in young children resulting in blood-Pb levels of approximately 30 to 50 μg/dl.

TABLE 13-10. SUMMARY OF LOWEST OBSERVED EFFECT LEVELS FOR KEY LEAD-INDUCED HEALTH EFFECTS IN CHILDREN

Lowest Observed Effect Level (PbB)	Heme Synthesis and Hematological Effects	Neurological Effects	Renal System Effects	Gastrointestinal Effects	Other Biochemical Effects
80-100 µg/dl		Encephalopathic signs and symptoms	Renal dys-function (aminoaciduria)	Colic, other overt gastrointestinal symptoms	
70 µg/dl	Frank anemia				
60 µg/dl					
50 µg/dl		?			
40 µg/dl	Reduced hemoglobin	Cognitive (CNS) deficts			
	Elevated coproporphyrin	Peripheral nerve dysfunction (slowed NCV's)			
	Increased urinary ALA				
30 µg/dl					Vitamin D metabolism interference
15-20 µg/dl	Erythrocyte protoporphyin elevation	CNS electrophysiological deficits			
10	ALA-D inhibition	?			Py-5-N activity inhibition
	?				

Abbreviations: PbB = blood lead concentrations; Py-5-N = pyrimidine-5'-nucleotidase.

PRELIMINARY DRAFT

However, due to specific methodological problems with each of the various studies (as noted in Chapter 12), much caution is warranted that precludes conclusive acceptance of the observed effects being due to Pb rather than other (at times uncontrolled for) potentially confounding variables.

Also of considerable importance are studies by by Benignus et al. (1981) and Otto et al. (1981, 1982a,b), which provide evidence of changes in EEG brain wave patterns and CNS evoked potential responses in non-overtly lead intoxicated children experiencing relatively low blood-Pb levels. Sufficient exposure information was provided by Otto et al. (1981, 1982a,b); and appropriate statistical analyses were carried out which demonstrated clear, statistically significant associations between electrophysiological (SW voltage) changes and blood-Pb levels in the range of 30 to 55 μg/dl and probable analogous associations at blood-Pb levels below 30 μg/dl (with no evident threshold down to 15 μg/dl). In this case, the continued presence of such electrophysiological changes upon follow-up two years later, suggests persistence of such effects even in the face of later declines in blood-Pb levels and, therefore, possible non-reversibility of the observed electrophysiological CNS changes. However, the reported electrophysiological effects were not found to be significantly associated with IQ decrements.

The precise medical or health significance of the neuropsychological and electrophysiological effects found by the above studies to be associated with low-level Pb-exposures is difficult to state with confidence at this time. The IQ deficits and other behavioral changes, although statistically significant, are generally relatively small in magnitude as detected by the reviewed studies, but nevertheless may still impact the intellectual development, school performance, and social development of the affected children sufficiently so as to be regarded as adverse. This would be especially true if such impaired intellectual development or school performance and disrupted social development were reflective of persisting, long-term effects of low-level lead exposure in early childhood. The issue of persistence of such lead effects, however, remains to be more clearly resolved, with some study results reviewed in Chapter 12 and mentioned above suggesting relatively short-lived or markedly decreasing Pb-effects on neuropsychological functions over a few years from early to later childhood and other studies suggesting that significant low-level Pb-induced neurobehavioral and EEG effects may, in fact, persist into later childhood.

In regard to additional studies reviewed in Chapter 12 concerning the neurotoxicity of lead, certain evidence exists which suggests that neurotoxic effects may be associated with Pb-induced altered heme synthesis, which results in an accumulation of ALA in brain affecting CNS GABA synthesis, binding, and/or inactivation by neuronal reuptake after synaptic release. Also, available experimental data suggest that these effects may have functional significance in the terms of this constituting one mechanism by which lead may increase the sensitivity of

rats to drug-induced seizures and, possibly, by which GABA-related behavioral or physiological control functions are disrupted. Unfortunately, the available research data do not allow credible direct estimates of blood-Pb levels at which such effects might occur in rats, other non-human mammalian species, or man. Inferentially, however, one can state that threshold levels for any marked Pb-induced ALA impact on CNS GABA mechanisms are most probably at least as high as blood-Pb levels at which significant accumulations of ALA have been detected in erythrocytes or non-blood soft tissues (see below). Regardless of any dose-effect levels inferred, though, the functional and/or medical significance of Pb-induced ALA effects on CNS mechanisms at low-levels of Pb-exposure remains to be more fully determined and cannot, at this time, be unequivocably seen as an adverse health effect.

Research concerning Pb-induced effects on heme synthesis, also provides information of importance in evaluating whether significant health effects in children are associated with blood-Pb levels below 30 μg/dl. As discussed earlier, in Chapter 12, Pb affects heme synthesis at several points in its metabolic pathway, with consequent impact on the normal functioning of many body tissues. The activity of the enzyme, ALA-S, catalyzing the rate-limiting step of heme synthesis does not appear to be significantly affected until blood-Pb levels reach or exceed approximately 40 μg/dl. The enzyme ALA-D, which catalizes the conversion of ALA to porphobilinogen as a further step in the heme biosynthetic pathway, appears to be affected at much lower blood-Pb levels as indexed directly by observations of ALA-D inhibition or indirectly in terms of consequent accumulations of ALA in blood and non-blood tissues. More specifically, inhibition of erythrocyte ALA-D activity has been observed in humans and other mammalian species at blood-Pb levels even below 10 to 15 μg/dl, with no clear threshold evident. Correlations between erythrocyte and hepatic ALA-D activity inhibition in lead workers at blood-Pb levels in the range of 12 to 56 μg/dl suggest that ALA-D activity in soft tissues (eg. brain, liver, kidney, etc.) may be inhibited at similar blood-Pb levels at which erythrocyte ALA-D activity inhibition occurs, resulting in accumulations of ALA in both blood and soft tissues.

It is now clear that significant increases in both blood and urinary ALA occur below the currently commonly-accepted blood-Pb level of 40 μg/dl and, in fact, such increases in blood and urinary ALA are detectable in humans at blood-Pb levels below 30 μg/dl, with no clear threshold evident down to 15 to 20 μg/dl. Other studies have demonstrated significant elevations in rat brain, spleen and kidney ALA levels consequent to acute or chronic Pb-exposure, but no clear blood-Pb levels can yet be specified at which such non-blood tissue ALA increases occur in humans. It is reasonable to assume, however, that ALA increases in non-blood tissues likely begin to occur at roughly the same blood-Pb levels associated with increases in erythrocyte ALA levels.

Lead also affects heme synthesis beyond metabolic steps involving ALA, leading to the accumulation of protoporphyrin in erythrocytes as the result of impaired iron insertion into the porphyrin moiety to form heme. The porphyrin acquires a zinc ion in lieu of the native iron, and the resulting accumulation of blood zinc protoporphyrin (ZPP) tightly bound to erythrocytes for their entire life (120 days) represents a commonly employed index of Pb-exposure for medical screening purposes. The threshold for elevation of erythrocyte protoporphyrin (EP) levels is well-established as being 25 to 30 μg/dl in adults and approximately 15 μg/dl for young children, with significant EP elevations (>1 to 2 standard deviations above reference normal EP mean levels) occurring in 50 percent of all children studied as blood-Pb approaches or moderately exceeds 30 μg/dl.

Medically, small increases in EP levels have generally not been viewed as being of great concern at initial detection levels around 15 to 20 μg/dl in children, but EP increases become more worrisome as markedly greater, significant EP elevations occur as blood-Pb levels approach and exceed 30 μg/dl and additional signs of significantly deranged heme synthesis begin to appear along with indications of functional disruption of various organ systems. Previously, such other signs of significant organ system functional disruptions had only been credibly detected at blood-Pb levels somewhat in excess of 30 μg/dl, e.g., hemoglobin synthesis inhibition starting at 40 μg/dl and significant nervous system effects at 50-60 μg/dl. This served as a basis for CDC establishment of 30 μg/dl blood-Pb as a criteria level for undue Pb exposure for young children and adoption by EPA of it as the "maximum safe" blood-Pb level (allowing some margin(s) of safety before reaching levels associated with inhibition of hemoglobin synthesis or nervous system deficits) in setting the 1978 NAAQS for lead.

To the extent that new evidence is now available, indicative of probable Pb effects on nervous system functioning or other important physiological processes at blood-Pb levels below 30 to 40 μg/dl, then the rationale for continuing to view 30 μg/dl as a "maximum safe" blood-Pb level is called into question and substantial impetus is provided for revising the criteria level downward, i.e., to some blood-Pb level below 30 μg/dl. At this time, such impetus toward revising the blood-Pb criteria level downward is gaining momentum not only from new neuropsychologic and electrophysiological findings of the type summarized above, but also from growing evidence for Pb effects on other functional systems. These include, for example, the: (1) disruption of formation of the heme-containing protein, cytochrome c, of considerable importance in cellular energetics involved in mediation of the normal functioning of many different mammalian (including human) organ systems and tissues; (2) inhibition by Pb of the biosynthesis of globin, the protein moiety of hemoglobin, in the presense of Pb at concentrations corresponding to a blood-Pb level of 20 μg/dl; (3) observations of significant inhibition of pyrimidine-5'-nucleotidase (Py-5-N) activity in adults at blood-Pb levels ≧44 μg/dl and in

children down to blood-Pb levels of 10 μg/dl; and (4) observations of Pb interference with vitamin D metabolism in children across a blood-Pb level range of 33 to 120 μg/dl, with consequent increasingly enhanced Pb uptake due to decreased vitamin D metabolism and likely associated increasingly cascading effects on nervous system and other functions at sequentially higher blood-Pb levels. Certain additional evidence for Pb effects on hormonal systems and immune system components, thus far detected only at relatively high blood-Pb levels or at least not credibly associated with blood-Pb levels as low as 30 to 40 μg/dl, also contributes to concern as blood-Pb levels exceed 30 μg/dl.

Also adding to the concern about relatively low lead exposure levels are the results of an expanding array of animal toxicology studies which demonstrate: (1) persistence of lead-induced neurobehavioral alterations well into adulthood long after termination of perinatal lead exposure early in development of several mammalian species; (2) evidence for uptake and retention of lead in neural and non-neuronal elements of the CNS, including long-term persistence in brain tissues after termination of external lead exposure and blood lead levels return to "normal"; and (3) evidence from various in-vivo and in-vitro studies indicating that, at least on a subcellular-molecular level, no threshold may exist for certain neurochemical effects of lead.

13.6 DOSE-RESPONSE RELATIONSHIPS FOR LEAD EFFECTS IN HUMAN POPULATIONS

Information summarized in the preceding section dealt with the various biological effects of lead germane to the general population and included comments about the various levels of blood lead observed to be associated with the measurable onset of these effects in various populations groups.

As indicated above, inhibition of ALA-D activity by lead occurs at virtually all blood lead levels measured in subjects residing in industrialized countries. If any threshold for ALA-D inhibition exists, it lies somewhere below 10 μg Pb/dl in blood lead.

Elevation in erythrocyte porphyrin for a given blood lead level is greater in children and women than in adult males, children being somewhat more sensitive than women. The threshold for currently detectable EP elevation in terms of blood lead levels for children was estimated at ca. 16 to 17 μg/dl in the recent studies of Piomelli et al. (1982). In adult males, the corresponding blood lead value is 25 to 30 μg/dl.

Statistically significant reduction in hemoglobin production occurs at a lower blood lead level in children, 40 μg/dl, than in adults, 50 μg/dl.

It appears that urinary ALA shows a correlation with blood lead levels to below 40 μg/dl, but since there is no clear agreement as to the meaning of elevated ALA-U below 40 μg/dl, this

value is taken as the threshold for pronounced excretion of ALA into urine. This value appears to apply to both children and adults. Whether this blood lead level represents a threshold for the potential neurotoxicity of circulating ALA cannot now be stated and requires further study.

Coproporphyrin elevation in urine first occurs at a blood lead level of 40 μg/dl and this threshold appears to apply for both children and adults.

A number of investigators have attempted to quantify more precisely dose-population response relationships for some of the above lead effects in human populations. That is they have attempted to define the proportion of a population exhibiting a particular effect at a given blood lead level. To date, such efforts at defining dose-response relationships for lead effects have been mainly limited to the following effects of lead on heme biosynthesis: inhibition of ALA-D activity; elevation of EP; and urinary excretion of ALA.

Dose-population response relationships for EP in children has been analyzed in detail by Piomelli and et al. (1982) and the corresponding plot at 2 levels of elevation (>1 S.D., >2 S.D.) is shown in Figure 13-3 using probit analysis. It can be seen that blood lead levels in half of the children showing EP elevations at >1 and 2 S.D.'s closely bracket the blood lead level taken as the high end of "normal" (i.e., 30 μg/dl). Dose-response curves for adult men and women as well as children prepared by Roels et al. (1976) are set forth in Figure 13-4. In Figure 13-4, it may be seen that the dose-response for children remains greater across the blood-lead range studied, followed by women, then adult males.

Figure 13-5 presents dose-population response data for urinary ALA exceeding two levels (at mean + 1 S.D. and mean + 2 S.D.), as calculated by EPA from the data of Azar et at. (1975). The percentages of the study populations exceeding the corresponding cut-off levels as calculated by EPA for the Azar data are set forth in Table 13-11. It should be noted that the measurement of ALA in the Azar et al. study did not account for amino acetone, which may influence the results observed at the lowest blood lead levels.

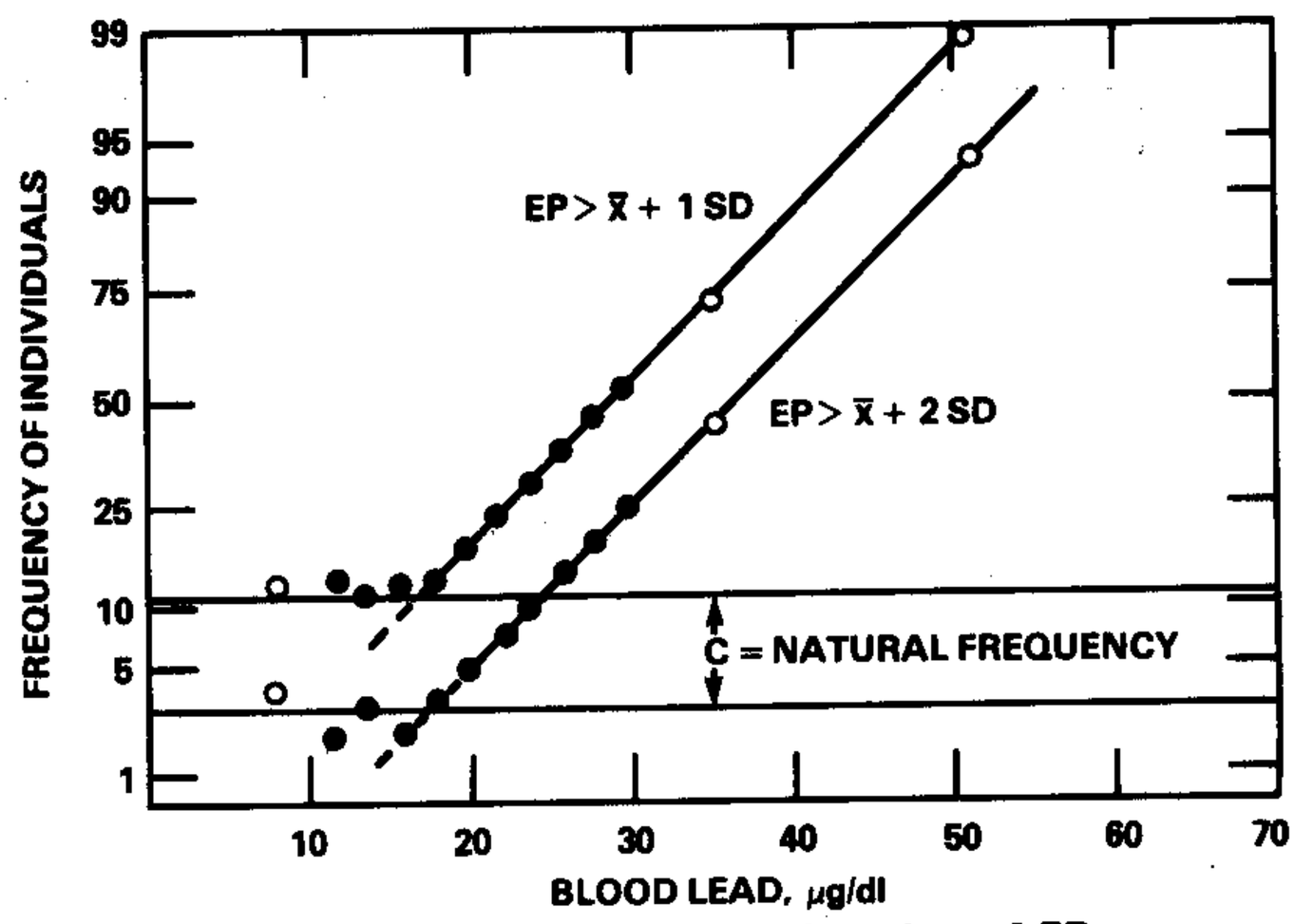

Figure 13-3. Dose-response for elevation of EP as a function of blood lead level using probit analysis. Geometric mean plus 1 S.D. = 33 μg/dl; geometric mean plus 2 S.D. = 53 μg/dl.

Source: Piomelli et al. (1982).

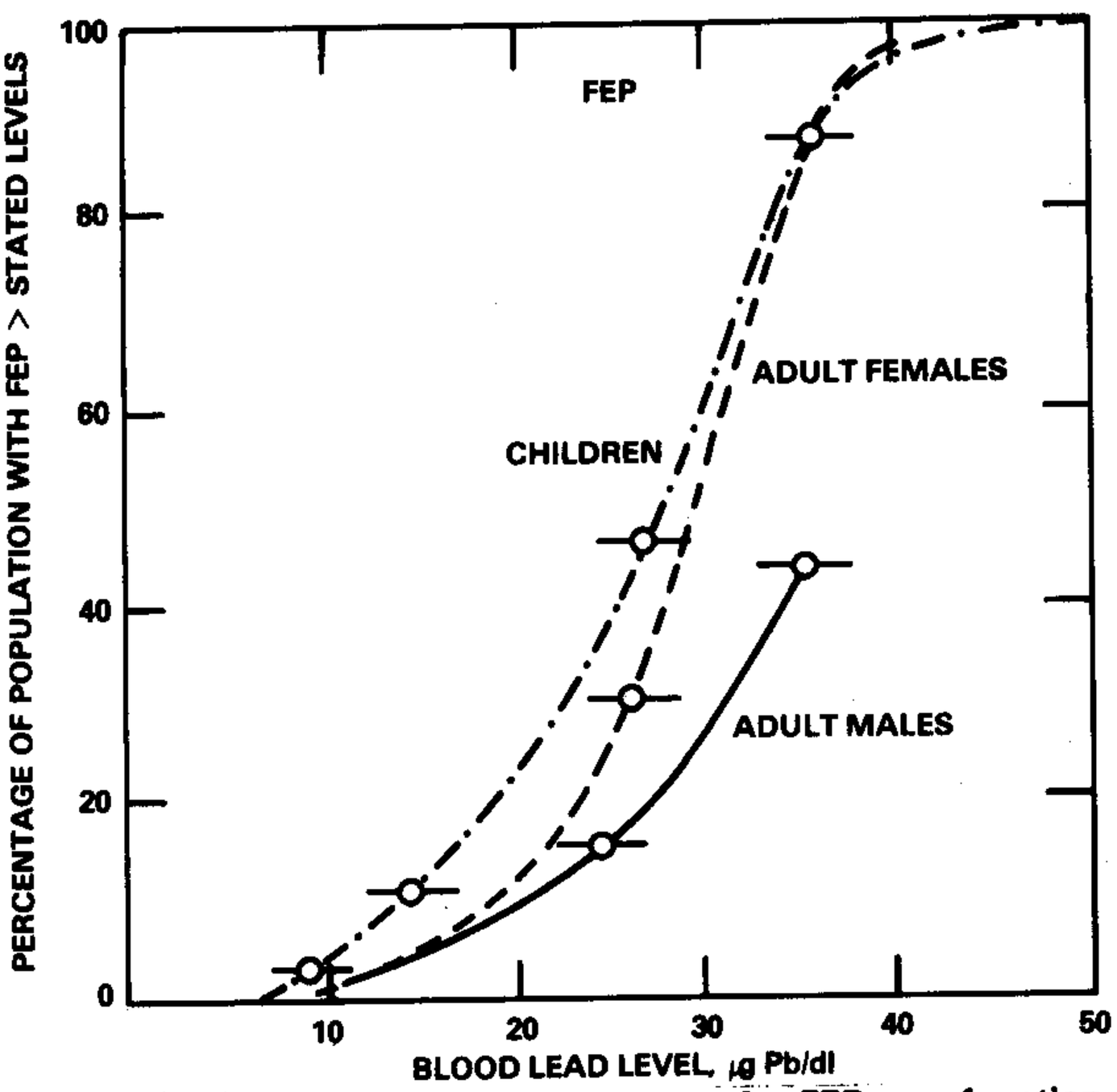

Figure 13-4. Dose-response curve for FEP as a function of blood lead level: in subpopulations.

Source: Roels et al. (1976).

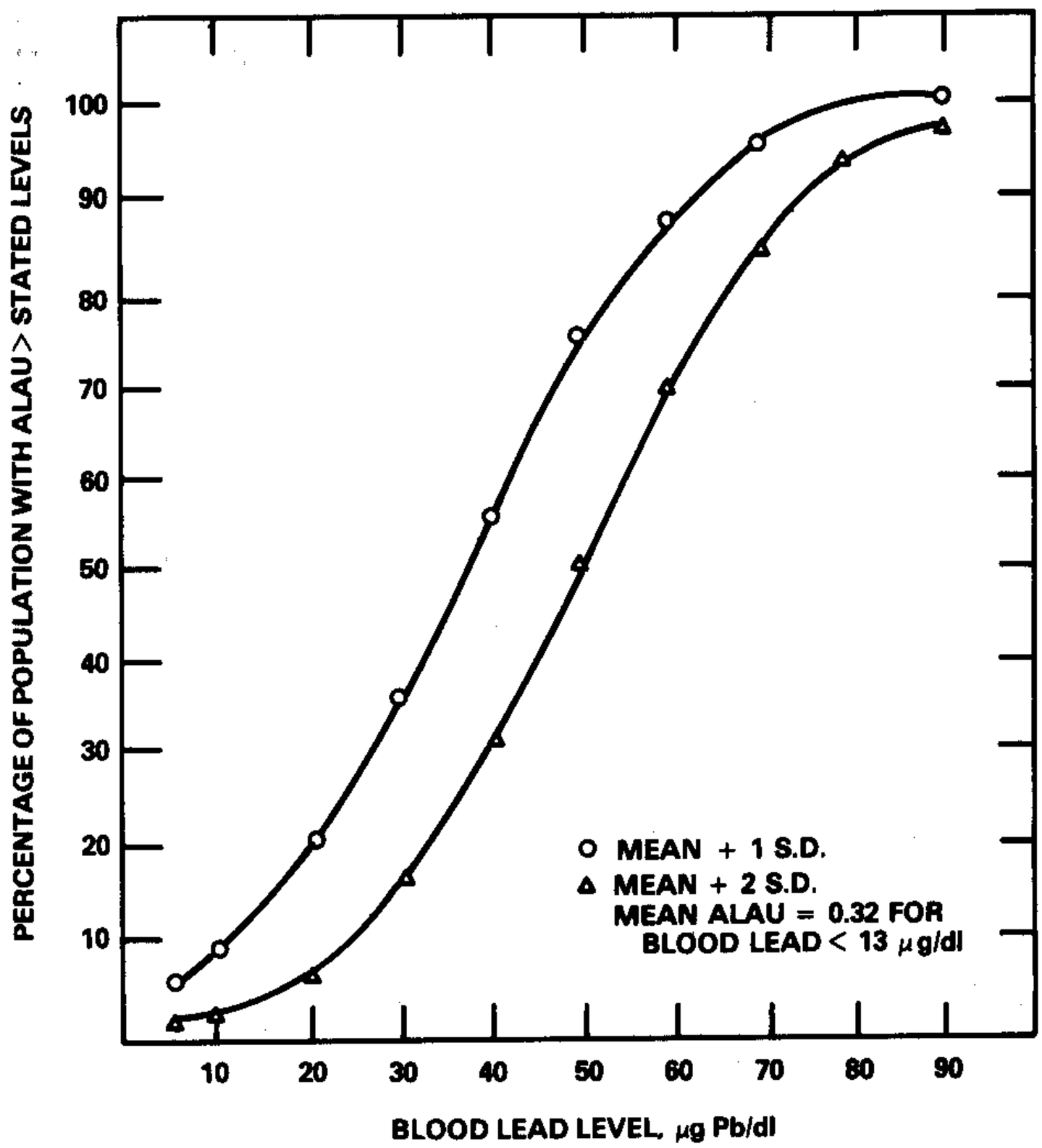

Figure 13-5. EPA calculated dose-response curve for ALA-U.

Source: Azar et al. (1975).

TABLE 13-11. EPA-ESTIMATED PERCENTAGE OF SUBJECTS WITH ALA-U EXCEEDING LIMITS FOR VARIOUS BLOOD LEAD LEVELS

Blood lead levels (μg/dl)	Azar et al. (1975) (Percent Population)
10	2
20	6
30	16
40	31
50	50
60	69
70	84

13.7 POPULATIONS AT RISK

Population at risk is a segment of a defined population exhibiting characteristics associated with significantly higher probability of developing a condition, illness, or other abnormal status. This high risk may result from either (1) greater inherent susceptibility or (2) from exposure situations peculiar to that group. What is meant by inherent susceptibility is a host characteristic or status that predisposes the host to a greater risk of heightened response to an external stimulus or agent.

In regard to lead, two such populations are definable. They are preschool age children, especially those living in urban settings, and pregnant women, the latter group owing mainly to the risk to the conceptus. Children are such a population for both of the reasons stated above, whereas pregnant women are at risk primarily due to the inherent susceptibility of the conceptus.

13.7.1 Children as a Population at Risk

Children are developing and growing organisms exhibiting certain differences from adults in terms of basic physiologic mechanisms, capability of coping with physiologic stress, and their relative metabolism of lead. Also, the behavior of children frequently places them in different relationship to sources of lead in the environment, thereby enhancing the opportunity for them to absorb lead. Furthermore, the occurrence of excessive exposure often is not realized until serious harm is done. Young children do not readily communicate a medical history of lead exposure, the early signs of such being common to so many other disease states that lead is frequently not recognized early on as a possible etiological factor contributing to the manifestation of other symptoms.

13.7.1.1 Inherent Susceptibility of the Young. Discussion of the physiological vulnerability of the young must address two discrete areas. Not only should the basic physiological differences be considered that one would expect to predispose children to a heightened vulnerability to lead, but also the actual clinical evidence must be considered that shows such vulnerability does indeed exist.

In Chapter 10 and Section 13.2 above, differences in relative exposure to lead and body handling of lead for children versus adults were pinpointed throughout the text. The significant elements of difference include: (1) greater intake of lead by infants and young children into the respiratory and gastro-intestinal tracts on a body weight basis compared to adults; (2) greater absorption and retention rates of lead in children; (3) much greater prevalence of nutrient deficiency in the case of nutrients which affect lead absorption rates from the GI tract; (4) differences in certain habits, i.e., normal hand to mouth activity as well as pica resulting in the transfer of lead-contaminated dust and dirt to the GI tract; (5) differences

in the efficiency of lead sequestration in the bones of children, such that not only is less of the body burden of lead in bone at any given time but the amount present may be relatively more labile. Additional information discussed in Chapter 12 suggests that the blood-brain barrier in children is less developed, posing the risk for greater entry of lead into the nervous system.

Hematological and neurological effects in children have been demonstrated to have lower thresholds in terms of blood lead levels than in adults. The extent of reduced hemoglobin production and EP accumulation occur at relatively lower exposure levels in children than in adults, as indexed by blood lead thresholds. With reference to neurologic effects, the onset of encephalopathy and other injury to the nervous system appears to vary both regarding likely lower thresholds in children for some effects and in the typical pattern of neurologic effects presented, e.g., in encephalopathy or other CNS deficits being more common in children versus peripheral neuropathy being more often seen in adults. Not only are the effects more acute in children than in adults, but also the neurologic sequelae are usually much more severe in children.

13.7.1.2 Exposure Consideration. The dietary habits of children as well as the diets themselves differ markedly from adults and, as a result, place children in a different relationship to several sources of lead. The dominance of canned milk and processed baby food in the diet of many young children is an important factor in assessing their exposure to lead since both those foodstuffs have been shown to contain higher amounts of lead than components of the adult diet. The importance of these lead sources is not their relationship to airborne lead directly but, rather, their role in providing a higher baseline lead burden to which the airborne contribution is added.

Children ordinarily undergo a stage of development in which they exhibit normal mouthing behavior, as manifested, for example, in the form of thumbsucking. At this time they are at risk for picking up lead-contaminated soil and dust on their hands and hence into their mouths where it can be absorbed. Scientific evidence documenting at least the first part of the chain is available.

There is, however, an abnormal extension of mouthing behavior, called pica, which occurs in some children. Although diagnosis of this is difficult, children who exhibit this trait have been shown to purposefully eat nonfood items. Much of the lead-based paint problem is known to occur because children actively ingest chips of leaded paint.

13.7.2 Pregnant Women and the Conceptus as a Population at Risk

There are some rather inconculsive data indicating that women may in general be somewhat higher risk to lead than men. However, pregnant women and their concepti as a subgroup are

demonstrably at higher risk. It should be pointed out that, in fact, it really is not the pregnant woman per se who is at greatest risk but, rather, the unborn child she is carrying. Because of obstetric complications, however, the mother herself can also be at somewhat greater risk at the time of delivery of her child.

Studies have demonstrated that women in general, like children, tend to show a heightened response of erythorcyte protoporphyrin levels upon exposure to lead. The exact reason for this heightened response is not known but may relate to endocrine differences between men and women.

As stated above, the primary reason pregnant women are a high-risk group is because of the fetus each is carrying. In addition, there is some suggestive evidence that lead exposures may also affect maternal complications at delivery. With reference to maternal complication at delivery, information in the literature suggests that the incidence of preterm delivery and premature membrane rupture relates to maternal blood lead level. Further study of this relationship as well as studies relating to discrete health effects in the newborn are needed.

Vulnerability of the developing fetus to lead exposure arising from transplacental transfer of maternal lead was discussed in Chapter 10. This process starts at the end of the first trimester. Umbilical cord blood studies involving mother-infant pairs have repeatedly shown a correlation between maternal and fetal blood lead levels.

Further suggestive evidence, cited in Chapter 12, has been advanced for prenatal lead exposures of fetuses possibly leading to later higher instances of postnatal mental retardation among the affected offspring. The available data are insufficient to state with any certainty that such effects occur or to determine with any precision what levels of lead exposure might be required prior to or during pregnancy in order to produce such effects.

13.7.3 Description of the United States Population in Relation to Potential Lead Exposure Risk

In this section, estimates are provided of the number of individuals in those segments of the population which have been defined as being potentially at greatest risk for lead exposures. These segments include pre-school children (up to 6 years of age), especially those living in urban settings, and women of child-bearing age (defined here as ages 15-44). These data, which are presented below in Table 13-12, were obtained from a provisional report by the U.S. Census Bureau (1982), which indicates that approximately 61 percent of the populace lives in urban areas (defined as central cities and urban fringe). Assuming that the 61 percent estimate for urban residents also applies to children of preschool age, then approximately 14,206,000 children of the total listed in Table 13-12 would be expected to be at greater risk

by virtue of higher lead exposures generally associated with their living in urban versus non-urban settings. (NOTE: The age distribution of the percentage of urban residents may vary between SMSA's.)

TABLE 13-12. PROVISIONAL ESTIMATE OF THE NUMBER OF INDIVIDUALS IN URBAN AND RURAL POPULATION SEGMENTS AT GREATEST POTENTIAL RISK TO LEAD EXPOSURE

Population Segment	Actual Age (year)	Total Number in U.S. Population (1981)	Urban Population[1]
Pre-school children	0-4	16,939,000	10,333,000
	5	3,201,000	1,953,000
	6	3,147,000	1,920,000
Total		23,287,000	14,206,000
Women of child-bearing age	15-19	10,015,000	6,109,000
	20-24	10,818,000	6,599,000
	25-29	10,072,000	6,144,000
	30-34	9,463,000	5,772,000
	35-39	7,320,000	4,465,000
	40-44	6,147,000	3,749,000
Total		53,835,000	32,838,000

Source: U.S. Census Bureau (1982), Tables 18 and 31.

[1]An urban/total ratio of 0.61 was used for all age groups. "Urban" includes central city and urban fringe populations.

The risk encountered with exposure to lead may be compounded by nutritional deficits (see Chapter 10). The most commonly seen of these is iron deficiency, especially in young children less than 5 years of age (Mahaffey and Michaelson, 1980). Data available from the National Center for Health Statistics for 1976-1980 (Fulwood et al., 1982) indicate that from 8 to 22 percent of children aged 3-5 may exhibit iron deficiency, depending upon whether this condition is defined as serum iron concentration (<40 μg/dl) or as transferrin saturation (<16 percent), respectively. Hence, of the 20,140,000 children ≤5 years of age (Table 13-12), as many as 4,431,000 would be expected to be at increased risk depending on their exposure to lead, due to iron deficiency.

As pointed out in Section 13.7.2, the risk to pregnant women is mainly due to risk to the conceptus. By dividing the total number of women of child-bearing age in 1981 (53,835,000) into the total number of live births in 1981 (3,646,000; National Center for Health Statistics, 1982), it may be seen that approximately 7 percent of this segment of the population may be at increased risk at any given time.

13.8 SUMMARY AND CONCLUSIONS

Among the most significant pieces of information and conclusions that emerge from the present human health risk evaluation are the following:

(1) Anthropogenic activity has clearly led to vast increases of lead input into those environmental compartments which serve as media (e.g., air, water, food, etc.) by which significant human exposure to lead occurs.

(2) Emission of lead into the atmosphere, especially through leaded gasoline combustion, is of major significance in terms of both the movement of lead to other environmental compartments and the relative impact of such emissions on the internal lead burdens in industrialized human populations. By means of both mathematical modeling of available clinical/epidemiological data by EPA and the isotopic tracing of lead from gasoline to the atmosphere to human blood of exposed populations, the size of atmospheric lead contribution can be confidently said to be 25-50 percent or probably somewhat higher.

(3) Given this magnitude of relative contribution to human external and internal exposure, reduction in levels of atmospheric lead would then result in significant widespread reductions in levels of lead in human blood (an outcome which is supported by careful analysis of the NHANES II study data). Reduction of lead in food (added in the course of harvesting, transport, and processing) would also be expected to produce significant widespread reductions in human blood lead levels in the United States.

(4) A number of adverse effects in humans and other species are clearly associated with lead exposure and, from a historical perspective, the observed "thresholds" for these various effects (particularly neurological and heme biosynthesis effects) continue to decline as more sophisticated experimental and clinical measures are employed to detect more subtle, but still significant effects. These include significant alterations in normal physiological functions at blood lead levels markedly below the currently accepted 30 μg/dl "maxim safe level" for pediatric exposures.

(5) Preceding chapters of this document demonstrate that young children are at greatest risk for experiencing lead-induced health effects, particularly in the urbanized, low income segments of this pediatric population. A second group at increased risk are pregnant women, because of exposure of the fetus to lead in the absence of any effective biological (e.g. placental) barrier during gestation.

(6) Dose-population response information for heme synthesis effects, coupled with information from various blood lead surveys, e.g. the NHANES II study, indicate that large numbers of American children (especially low income, urban dwellers) have blood lead levels sufficiently high (in excess of 15-20 μg/dl) that they are clearly at risk for deranged heme synthesis and, possibly, other health effects of growing concern as lead's role as a general systemic toxicant becomes more fully understood.

13.9 REFERENCES

Angle, C. R.; McIntire, M. S. (1979) Environmental lead and children: the Omaha study. J. Toxicol. Environ. Health 5: 855-870.

Azar, A.; Snee, R. D.; Habibi, K. (1975) An epidemiologic approach to community air lead exposure using personal air samplers. In: Lead. Environ. Qual. Saf. Suppl. 2: 254-288.

Benignus, V. A.; Otto, D. A.; Muller, K. E.; Seiple, K. J. (1981) Effects of age and body lead burden on CNS function in young children. II: EEG spectra. Electroencephalogr. Clin. Neurophysiol. 52: 240-248.

Burchfiel, J. L.; Duffy, F. H.; Bartels, P. H.; Needleman, H. L. (1980) The combined discriminating power of quantitative electroencephalography and neuropsychologic measures in evaluating central nervous system effects of lead at low levels. In: Needleman, H. L., ed. Low level lead exposure: the clinical implications of current research. New York, NY: Raven Press; pp. 75-90.

Chamberlain, A. C.; Heard, M. J.; Little, P.; Newton, D.; Wells, A. C.; Wiffen, R. D. (1978) Investigations into lead from motor vehicles. Harwell, United Kingdom: United Kingdom Atomic Energy Authority; report no. AERE-R9198.

Ernhart, C. B.; Landa, B.; Schell, N. B. (1981) Subclinical levels of lead and developmental deficit - a multivariate follow-up reassessment. Pediatrics 67: 911-919.

Facchetti, S.; Geiss, F. (1982) Isotopic lead experiment: status report. Luxembourg: Commission of the European Communities; Publication no. EUR 8352 EN.

Greathouse, D. G.; Craun, G. F.; Worth, D. (1976) Epidemiologic study of the relationship between lead in drinking water and blood lead levels. In: Hemphill, D. D., ed. Trace substances in environmental health-X: [proceedings of University of Missouri's 10th annual conference on trace substances in environmental health]; June; Columbia, MO. Columbia, MO: University of Missouri-Columbia; pp. 9-24.

Griffin, T. B.; Coulston, F.; Wills, H.; Russell, J. C.; Knelson, J. H. (1975) Clinical studies of men continuously exposed to airborne particulate lead. In: Griffin, T. B.; Knelson, J. H., eds. Lead. New York, NY: Academic Press; pp. 221-240. (Coulston, F.; Korte, F., eds. Environmental quality and safety: supplement v. 2).

Gross, S. B. (1979) Oral and inhalation lead exposures in human subjects (Kehoe balance experiments). New York, NY: Lead Industries Association.

Gross, S. B. (1981) Human oral and inhalation exposures to lead: summary of Kehoe balance experiments. J. Toxicol. Environ. Health 8: 333-377.

Hammond, P. B.; O'Flaherty, E. J.; Gartside, P. S. (1981) The impact of air-lead on blood-lead in man - a critique of the recent literature. Food Cosmet. Toxicol. 19: 631-638.

Kang, H. K.; Infante, P. F.; Carra, J. S. (1983) Determination of blood-lead elimination patterns of primary lead smelter workers. J. Toxicol. Environ. Health 11: 199-210.

Kehoe, R. A. (1961a) The metabolism of lead in man in health and disease: the normal metabolism of lead. (The Harben lectures, 1960). J. R. Inst. Public Health Hyg. 24: 81-97.

Kehoe, R. A. (1961b) The metabolism of lead in man in health and disease: the metabolism of lead under abnormal conditions. (The Harben lectures, 1960). J. R. Inst. Public Health Hyg. 24: 129-143.

Kehoe, R. A. (1961c) The metabolism of lead in man in health and disease: present hygienic problems relating to the absorption of lead. (The Harben lectures, 1960). J. R. Inst. Public Health Hyg. 24: 177-203.

Lucas, J. M. (1981) Effect of analytical variability on measurements of population blood lead levels. Am. Ind. Hyg. Assoc. J. 42: 88-96.

Mahaffey, K. R.; Michaelson, I. A. (1980) The interaction between lead and nutrition. In: Needleman, H. L., ed. Low level lead exposure: the clinical implications of current research. New York, NY: Raven Press; pp. 159-200.

National Academy of Sciences, Committee on Lead in the Human Environment. (1980) Lead in the human environment. Washington, DC: National Academy of Sciences.

National Center for Health Statistics. (1982). Advance report of final natality statistics for 1980. Vol. 31(8): Supplement for November 3.

Needleman, H. L.; Gunnoe, C.; Leviton, A.; Reed, R.; Peresie, H.; Maher, C.; Barrett, P. (1979) Deficits in psychological and classroom performance of children with elevated dentine lead levels. N. Engl. J. Med. 300: 689-695.

Nutrition Foundation, Inc. (1982) Assessment of the safety of lead and lead salts in food: a report of the Nutrition Foundation's Expert Advisory Committee. Washington, DC. The Nutrition Foundation.

O'Flaherty, E. J.; Hammond, P. B.; Lerner, S. I. (1982) Dependence of apparent blood lead half-life on the length of previous lead exposure in humans. Fundam. Appl. Toxicol. 2: 49-54.

Otto, D. A.; Benignus, V. A.; Muller, K. E.; Barton, C. N. (1981) Effects of age and body lead burden on CNS function in young children. I: Slow cortical potentials. Electroencephalogr. Clin. Neurophysiol. 52: 229-239.

Otto, D.; Benignus, V.; Muller, K.; Barton, C. (1983) Evidence of changes in CNS function at low-to-moderate blood lead levels in children: a challenge to currently accepted threshold limits. In: Rutter, M.; Jones, R. R., eds. Lead versus health: the effects of low level lead exposure. New York, NY: John Wiley & Sons; PAGES. (IN PRESS)

Otto, D.; Benignus, V.; Muller, K.; Barton, C.; Seiple, K.; Prah, J.; Schroeder, S. (1982) Effects of low to moderate lead exposure on slow cortical potentials in young children: two year follow-up study. Neurobehav. Toxicol. Teratol. 4: 733-737.

Perino, J.; Ernhart, C. B. (1974) The relation of subclinical lead level to cognitive and sensorimotor impairment in black preschoolers. J. Learn. Dis. 7: 616620.

Piomelli, S.; Seaman, C.; Zullow, D.; Curran, A.; Davidow, B. (1982) Threshold for lead damage to heme synthesis in urban children. Proc. Natl. Acad. Sci. U.S.A. 79: 3335-3339.

Pocock, S. J.; Shaper, A. G.; Walker, M.; Wale, C. J.; Clayton, B.; Delves, T.; Lacey, R. F.; Packham, R. F.; Powell, P. (1983) The effects of tap water lead, water hardness, alcohol, and cigarettes on blood lead concentrations. J. Epidemiol. Comm. Health 37: 1-7.

Rabinowitz, M. B.; Wetherill, G. W.; Kopple, J. D. (1973) Lead metabolism in the normal human: stable isotope studies. Science (London) 182: 725-727.

Rabinowitz, M,; Wetherill, G. W.; Kopple, J. D. (1974) Studies of human lead metabolism by using stable isotope tracers. Environ. Health Perspect. 7: 145-152.

Rabinowitz, M. B.; Wetherill, G. W.; Kopple, J. D. (1976) Kinetic analysis of lead metabolism in healthy humans. J. Clin. Invest. 58: 260-270.

Rabinowitz, M. B.; Wetherill, G. W.; Kopple, J. D. (1977) Magnitude of lead intake from respiration by normal man. J. Lab. Clin. Med. 90: 238-248.

Roels, H. A.; Buchet, J-P.; Lauwerys, R. R.; Bruaux, P.; Claeys-Thoreau, F.; Lafontaine, A.; Verduyn, G. (1980) Exposure to lead by the oral and the pulmonary routes of children living in the vicinity of a primary lead smelter. Environ. Res. 22: 81-94.

Roels, H.; Buchet, J-P.; Lauwerys, R.; Hubermont, G.; Bruaux, P.; Claeys-Thoreau, F.; Lafontaine, A.; Van Overschelde, J. (1976) Impact of air pollution by lead on the heme biosynthetic pathway in school-age children. Arch. Environ. Health 31: 310-316.

Ryu, J. E.; Ziegler, E. E.; Nelson, S. E.; Fomon, S. J. (1983) Dietary intake of lead and blood lead in early infancy. Pediatr. Res. VOL: PAGES. (IN PRESS)

Sherlock, J.; Smart, G.; Forbes, G. I.; Moore, M. R.; Patterson, W. J.; Richards, W. N.; Wilson, T. S. (1982) Assessment of lead intakes and dose-response for a population in Ayr exposed to a plumbsolvent water supply. Hum. Toxicol. 1: 115-122.

Stark, A. D.; Quah, R. F.; Meigs, J. W.; DeLouise, E. R. (1982) The relationship of environmental lead to blood-lead levels in children. Environ. Res. 27: 372-383.

U.S. Bureau of the Census. (1982a) Provisional estimate of social, economic and housing characteristics for the United States. (PHC80-S1-1).

U.S. Bureau of the Census. (1982b) 1980 census of population and housing: supplementary report: provisional estimates of social, economic, and housing characteristics: states and selected standard metropolitan statistical areas. Washington, DC: U.S. Department of Commerce; Bureau of the Census report no. PHC 80-S1-1. Available from: U.S. Department of Commerce, Bureau of the Census, Washington, DC.

U.S. Environmental Protection Agency, Health Effects Research Lab. (1977) Air quality criteria for lead. Research Triangle Park, NC: U.S. Environmental Protection Agency, Criteria and Special Studies Office; EPA report no. EPA-600/8-77-017. Available from: NTIS, Springfield, VA; PB 280411.

U.S. Public Health Service. (1982) Hematological and nutritional biochemistry reference data for persons 6 months - 74 years of age: United States, 1976-80. Hyattsville, MD: U.S. Department of Health and Human Services, National Center for Health Statistics; DHHS publication no. (PHS) 83-1682. (Vital and Health Statistics, series 11, no. 232.)

United Kingdom Central Directorate on Environmental Pollution. (1982) The Glasgow duplicate diet study (1979/1980): a joint survey for the Department of the Environment and the Ministry of Agriculture Fisheries and Food. London, United Kingdom: Her Majesty's Stationery Office; pollution report no. 11.

Walter, S. D.; Yankel, A. J.; von Lindern, I. H. (1980) Age-specific risk factors for lead absorption in children. Arch. Environ. Health 35: 53-58.

Yankel, A. J.; von Lindern, I. H.; Walter, S. D. (1977) The Silver Valley lead study: the relationship of childhood lead poisoning and environmental exposure. J. Air Pollut. Control Assoc. 27: 763-767.

Zielhuis, R. L. (1975a) Dose-response relationships for inorganic lead. I: Biochemical and haematological responses. Int. Arch. Occup. Environ. Health 35: 1-18.

Zielhuis, R. L. (1975b) Dose-response relationships for inorganic lead. II: Subjective and functional responses - chronic sequelae - no-response levels. Int. Arch. Occup. Health 35: 19-35.

★ U.S. GOVERNMENT PRINTING OFFICE: 1983 — 662-038/1003