PETITION TO THE
OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT (OEHHA)
CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY

Pursuant to California Government Code section 11340.6

to amend California Code of Regulations, title 27, section 27001(c)

to add delayed onset of puberty in females as a reproductive toxic endpoint

for which lead is known to cause reproductive toxicity

Submitted September 22, 2016

by

Mateel Environmental Justice Foundation
Californians for Alternatives to Toxics
and
Ecological Rights Foundation

REQUEST TO AMEND CAL. CODE REGS., TIT. 27 § 27001(c)

Pursuant to California Government Code section 11340.6, petitioners Mateel Environmental Justice Foundation (Mateel), Californians for Alternatives to Toxics (CATs), and Ecological Rights Foundation (ERF) (collectively "petitioners") petition the California Office of Environmental Health Hazard Assessment (OEHHA) to amend California Code of Regulations, title 27, section 27001(c) to list lead as a female reproductive toxicant, using as the basis for this listing findings by the U.S. Environmental Protection Agency ("EPA") and the National Toxicology Program ("NTP") that lead is known to cause delayed onset of puberty in females. OEHHA may contact William Verick, Klamath Environmental Law Center, 424 First Street, Eureka, CA 95501, (707) 268-8900, wverick@igc.org if it wishes to respond to this petition, discuss it, or if OEHHA needs clarification or more information.

(a) **Substance of the Amendment Requested:**

– Petitioners request that OEHHA amend California Code of Regulations, title 27, section 27001(c) to add, as an additional basis for listing lead as a reproductive toxicant, the formal findings by EPA and NTP (both authoritative bodies for purposes of Cal. Code Regs., tit. 27 § 25306(*l*)) that lead exposure causes delayed onset of puberty in females. This added basis for listing lead as a female reproductive toxicant would be in addition to the existing – required to be labeled – basis for listing lead as a developmental, female reproductive and male reproductive toxicant. Petitioners request that OEHHA do so no later than the next time OEHHA causes the list to be revised and republished in light of additional knowledge.

(b) **The Reason for the Request:**

– Lead is currently listed as a developmental, female reproductive and male reproductive toxicant based on lead being "required to be labeled" as causing these effects by U.S. Occupational Safety and Health Administration ("OSHA") Mateel maintains that delayed onset of puberty in females is one of the reproductive toxic endpoints currently covered under the rubric of lead's current listing as a "female reproductive toxicant." In the Final Statement of Reasons Cal. Code Regs., tit. 27 § 25803 at pp. 73-74, the HWA responded to a comment that asked how determine the toxic endpoints for which a chemical was listed, by answering that the person should consult the documents that provided the basis for listing the chemical – the transcript of the meeting of the SAP or the data HWA provided to the SAP. OSHA clearly stated that lead causes abnormal sterility and OEHHA has determined that delayed onset of puberty is one form of impaired reproductive performance (which includes sub-fertility and infertility). (See, Nov. 1993 Criteria for Recommending Chemicals for Listing as "Known to the State to Cause Reproductive Toxicity" at p. 2.) There is, however, some ambiguity on this issue. Does "sterility" mean the same as "sub-fertility and/or infertility?

Mateel would like to clear up that ambiguity by having OEHHA make it unambiguous that lead is listed for female reproductive toxicity based on the findings of EPA and NTP that lead

causes delayed onset of puberty in females. The data in the EPA ISA and the NTP 2012 Lead Monograph would provide the information required so that a person could be certain that lead has been listed as a female reproductive toxicant on the basis of its causing delayed onset of puberty in females. In June 2013 the U.S. Environmental Protection Agency ("EPA") issued its Integrated Science Assessment for Lead ("EPA ISA") The EPA ISA found that there was a "causal relationship" between lead exposure and delayed onset of puberty in females. (*See*, EPA ISA at pages *l*xxxvi, 1-35, 4-590-605, 4-708.) In 2012 the National Toxicology Program ("NTP") issued its Monograph on the Health Effects of Low-Level Lead (2012 NTP Lead Monograph"). In its 2012 Lead Monograph, NTP found that there was sufficient evidence that blood lead levels < 10 µg/dL are associated with delayed puberty in both boys and girls. (*See*, NTP 2012 Lead Monograph at pages 89, 91-94, 118.)[1] Health & Saf. Code § 25249.8(b) provides that a chemical is known to the state to cause reproductive toxicity "if a body considered to be authoritative" by the state's qualified experts has formally identified it as causing reproductive toxicity. Both the EPA and the NTP are such "authoritative bodies." (*See*, Cal. Code Regs., tit. 27 § 25306(*l*)(3) and (4).) Via the EPA ISA and the NTP 2012 Lead Monograph, both of these authoritative bodies have formally identified lead as causing delayed onset of puberty in females.

– The statutory, regulatory and scientific authority referenced herein demonstrates that – to the extent lead is not already so listed – OEHHA has a non-discretionary duty, pursuant to Health & Safety Code section 25249.8(b) and Cal. Code Regs, tit. 27 §§ 25306(a) and (*l*), to list lead as known to cause female reproductive toxicity for the reproductive toxic endpoint of delayed onset of puberty in females. As described herein, OEHHA clearly has the authority to take this regulatory action. Petitioners therefore request that OEHHA add lead to the California Code of Regulations, title 27 section 27001(c) list as known to cause reproductive toxicity on the basis of the findings of both EPA and NTP that lead causes female reproductive toxicity.

(c) Authority of the State Agency to Take the Action Requested

– Health & Safety Code section 25249.8(b) requires that a chemical be listed as known to the state to cause reproductive toxicity if that chemical has been formally identified as causing reproductive toxicity by a body considered authoritative by the state's qualified experts. Pursuant to California Code of Regulations, title 27 section 25306(*l*) both EPA and NTP are such an authoritative bodies. As the attached EPA ISA and NTP 2012 Lead Monograph show, both of these authoritative bodies have formally identified lead as causing delayed onset of puberty in females, which is a toxic endpoint the OEHHA considers to be a form of female reproductive toxicity. Health & Safety Code section 25249.8 commands the Governor to cause to be published "a list of those chemicals known to cause . . . reproductive toxicity . . . and he shall cause such list to be revised and republished in light of additional knowledge at least once per year thereafter." Health & Safety Code section 25249.12 commands the Governor to designate a lead agency that may be required to implement Proposition 65, and thus "adopt and modify regulations, standards,

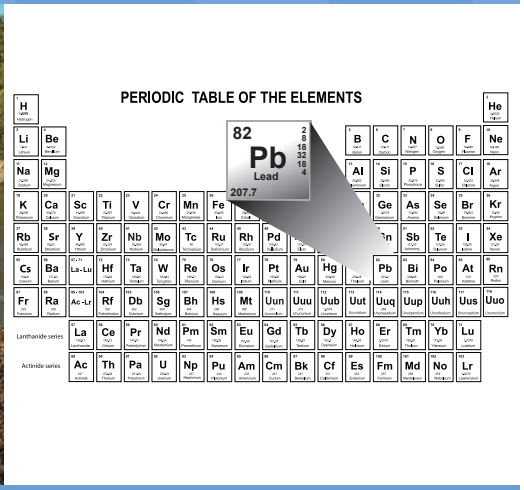
[1] The pages from the EPA ISA and the NTP 2012 Lead Monograph that are cited in this petition are attached and incorporated by reference.

and permits as necessary to conform with and implement [Proposition 65] and to further its purposes." Subparagraph (a) to California Code of Regulations, Division 4, Chapter 1, Article 1 states that the Office of Environmental Health Hazard Assessment is the "lead agency for implementing the Safe Drinking Water and Toxic Enforcement Act of 1986 (Health and Safety Code Section 25249.5 et seq.)." California Government Code section 11340.7(b) permits a state agency petitioned pursuant to Government Code section 11340.6 to "grant or deny the petition in part." OEHHA therefore has the authority to take the action requested in this petition.

EPA/600/R-10/075F | June 2013 | www.epa.gov

United States Environmental Protection Agency

Integrated Science Assessment for Lead



Office of Research and Development
National Center for Environmental Assessment, Research Triangle Park, NC

Table ES-1 (Continued): Summary of causal determinations for the relationship between exposure to Pb and health effects.

Health Outcome	Causality Determination[a] (Table with Key Evidence)
Hematologic Effects (Section 1.6.5)	
Decreased Red Blood Cell (RBC) Survival and Function	Causal Relationship (Table 4-35)
Animal toxicological studies demonstrate that exposures resulting in blood Pb levels relevant to humans (2-7 µg/dL) alter several hematological parameters (Hemoglobin [Hb], Hematocrit [Hct], and mean corpuscular volume [MCV]),increase measures of oxidative stress and increase cytotoxicity in red blood cell (RBC) precursor cells. Limited body of epidemiologic studies provides additional support for the association of Pb exposure with these endpoints. Plausible MOAs are demonstrated in experimental animals.	
Altered Heme Synthesis	Causal Relationship (Table 4-35)
Consistent findings from studies in experimental adult animal studies report that relevant exposures (e.g. blood Pb levels of 6.5 µg/dL) cause decreased ALAD and ferrochelatase activities. Additional support is garnered from a larger body of ecotoxicological studies demonstrating decreased ALAD activity across a wide range of species and a limited body of epidemiologic studies. Plausible MOAs are demonstrated in experimental animals.	
Reproductive and Developmental Effects (Section 1.6.6)	
Development	Causal Relationship (Table 4-48)
Multiple cross-sectional epidemiologic studies report associations between concurrent blood Pb levels and delayed pubertal onset for girls (6-18 years) and boys (8-15 years). These associations are consistently observed in populations with concurrent blood Pb levels 1.2-9.5 µg/dL. Few studies consider confounding by nutrition. Uncertainties remain regarding the timing, frequency, duration and level of Pb exposures contributing to the effects observed in epidemiologic studies of older children. Experimental animal studies demonstrate delayed onset of puberty in female pups with blood Pb levels of 1.3-13 µg/dL and delayed male sexual maturity at blood Pb levels of 34 µg/dL.	
Birth Outcomes (e.g., low birth weight, spontaneous abortion)	Suggestive of Causal Relationship (Table 4-48)
Some well-conducted epidemiologic studies report associations of maternal Pb biomarkers or cord blood Pb with preterm birth and low birth weight/fetal growth; however, the epidemiologic evidence is inconsistent overall and findings from experimental animal studies are mixed.	
Male Reproductive Function	Causal Relationship (Table 4-48)
Key evidence is provided by toxicological studies in rodents, non-human primates, and rabbits showing detrimental effects on semen quality, sperm and fecundity/fertility with supporting evidence in epidemiologic studies. Toxicological studies with relevant Pb exposure routes leading to blood Pb concentrations ranging from 5-43 µg/dL reported effects on sperm quality and sperm production rate, sperm DNA damage, and histological or ultrastructural damage to the male reproductive organs. Consistent associations in studies of occupational populations with concurrent blood Pb levels of 25 µg/dL and greater, report detrimental effects of Pb on sperm; however, uncertainties remain regarding the timing, frequency, duration and level of Pb exposures contributing to the effects observed in epidemiologic studies.	
Female Reproductive Function	Suggestive of Causal Relationship (Table 4-48)
Although findings are mixed overall, the body of evidence include some high-quality epidemiologic and toxicological studies, suggesting that Pb may affect some aspects of female reproductive function (hormone level, placental pathology).	

puberty onset (U.S. EPA, 2006b). There was recognition that Pb is transferred across the placenta and through the breast milk, contributing to exposure during development. The 2006 Pb AQCD reported delayed puberty onset in both male and female populations in animal toxicology studies showing associations with dam blood Pb levels of ~40 µg/dL and pup blood Pb levels of 26 µg/dL. The research reported in this ISA continues to find delayed puberty onset with Pb exposure at even lower Pb doses in animal toxicology studies as is detailed below. Mechanistic understanding of delayed puberty onset is also reported in this ISA. Lower dose Pb exposure studies in animal toxicology are also reported in studies of retinal function and postnatal growth in this ISA. Studies included in this ISA expand upon evidence reported in previous Pb AQCDs for the aforementioned systems sensitive to developmental effects with recent studies showing effects at lower doses of Pb. This section does not cover associations between Pb and neurodevelopmental impacts, which are discussed in detail in Section 4.3. The studies presented in the following text and tables are grouped by study design and methodological strength.

4.8.1.1 Effects on Puberty among Females

Recent toxicological studies of rodents have examined the effects of Pb on pubertal and reproductive organ development and on biomarkers of pubertal development among females. There have also been recent epidemiologic studies examining associations between blood Pb levels and onset of puberty among girls, which are summarized in Table 4-36 and in the text below. All of the epidemiologic studies examined concurrently measured blood Pb and puberty and are reported below. Additionally, while there was a longitudinal investigation by Naicker et al. (2010), who followed girls to determine their age of menarche, blood Pb levels were measured once at 13 years of age.

Table 4-36 Summary of epidemiologic studies of associations between blood Pb levels and puberty for females.

Reference[a]	Outcome	Study Population Study Location Study Years	Methodological Details	Blood Pb Data in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Selevan et al. (2003)	Tanner staging and age at menarche	Girls ages 8-18 yr from the NHANES III study $N_{NH\ White}$=600 $N_{NH\ Black}$=805 $N_{Mexican\text{-}American}$=781 U.S.A. 1988-1994	Cross-sectional study using ordinal logistic regression and Cox proportional hazards	Geometric mean Non-Hispanic (NH) Whites: 1.4 NH Blacks: 2.1 Mexican-Americans: 1.7 Blood Pb levels>5µg/dL: NH Whites: 2.7% NH Blacks: 11.6% Mexican-Americans: 12.8% Blood Pb levels >10 µg/dL: NH Whites: 0.3% NH Blacks: 1.6% Mexican-Americans: 2.3%	OR (95% CI) Breast development NH Whites 1 µg/dL: 1.00 (Ref) 3 µg/dL: 0.82 (0.47, 1.42) NH Blacks: 1 µg/dL: 1.00 (Ref) 3 µg/dL: 0.64 (0.42, 0.97) Mexican Americans: 1 µg/dL: 1.00 (Ref) 3 µg/dL: 0.76 (0.63, 0.91) Pubic hair development NH Whites: 1 µg/dL: 1.00 (Ref) 3 µg/dL: 0.75 (0.37, 1.51) NH Blacks: 1 µg/dL: 1.00 (Ref) 3 µg/dL: 0.62 (0.41, 0.96) Mexican Americans: 1 µg/dL: 1.00 (Ref) 3 µg/dL: 0.70 (0.54, 0.91)	For breast development: Age, age^2, height, BMI, family income, ever smoked>100 cigarettes, dietary Fe, dietary vitamin C, dietary Ca^{2+}. For pubic hair development: Age, age^2, height, family income, ever smoked>100 cigarettes, anemia, dietary Fe, dietary vitamin C Considered in all models: age, smoking, dietary Ca^{2+}, dietary Fe, dietary vitamin C, dietary total fat, anemia, urban residence, family income

Table 4-36 (Continued): Summary of epidemiologic studies of associations between blood Pb levels and puberty for females.

Reference[a]	Outcome	Study Population Study Location Study Years	Methodological Details	Blood Pb Data in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Selevan et al. (2003) (Continued)	Tanner staging and age at menarche	Girls ages 8-18 yr from the NHANES III study $N_{NH\ White}$=600 $N_{NH\ Black}$=805 $N_{Mexican\text{-}American}$=781 U.S.A. 1988-1994	Cross-sectional study using ordinal logistic regression and Cox proportional hazards	Geometric mean Non-Hispanic (NH) Whites: 1.4 NH Blacks: 2.1 Mexican-Americans: 1.7 Blood Pb levels>5µg/dL: NH Whites: 2.7% NH Blacks: 11.6% Mexican-Americans: 12.8% Blood Pb levels >10 µg/dL: NH Whites: 0.3% NH Blacks: 1.6% Mexican-Americans: 2.3%	**HR (95% CI)** *included only girls ages 8-16 yr Age at menarche NH Whites: 1 µg/dL: 1.00 (Ref) 3 µg/dL: 0.74 (0.55, 1.002) NH Blacks: 1 µg/dL: 1.00 (Ref) 3 µg/dL: 0.78 (0.63, 0.98) Mexican Americans: 1 µg/dL: 1.00 (Ref) 3 µg/dL: 0.90 (0.73, 1.11)	For age at menarche: Height, BMI, family income, anemia, dietary Ca^{2+}. Considered in all models: age, smoking, dietary Ca^{2+}, dietary Fe, dietary vitamin C, dietary total fat, anemia, urban residence, family income

Table 4-36 (Continued): Summary of epidemiologic studies of associations between blood Pb levels and puberty for females.

Reference[a]	Outcome	Study Population Study Location Study Years	Methodological Details	Blood Pb Data in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Wu et al. (2003b)	Tanner staging and age at menarche	Girls ages 8-16 yr from the NHANES III study N=1706 U.S.A. 1988-1994	Cross-sectional study using logistic regression with weighting	Mean (SD): 2.5 (2.2) Weighted proportion of the sample with blood Pb 5.0-21.7: 5.9%	OR (95% CI) Breast development 0.7-2.0 µg/dL: 1.00 (Ref) 2.1-4.9 µg/dL: 1.51 (0.90, 2.53) 5.0-21.7 µg/dL: 1.20 (0.51, 2.85) Pubic hair development 0.7-2.0 µg/dL: 1.00 (Ref) 2.1-4.9 µg/dL: 0.48 (0.25, 0.92) 5.0-21.7 µg/dL: 0.27 (0.08, 0.93) Menarche 0.7-2.0 µg/dL: 1.00 (Ref) 2.1-4.9 µg/dL: 0.42 (0.18, 0.97) 5.0-21.7 µg/dL: 0.19 (0.08, 0.43)	Race/ethnicity, age, family size, residence, poverty income, ratio, BMI

Table 4-36 (Continued): Summary of epidemiologic studies of associations between blood Pb levels and puberty for females.

Reference[a]	Outcome	Study Population Study Location Study Years	Methodological Details	Blood Pb Data in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Gollenberg et al. (2010)	Luteinizing hormone (LH) and inhibin B	Girls ages 6-11 yr from the NHANES III study N=705 U.S.A. 1988-1994	Cross-sectional study using survey logistic regression	Median 2.5 (range 0.07-29.4) Blood Pb >10 µg/dL: 5%	OR (95% CI) Exceeding pubertal inhibin B cutoff (>35pg/mL) <1 µg/dL: 1.00 (Ref) 1-4.9 µg/dL: 0.38 (0.12, 1.15) ≥ 5 µg/dL: 0.26 (0.11, 0.60) Exceeding pubertal LH cutoff (>0.4 mIU/mL) <1 µg/dL: 1.00 (Ref) 1-4.9 µg/dL: 0.98 (0.48, 1.99) ≥ 5 µg/dL: 0.83 (0.37, 1.87) *Note: a sensitivity analysis including only those with blood Pb <10 µg/dL had similar results but ORs were slightly attenuated	Age, race/ethnicity, BMI, census region, poverty-income ratio

Table 4-36 (Continued): Summary of epidemiologic studies of associations between blood Pb levels and puberty for females.

Reference[a]	Outcome	Study Population Study Location Study Years	Methodological Details	Blood Pb Data in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Denham et al. (2005)	Age at menarche	10- to 16.9-yr-old girls in the Akwesasne community N=138 Akwesasne Mohawk Nation (boundaries of New York, Ontario, and Quebec Years not reported	Cross-sectional study using probit and logistic regression	Mean (SD): 0.49 (0.905) Median: 1.2	Coefficients for binary logistic regression predicting menarche with Pb centered at the mean: **log blood Pb** -1.29 (p-value 0.01) **log blood Pb -squared**: -1.01 (p-value 0.08) Non-linear relationship observed and Pb below the mean did not appear to affect the odds of menarche. Increasing blood Pb from 0.49 to 0.98 µg/dL decreased the odds of menarche attainment by 72%	Age, SES, BMI
Naicker et al. (2010)	Self-reported Tanner staging at age 13 and age at menarche	Girls of black or mixed ancestry who were enrolled in the Birth to Twenty (Bt20) cohort (born in 1990) that lived in Johannesburg/ Soweto for at least 6 mo after birth N=682 Johannesburg/ Soweto, South Africa Born in 1990	Cross-sectional and longitudinal study using logistic regression	Mean (SD): 4.9 (1.9) Blood Pb levels ≥ 10 µg/dL: 1%	OR (95% CI) Delay in breast development at age 13 <5 µg/dL: 1.00 (Ref) ≥ 5 µg/dL: 2.34 (1.45, 3.79) Delay in pubic hair development at age 13 <5 µg/dL: 1.00 (Ref) ≥ 5 µg/dL: 1.81 (1.15, 2.84) Delay in attainment of menarche at age 13 <5 µg/dL: 1.00 (Ref) ≥ 5 µg/dL: 2.01 (1.38, 2.94)	BMI

Table 4-36 (Continued): Summary of epidemiologic studies of associations between blood Pb levels and puberty for females.

Reference[a]	Outcome	Study Population Study Location Study Years	Methodological Details	Blood Pb Data in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Den Hond et al. (2011)	Tanner staging, age at menarche, regular menses	Girls ages 14 and 15 yr, in their 3rd year of secondary education and living in the same study areas for at least 5 years N=792 Flanders, Belgium 2003-2004	Cross-sectional study using logistic regression	Median: 1.81 10th percentile: 0.88 90th percentile: 3.81	OR (95% CI) Pubic hair development with doubling of exposure: 0.65 (0.45, 0.93) *Association was no longer statistically significant when PCB marker included in the model No association between Pb and breast development (results not given)	Age, BMI, smoking, oral contraceptive use Considered but did not include: food intake and lifestyle parameters

Table 4-36 (Continued): Summary of epidemiologic studies of associations between blood Pb levels and puberty for females.

Reference[a]	Outcome	Study Population Study Location Study Years	Methodological Details	Blood Pb Data in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Tomoum et al. (2010)	Hormones and pubertal development using Tanner staging	Healthy children aged 10-13 yr; seeking treatment for minor health problems and living in one of two designated areas (one with high-risk for Pb contamination and one with no Pb source) N=20 Cairo, Egypt 2007	Cross-sectional study using Chi-square	Blood Pb Not stated for girls only (combined with boys in the study the mean was 9.46 [3.08])	**Breast Development** **<10 µg/dL:** Stage 2: 36.4% Stage 3: 63.6% **≥ 10 µg/dL:** Stage 2: 100% Stage 3: 0% Chi-square p-value<0.01 **Pubic Hair Development** **<10 µg/dL:** Stage 2: 36.4% Stage 3: 63.6% **≥ 10 µg/dL:** Stage 2: 77.8% Stage 3: 22.2% Chi-square p-value>0.05	None

Table 4-36 (Continued): Summary of epidemiologic studies of associations between blood Pb levels and puberty for females.

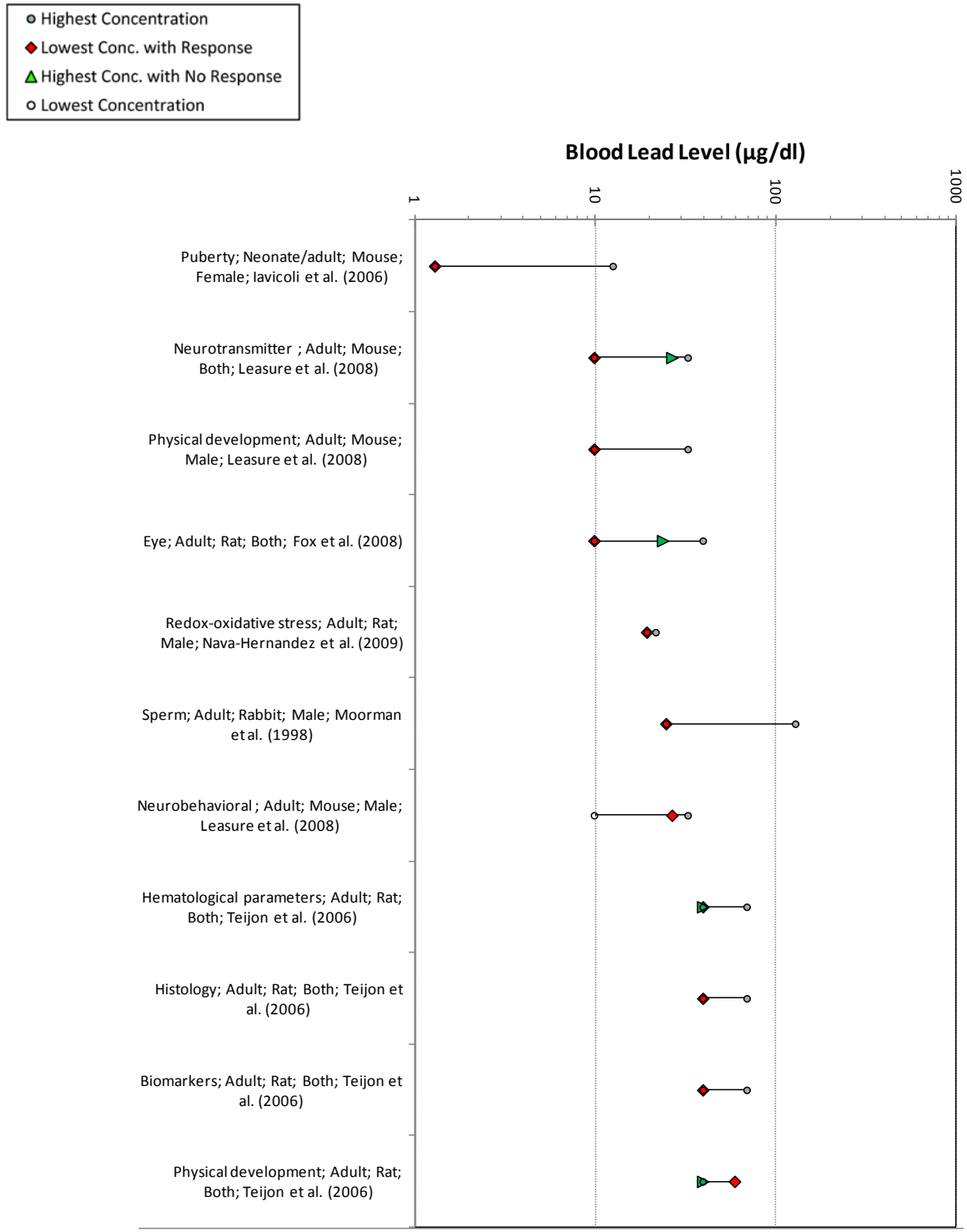
Reference[a]	Outcome	Study Population Study Location Study Years	Methodological Details	Blood Pb Data in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Tomoum et al. (2010) (Continued)	Hormones and pubertal development using Tanner staging	Healthy children aged 10-13 yr; seeking treatment for minor health problems and living in one of two designated areas (one with high-risk for Pb contamination and one with no Pb source) N=20 Cairo, Egypt 2007	Cross-sectional study using Chi-square	Blood Pb Not stated for girls only (combined with boys in the study the mean was 9.46 [3.08])	**Median FSH level** **<10 µg/dL:** 7.3 (IQR 7.9) mIU/mL **≥ 10 µg/dL:** 3.17 (IQR 2.6) mIU/mL Mann-Whitney U-test p-value <0.05 **Median LH level** **<10 µg/dL:** 8.9 (IQR 6.1) mIU/mL **≥ 10 µg/dL:** 1.23 (IQR 2.5) mIU/mL Mann-Whitney U-test p-value<0.05 Median Estradiol levels were similar among girls with blood Pb levels of <10 µg/dL and ≥ 10 µg/dL (quantitative results not given).	None
Wolff et al. (2008); Wolf and Daley (2007)	Pubertal stages defined using standard drawings	9-yr old girls from the study hospital and nearby pediatric offices N=192 New York City, NY 1996-1997	Cross-sectional study using Poisson multivariate regression with robust error variance	Median: 2.4	**PR (95% CI)** (unit not given, assume results are per 1 µg/dL) **Breast stage**: 1.01 (0.79, 1.30) **Pubic hair stage:** 1.25 (0.83, 1.88)	For breast development: Age, BMI, race For hair stage: Height, private clinic, race

[a]Studies are presented in order of first appearance in the text of this section, which is based on study design and methodological strength.

Multiple studies have been performed examining blood Pb levels and puberty using NHANES III data (Gollenberg et al., 2010; Selevan et al., 2003; Wu et al., 2003b). A study included girls 8-18 years of age and reported the results stratified by race (Selevan et al., 2003). This study also included many important potential confounders, such as nutritional factors. Higher blood Pb levels were associated with lower Tanner stage of breast and pubic hair development and later age at menarche among African Americans and with lower stage of breast and pubic hair development among Mexican Americans. For whites, the associations were in the same directions, but none reached statistical significance. Another NHANES III study included girls aged 8-16 years and did not stratify the results by race/ethnicity (Wu et al., 2003b). The study, which also controlled for a variety of potential confounders (although not nutritional factors), reported an association between increased blood Pb and delayed attainment of menarche and pubic hair development but not breast development. The associations were observed even at blood Pb levels of 2.1-4.9 µg/dL compared to girls with blood Pb levels <2.1 µg/dL. In a study of girls aged 6-11 years old from NHANES III data, higher blood Pb levels were associated with lower inhibin B, a protein that inhibits follicle stimulating hormone (FSH) production, but no association was observed for luteinizing hormone (LH). (Gollenberg et al., 2010). The inverse association between blood Pb and inhibin B was greater among girls with Fe deficiency compared to those with high Pb but sufficient Fe levels. Inhibin B and LH were chosen for this study because, as the authors indicated, these hormones are, "believed to be relevant for younger girls… near the onset of puberty and…serve as markers for hypothalamic-pituitary-gonadal functioning."

A study of girls aged 10-16.9 years of age in the Akwesasne Mohawk Nation reported a nonlinear association between higher blood Pb and greater age at menarche (Denham et al., 2005). No association was observed below blood Pb of 0.49 µg/dL in a nonlinear model of the Pb-menarche relationship. A study conducted in South Africa reported an association between increased blood Pb levels and older age at first menarche and pubertal development (Naicker et al., 2010). Another study reporting on girls with low blood Pb concentrations observed an association between higher blood Pb and less pubic hair development but not breast development (Den Hond et al., 2011). The association was no longer statistically significant when a marker for polychlorinated biphenyl exposure was included in the model. A study among girls aged 10-13 years (median: 12 years) reported lower levels of FSH and LH levels in the group with blood Pb of at least 10 µg/dL compared to the group with blood Pb less than 10 µg/dL (Tomoum et al., 2010). In addition, there were some indications of lower Tanner stages of breast development associated with Pb levels of at least 10 µg/dL, but this relationship was not present for stages of pubic hair development and there was no control for potential confounders. A study performed in NYC among 9-year old girls reported no association

between Pb levels and pubertal development (Wolff et al., 2008), but this age group may be too young to study when investigating delayed puberty as the outcome.



Note: This figure illustrates reproductive and developmental effects associated with Pb exposure in studies that examined multiple exposure concentrations. Dosimetric representation reported by blood Pb level. (Studies are described in Table 4-37).

Figure 4-36 Toxicological concentration-response array for reproductive and developmental effects of Pb.

Table 4-37 Toxicological concentration-response array summary for reproductive and developmental effects of Pb presented in Figure 4-36.

Reference	Exposure	Blood Pb level with Effect (µg/dL)	Altered Outcome
Iavicoli et al. (2006a)	Lifetime Pb exposure starting in utero. Dam exposure during gestation (0.2- 40 ppm daily dietary Pb); offspring continuous dietary exposure until the termination of the experiment at puberty	1.3 & 13	Delayed onset female puberty
Leasure et al. (2008)	Gestational and Lactational Exposure to one of 3 Pb acetate doses (27, 55, or 109 ppm) in dam drinking water, 2 weeks before mating, through gestation out to PND10.	10 & 42	Neurotransmitter, Dopamine homeostasis
		10, 24 & 42	Physical Development, Adult obesity (males)
		10 & 42	Aberrant response to amphetamine
Fox et al. (2008)	Dam exposure 2 weeks before mating, throughout gestation, and until PND10 (27, 55, or 109 ppm Pb acetate in drinking water)	12	Retinal aberrations
Nava-Hernandez et al. (2009)	Adult male rats, 10 mg/kg BW Pb chloride i.p. once daily for 8 weeks	19.5	Sperm affected via redox imbalance
Moorman et al. (1998)	Adult male rabbits, chronic exposure: subcutaneous injection, loading dose of 0.2-3.85 mg/kg BW Monday (M), Wednesday (W) and Fridays (F) weeks 6-10, followed by maintenance dose of 0.13-2.0 mg/kg BW Pb acetate MWF over weeks 11-20 of the study	25-130	Semen quality affected
Teijon et al. (2006)	Wistar rats, 200 ppm or 400 ppm Pb acetate in drinking water to dams from GD1 through lactation & 1 and 3 months postweaning to pups	40 & 100	Hematology
		40 & 100	Histology: Offspring renal & hepatic
		40 & 100	Biomarker: Offspring renal function
		100	Physical development: Birth weight

Table 4-37 (Continued): Toxicological concentration-response array summary for reproductive and developmental effects of Pb presented in Figure 4-36.

Reference	Exposure	Blood Pb level with Effect (µg/dL)	Altered Outcome
Fox et al. (2008)	Dam exposure 2 weeks before mating, throughout gestation, and until PND10 (27, 55, or 109 ppm Pb acetate in drinking water)	12	Retinal aberrations
Nava-Hernandez et al. (2009)	Male adult albino rats, 10 mg/kg BW Pb chloride i.p. once daily for 8 weeks	19.5	Sperm affected via redox imbalance
Moorman et al. (1998)	Adult male rabbits, chronic exposure: subcutaneous injection, loading dose of 0.2-3.85 mg/kg BW Monday (M), Wednesday (W) and Fridays (F) weeks 6-10, followed by maintenance dose of 0.13-2.0 mg/kg BW Pb acetate MWF over weeks 11-20 of the study	25-130	Semen quality affected
Teijon et al. (2006)	Wistar rats, 200 ppm or 400 ppm Pb acetate in drinking water to dams from GD1 through lactation & 1 and 3 months postweaning to pups	40 & 100	Hematology
		40 & 100	Histology: Offspring renal & hepatic
		40 & 100	Biomarker: Offspring renal function
		100	Physical development: Birth weight
Fox et al. (2008)	Dam exposure 2 weeks before mating, throughout gestation, and until PND10 (27, 55, or 109 ppm Pb acetate in drinking water)	12	Retinal aberrations

Earlier studies showed that prenatal and lactational exposures to Pb can cause a delay in the onset of female puberty in rodents. Recent studies corroborate these findings and show that puberty onset is one of the more sensitive markers of reproductive or developmental effects of Pb exposure as is demonstrated in the exposure response array (Figure 4-36 and Table 4-37; including outcomes described in sections that follow). Dumitrescu et al. (2008b) exposed adult Wistar female rats to varying doses of Pb acetate (50-150 ppb) in drinking water for 3 months before mating and during pregnancy. Vaginal opening, an indicator of sexual maturation, was statistically significantly delayed in pups from all Pb treated groups when compared to pups from non-treated dams. The age at vaginal opening in female pups from the Pb treated groups increased, in a concentration-dependent manner, from 39 days to 43-47 days. The authors also observed

a correlation between body weight and age at vaginal opening meaning that as body weight decreased the age at vaginal opening increased. This effect also exhibited a concentration-dependent relationship.

In recent studies by one lab, Iavicoli et al. (Iavicoli et al., 2006a; 2004) reported a statistically significant delay in several indicators of sexual maturity in female offspring (Swiss mice, F1 generation) born to dams that ingested 0.02- 40 ppm Pb in their daily diet during pregnancy; offspring had continuous dietary exposure until the termination of the experiment at puberty. Maternal ingestion of Pb at the various doses resulted in female pup blood Pb levels of 0.7, 1.3, 1.6, 1.9, 3.5, 3.9, 8 and 13 µg/dL. There was a dose-dependent delay in age at vaginal opening, age of first estrus, age of vaginal plug formation, and age of first parturition . Vaginal opening was significantly different between the following groups at blood Pb levels of 0.7, 1.3, 1.6, 1.9-3.9, 8 and 13 µg/dL. Estrus cyclicity, age of first vaginal plug detection and age of first parturition were significantly different between the groups with blood Pb levels of 0.7, 1.3-1.6, 1.9-3.9, 8 and 13 µg/dL. After in utero Pb exposure, sexual development was delayed in a concentration-dependent fashion in Pb-exposed female offspring.

In addition, Pb-induced shifts in sexual maturity were observed in the subsequent generation (F_2 generation) across the same dose range (Iavicoli et al., 2006a). These F_2 animals continued to be exposed to the aforementioned concentrations of Pb over multiple generations through the diet. Results in the F_2 generation including blood Pb levels and markers of delayed sexual maturity in female offspring closely resembled those of the F_1 generation.

In the 2006 Pb AQCD (U.S. EPA, 2006b), it was reported that a statistically significant reduction in the circulating levels of insulin-like growth factor 1 (IGF-1), LH, and estradiol (E2) was associated with Pb-induced delayed puberty onset in Fischer 344 pups, providing evidence for the effect of Pb on a mode of action for delayed puberty onset. Subsequently, Pine et al. (2006) evaluated whether IGF-1 replacement could reverse the effects of Pb on delayed female puberty onset, providing more evidence for the effect on a mode of action associated with Pb on delayed puberty onset. The authors reported that offspring from dams exposed to Pb during gestation and lactation (daily oral gavage of dam with 1.0 mL solution of Pb acetate 12 mg/mL; mean maternal blood Pb level 40 µg/dL) exhibited a marked increase in LH and luteinizing hormone releasing hormone (LHRH) secretion after IGF-1 administration (200 $ng^3/\mu L$ i.p. injection twice daily from PND23 until the appearance of vaginal opening which appears in control animals at ~PND40) resulting in restored timing of vaginal opening to that of control animals. It should be noted that, IGF-1 replacement in Pb-exposed animals did not cause advanced puberty onset over non-Pb-exposed controls. The results of this study provide support to

the theory that Pb-induced delayed onset of puberty may be due to disruption of pulsatile release of sex hormones (U.S. EPA, 2006b) and not necessarily due to a direct toxic effect on the hypothalamic-pituitary-gonadal axis (Salawu et al., 2009), and IGF-1 may play a prominent role in the process.

In summary, epidemiologic studies consistently show an association between higher concurrent blood Pb and delayed pubertal development in girls. This association is apparent even at low blood Pb levels (mean and/or median concurrent blood Pb levels of 1.2 to 9.5 µg/dL). Most of the studies had good sample sizes and controlled for some potential confounders. Nutritional information was rarely controlled for although this could be important, especially in populations where malnutrition is prevalent. These epidemiologic studies are cross-sectional, which does not allow for the study of temporality between Pb exposure and pubertal onset nor does it consider the influence of past blood Pb levels. Recent evidence from the toxicology literature continues to indicate Pb-induced delays in the onset of puberty. Further, the biological plausibility of delayed puberty is expanded with the toxicological literature that shows this pathway is mediated by IGF-1.

4.8.1.2 Effects on Puberty among Males

Recent epidemiologic studies examining the association between blood Pb and onset of puberty in males are summarized in Table 4-38. The majority of studies used concurrent measures of blood Pb and puberty (Den Hond et al., 2011; Tomoum et al., 2010; Hauser et al., 2008), but Williams et al. (2010) performed a longitudinal analysis of blood Pb levels measured at ages 8-9 years and pubertal onset, following the participants for 3 years. Little epidemiologic information was available regarding pubertal onset in the 2006 Pb AQCD (U.S. EPA, 2006b).

Table 4-48 Summary of evidence supporting reproductive and developmental causal determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Exposure or Blood Pb Levels Associated with Effects[c]
Effects on Development – Causal			
Delayed Puberty Onset			
Consistent associations with relevant blood Pb levels in high-quality epidemiologic studies that help rule out chance, bias, and confounding with reasonable confidence	Consistent evidence in multiple large cross-sectional epidemiologic studies for females and males plus a longitudinal study for males. Most of these studies have large sample sizes and controlled for potential confounding by covariates such as age, BMI, and/or education/SES. There is uncertainty related to exposure patterns resulting in likely higher past Pb exposure	Tomoum et al. (2010), Hauser et al. (2008), Williams et al. (2010), Denham et al .(2005), Naicker et al. (2010), Wu et al. (2003b), Gollenberg et al. (2010) Sections 4.8.1.1 and 4.8.1.2	Concurrent blood Pb levels: 1.2-9.5 µg/dL
	A large study using females aged 8-18 years from the NHANES III study also controlled for various dietary factors and reported associations between blood Pb levels and delayed puberty onset	Selevan et al. (2003)	
Consistent toxicological evidence with relevant Pb exposures to rule out change, bias, and confounding with reasonable confidence.	Consistent toxicological evidence from multiple laboratories of delayed male and female puberty onset with Pb exposure via diet or oral gavage in rodents	Dumitrescu et al. (2008b), Iavicoli et al. (2006a), Pine et al. (2006) Sections 4.8.1.1 and 4.8.1.2	Blood Pb level after dietary exposure, or gavage, from gestation to estrus: 1.3 - 13 µg/dL
Evidence clearly describes mode of action.	Toxicological evidence describes HPG axis dysfunction and. Mode of action changes in IGF-1 contributing to Pb-induced delay in puberty onset.	Section 4.8.1.1	
Postnatal Growth			
Available toxicological evidence is mixed	There are mixed findings in the toxicological literature on Pb exposure and postnatal growth with some studies showing stunted growth in animals exposed to Pb and some showing no effects.	Section 4.8.1.3	22.8 µg/dL
	Recent toxicological evidence of effect of Pb on postnatal growth: obesity in adult male offspring after gestational + lactational Pb exposure		10 µg/dL 42 µg/dL

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NTP Monograph

Health Effects of Low-Level Lead

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8.0 REPRODUCTIVE/ DEVELOPMENTAL EFFECTS

8.1 Conclusions

The NTP concludes there is *sufficient* evidence that blood Pb levels <10 µg/dL are associated with adverse health effects on development in children and that blood Pb levels <5 µg/dL are associated with adverse health effects on reproduction in adult women.

Because the database of human studies on most reproductive endpoints is limited to occupational exposure studies, many of the available studies are for blood Pb levels >10 µg/dL. Given this fact and the focus of the original nomination on reproductive and developmental effects, higher blood Pb levels were included in the evaluation of these health effects, unlike other sections of this document. Consideration of blood Pb levels >10 µg/dL resulted in several conclusions for Pb-related reproductive effects in men but did not affect the conclusions for women or children.

Unlike the data set for most other health effects, a number of prospective studies of developmental effects include prenatal measures of exposure (either maternal blood or umbilical cord blood). Maternal blood Pb <10 µg/dL is associated with decreased head circumference in children through 4 years of age, providing evidence that prenatal exposure is associated with reduced postnatal growth in children. Conclusions on effects of prenatal exposure for outcomes evaluated as children are complicated by the high degree of correlation in childhood blood Pb levels over time, and as described below, concurrent blood Pb levels <10 µg/dL in children are also associated with reduced postnatal growth.

Children

In children, there is *sufficient* evidence that blood Pb levels <10 µg/dL are associated with delayed puberty in both boys and girls. Nine studies with mean blood Pb levels <10 µg/dL support the relationship between Pb and delayed puberty (see **Table 8.3**); although several studies report effects on puberty at blood Pb levels <5 µg/dL, there is also evidence indicating no effect of blood Pb <5 µg/dL. Therefore, there is *limited* evidence that delayed puberty is associated with blood Pb levels <5 µg/dL. There is *sufficient* evidence that decreased postnatal growth is associated with blood Pb levels <10 µg/dL in children. Epidemiological studies consistently report an inverse relationship between blood Pb levels <10 µg/dL and postnatal growth (see **Table 8.4**). Developmental effects on neurological, immunological, renal, and cardiovascular systems are not covered in this section because they are reviewed in individual chapters.

Women

In adult women, there is *sufficient* evidence that maternal blood Pb levels <5 µg/dL are associated with reduced fetal growth or lower birth weight. The association between maternal Pb exposure and reduced fetal growth is supported by several prospective studies with maternal blood Pb data during pregnancy, a large retrospective cohort of over 43,000 mother-infant pairs that reports a mean maternal blood Pb level of 2.1 µg/dL, and a number of cross-sectional studies with maternal or umbilical cord blood Pb at delivery. Although maternal or paternal bone Pb data are not available in studies of most reproductive health outcomes, a set of studies from a single group reported that maternal bone Pb is associated with lower birth weight, birth length, and head circumference. There is *limited* evidence that maternal blood Pb levels <10 µg/dL are associated with spontaneous abortion and preterm birth or reduced gestational age. Although a number of prospective studies with maternal blood Pb levels during pregnancy and cross-sectional studies with umbilical cord blood Pb levels at delivery reported an association between prenatal blood Pb levels <10 µg/dL and preterm birth, the conclusion of *limited* evidence is based on the inconsistent results and because a retrospective study with a large cohort of over 43,000 mother-infant pairs did not find an association between maternal blood Pb levels and preterm birth. The conclusion of *limited* evidence for an association with spontaneous abortion in women is based principally on the Borja-Aburto *et al.* (1999) study, which has the strength of its prospective nested case-control design, with additional support provided by occupational studies that reported an association with Pb exposure but lack blood Pb measurements. There is *inadequate* evidence for other reproductive and effects of Pb associated with blood Pb levels <10 µg/dL in women.

Men

In adult men, there is *inadequate* evidence that blood Pb levels <10 µg/dL are associated with

Table 8.1: Major reproductive/developmental effects considered

Effect	Description
Delayed puberty	Delay in measures of puberty (e.g., Tanner staging of genitalia, pubic hair, and breast development)
Postnatal growth	Slower growth (as indicated by height, head circumference, etc., for age)
Sperm parameters	Numerous sperm or semen measures (sperm count, motility, morphology, etc.)
Conception	Greater time to pregnancy or lower fecundity
Pregnancy loss	Spontaneous abortion (fetal loss <20 weeks gestation), stillbirth (fetal loss ≥20 weeks)
Gestation length	Shorter length of gestation (as a continuous measure), preterm birth (<37 weeks)
Fetal growth	Lower birth weight, often adjusted for gestational age
Birth defects	Congenital malformations

(see **Table 8.2** for principal conclusions and original documents for complete conclusions). Although the 2006 EPA AQCD for Lead (U.S. EPA 2006) cited the Borja-Aburto *et al.* study (1999) as a well-conducted prospective case-control study supporting a significant relationship between maternal blood Pb level at 10 µg/dL to 12 µg/dL and spontaneous abortion, the EPA concluded that, collectively, there is little evidence to support an association between maternal or paternal Pb exposure and incidence of spontaneous abortion. The 2006 EPA AQCD for Lead (U.S. EPA 2006) concluded that the data are inadequate to evaluate reproductive effects in females, and studies of developmental effects suggest at most a small association between Pb exposure and preterm birth, congenital abnormalities, birth weight, or fetal growth. The EPA is in the process of revising the AQCD, and the conclusions of the external draft (U.S. EPA 2012) are largely in line with the 2006 AQCD for Lead (U.S. EPA 2006), plus additional review of the evidence for delayed pubertal onset.

Table 8.2: Main conclusions for reproductive/developmental effects in the 2006 EPA AQCD for Lead and the 2007 ATSDR Toxicological Profile for Lead

"The epidemiologic evidence suggests small associations between exposure to Pb and male reproductive outcomes, including perturbed semen quality and increased time to pregnancy. These associations appear at blood Pb levels >45 µg/dL, as most studies have only considered exposure in the occupational setting. There are no adequate data to evaluate associations between Pb exposure and female fertility." (U.S. EPA 2006, pg 6-271)
"Studies of children also have shown associations between PbB [blood Pb level] and growth, delayed sexual maturation in girls, and decreased erythropoietin production. Some studies of humans occupationally or environmentally exposed to Pb have observed associations between PbB and abortion and preterm delivery in women and alterations in sperm and decreased fertility in men. On the other hand, there are several studies that found no significant association between Pb exposure and these end points. At least for the effects in males, the threshold PbB appears to be in the range of 30-40 µg/dL." (ATSDR 2007, pg 23)

The NTP considered the conclusions and data summaries from the EPA's 2006 AQCD for Lead (U.S. EPA 2006) and ATSDR's 2007 Toxicological Profile for Lead (ATSDR 2007). In general, the NTP concurred with the conclusions and agreed that the data support them. Differences from the ATSDR and EPA documents are identified for specific endpoints in this document.

8.3 Evidence for Pb-Related Effects on Reproductive and Developmental Outcomes

8.3.1 Delayed Puberty

There is *sufficient* evidence that blood Pb levels <10 µg/dL are associated with a delay in sexual maturation or puberty onset in children 8-17 years of age (see **Table 8.3** and the Puberty section of Appendix E: Reproductive and Developmental Effects). An inverse association between blood Pb level and markers of sexual maturation has been reported in eight cross-sectional studies and a single prospective study involving children with blood Pb levels from 10 to <1 µg/dL from seven different populations in North America, Europe, and Africa. Pb-related developmental delay in a several biological markers of puberty (e.g., age

Table 8.3: Studies of biomarkers of puberty associated with low-level Pb exposure used to develop conclusions

Relevance to Conclusions	Study Description	Study Design	Key Reproductive/Developmental Findings	Reference
Chapaevsk, Russia				
Effect	Boys 11-12; n=481	Prospective	Delayed puberty onset in boys with blood Pb ≥5 µg/dL. Puberty measures differed by testicular volume and Tanner genital and pubic hair staging.	Williams (2010) *(same population as Hauser)*
	Boys 8-9; n=489	Cross-sectional	Delayed puberty onset in boys with blood Pb ≥5 µg/dL. Puberty determined by Tanner genital staging and testicular volume; effects not significant for pubic hair staging.	Hauser (2008)
NHANES III				
Effect	Girls 8-16 years of age; n=1,235	Cross-sectional	Delayed puberty onset in girls with blood Pb ≥2 µg/dL compared to those with blood Pb <2 µg/dL. Puberty differed by Tanner pubic hair developmental stage and age at menarche; no effect on developmental stage of breasts.	Wu (2003) *(population overlap with Gollenberg, Selevan)*
	Girls 8-11 years old; n=705	Cross-sectional	Girls with higher blood Pb (blood Pb ≥5 µg/dL compared with those <1 µg/dL) had lower likelihood of having inhibin B levels >35 pg/mL, a level the authors report to be associated with puberty and development of pubic hair and breasts.	Gollenberg (2010)
	Girls 8-16 years old; n=600-805 per race/ethnicity	Cross-sectional	Delayed puberty onset in African American girls (age at menarche, Tanner breast and pubic hair developmental stage) and Mexican American girls (breast and pubic hair stage) at blood Pb >3 µg/dL compared to blood Pb <1 µg/dL; not in non-Hispanic whites.	Selevan (2003)
Effect	Girls 13 years old in South Africa; n=682-712, by endpoint	Cross-sectional	Delayed puberty onset (determined by Tanner pubic hair and breast developmental stage and age at menarche) in girls with blood Pb ≥5 µg/dL, and significant association with blood Pb by trend analysis across for stage or age at menarche.	Naicker (2010)
Effect	Children aged 17 in Belgium; n=100 Pb and 100 referent	Cross-sectional	Testicular volume was lower in boys living in areas with higher blood Pb (1.8-2.7 µg/dL) compared to referents (1.5 µg/dL). Genital and breast stage did not differ consistently and significantly between referent and exposed; comparison by blood Pb not reported.	Staessen (2001)
Effect	Girls aged 10-17 in Akwesasne Mohawk Nation; n=138	Cross-sectional	Delayed puberty onset (age at menarche) in girls with blood Pb above mean (≥0.49 µg/dL) and a predicted delay in age at menarche of 10 months with blood Pb above median (1.2 µg/dL).	Denham (2005)
No effect	Girls 9 years old in New York; n=139	Cross-sectional	Blood Pb had no effect on puberty onset in girls (by breast and pubic hair stage) with median blood Pb level of 2 µg/dL.	Wolff (2008)
Effect	Children 10-13 years old in Egypt; n=41	Cross-sectional	Delayed puberty onset in boys and girls with blood Pb ≥10 µg/dL. Puberty measures differed for testes size, Tanner pubic hair and penile stage in boys, and Tanner developmental stage of breasts in girls, but not pubic hair in girls.	Tomoum (2010)

Epidemiological studies of Pb exposure and puberty are listed by decreasing cohort size and grouped together for overlapping or shared study groups.

Abbreviations: NHANES: National Health and Nutrition Examination Survey.

at menarche and Tanner developmental staging of breasts) have been reported in cross-sectional studies of girls, although there is no single measure that is consistently associated with blood Pb levels in all analyses. For boys, a Pb-related decrease in testicular volume was observed in all four publications, suggesting that testicular volume may be a reliable indicator of the effects of Pb on puberty in boys. The reported delay in sexual maturation with increasing blood Pb was significant across multiple studies, in various endpoints, and from different groups in analyses that adjusted for factors known to effect puberty such as race, BMI, and socioeconomic status. A conclusion of *limited* evidence that delayed puberty is associated with blood Pb levels <5 µg/dL is based on the four studies (three in girls and one in boys) that report delay in markers of puberty associated with blood Pb levels <5 µg/dL and the lack of association with blood Pb among girls in the Wolff *et al.* (2008) study with a median blood Pb level of 2 µg/dL.

Three cross-sectional studies using the NHANES III data set reported delayed puberty onset in girls. Wu *et al.* (2003) reported delayed Tanner pubic hair stage and age at menarche in girls 8-16 years of age with blood Pb levels ≥2 µg/dL, with no effect on developmental stage of breasts. In a separate analysis divided by race and ethnicity, Selevan *et al.* (2003) reported an association between blood Pb >3 µg/dL and delayed puberty onset in African American and Mexican American girls as determined by Tanner pubic hair and breast stages. Age at menarche was also delayed at blood Pb levels >3 µg/dL in African American girls; however, there was no Pb-related effect on puberty in non-Hispanic whites. Gollenberg *et al.* (2010) reported that girls with blood Pb levels ≥5 µg/dL were less likely to have levels of inhibin B of >35 pg/mL, a level that the authors suggest is associated with pubic hair and breast development. A similar delay in puberty onset indicated by age at menarche was reported in 13-year-old girls in South Africa (Naicker *et al.* 2010) and 10- to 17-year-old girls from the Mohawk Nation (Denham *et al.* 2005). Naicker *et al.* (2010) also found an association with blood Pb ≥5 µg/dL and delays pubic hair and breast development stage. In contrast, Tomoum *et al.* (2010) reported decreased Tanner breast developmental stage and no effect on pubic hair in girls 10-13 years of age with blood Pb>10 µg/dL in Egypt. Staessen *et al.* (2001) reported significant (p=0.04) differences in breast development in 17-year-old girls among three groups: two study groups with blood Pb levels of 1.8 and 2.7 µg/dL and a reference group with blood Pb of 1.5 µg/dL. However, a direct comparison of breast stage by blood Pb levels was not reported, and the delay in breast stage was significant only when comparing the study group with mean blood Pb of 1.8 µg/dL to the reference group. One of the eight studies reporting data on girls did not detect a delay in any marker of puberty onset associated with blood Pb; mean blood Pb was 2 µg/dL in the 9-year-old girls from New York in that study (Wolff *et al.* 2008).

All four of the studies addressing boys report Pb-associated decreases in testicular volume or Tanner genital or pubic hair staging (Staessen *et al.* 2001, Hauser *et al.* 2008, Tomoum *et al.* 2010, Williams *et al.* 2010). In a paired cross-sectional and follow-up prospective study, blood Pb ≥5 µg/dL was associated with delayed puberty that was significant by Tanner genital staging ($p<0.05$) and reduced testicular volume ($p\leq0.05$) in Russian boys when they were 8-9 years of age and again at 11-12 years of age (Hauser *et al.* 2008, Williams *et al.* 2010). Testicular volume was significantly lower in 17-year-old boys living in areas with higher blood levels (1.8-2.7 µg/dL) compared to a reference group (1.5 µg/dL), although the authors do not include a direct comparison of testicular volume by blood Pb levels (Staessen *et al.* 2001). Blood Pb ≥10 µg/dL was associated with decreased testes size and developmental delay in Tanner pubic hair and penile stages in 10- to 13-year-old boys in Egypt (Tomoum *et al.* 2010).

Summary of Support for Conclusions

Animal data supports a Pb-associated developmental delay in sexual maturation, indicated by biomarkers of puberty such as reduced prostate weight and delay in vaginal opening, at high blood Pb levels in some studies (i.e., 40 to >300 µg/dL) in Fisher and Sprague Dawley rats and at blood Pb levels similar to the human studies (i.e., 3-13 µg/dL) in Swiss mice (see U.S. EPA 2006 for recent reviews of the animal data, ATSDR 2007). The mouse data from Iavicoli *et al.* (2004) are interesting because the exposure that resulted in blood Pb from 3 to 13 µg/dL is associated with delayed puberty in female mice similar to the human data, whereas blood Pb <3 µg/dL was associated with accelerated time to puberty in mice, which suggests the possibility of a different mechanism of action in mice at

blood Pb levels <3 µg/dL. The human data supporting Pb-associated delay in puberty include the one prospective study in boys discussed above, with the rest being cross-sectional studies. The determination of causation from cross-sectional studies has a serious limitation because cross-sectional studies rely on concurrent blood Pb measurements and provide no information on cumulative Pb or Pb exposure at earlier time points that may be critical for sexual maturation. However, the consistency of effects across studies, among multiple groups, and in multiple measures of puberty in both males and females lends weight to the evidence for developmental delay in puberty at blood Pb concentrations from 10 to <1 µg/dL. The conclusion of *sufficient* evidence for developmental delay in puberty in children at blood Pb levels <10 µg/dL is based on the prospective study and eight cross-sectional studies that report effects above the mean blood Pb level (0.49 µg/dL to 5 µg/dL) of the group under study. A lower effect level and the conclusion of *limited* evidence for delayed puberty at blood Pb <5 µg/dL is supported by two of the studies (Selevan *et al.* 2003, Wu *et al.* 2003) that use the NHANES III data to examine puberty onset in girls from 8-16 years of age, the Denham *et al.* (2005) study of 10- to 17-year-old girls in the Mohawk Nation, and the Staessen *et al.* (2003) study reporting lower testicular volume in boys. The Tomoum *et al.* (2010) study provides additional support but reported delay in measures of puberty in boys and girls at blood Pb levels ≥10 µg/dL. The NTP's conclusions for *sufficient* evidence for delay in sexual maturation in boys and girls at blood Pb levels <10 µg/dL and *limited* evidence at blood Pb <5 µg/dL expands the conclusion from ATSDR's 2007 Toxicological Profile for Lead (ATSDR 2007), which was limited to girls at blood Pb levels <10 µg/dL; the EPA's 2006 AQCD for Lead (U.S. EPA 2006) did not present specific conclusions on sexual maturation.

8.3.2 Postnatal Growth

There is *limited* evidence that maternal Pb <10 µg/dL is associated with decreased head circumference in children up to 4 years of age and *sufficient* evidence that concurrent blood Pb <10 µg/dL in children is associated with decreased postnatal growth. Prospective studies in two groups (Rothenberg *et al.* 1993, Rothenberg *et al.* 1999, Schell *et al.* 2009) report an inverse association between maternal blood Pb and head circumference, and one study reports the lack of an association between maternal blood Pb and height or weight through 10 years of age (Lamb *et al.* 2008). The data from prospective studies (see **Table 8.4** and Postnatal Growth section of Appendix E: Reproductive and Developmental Effects) in two groups support an association between higher blood Pb levels in children and lower subsequent growth (Greene and Ernhart 1991, Rothenberg *et al.* 1993, Rothenberg *et al.* 1999). Numerous cross-sectional studies report an association between higher blood Pb in children and smaller head circumference, height, or other indicators of growth (e.g., weight, chest circumference, etc.). The clear majority of cross-sectional studies, including studies with large numbers of subjects, such as the NHANES data sets, demonstrate an association between higher concurrent blood Pb (means from 2 to 15 µg/dL) and lower height or other indicators of postnatal growth in children from 1 to 16 years of age. This strong evidence for an association between growth and concurrent blood Pb is based on exposure data that is largely outside the relevant time window – the relevant timing of Pb exposure to effect growth is during or before the growth that results in differences in height. Therefore, the conclusion of *sufficient* evidence that blood Pb <10 µg/dL in children is associated with decreased growth is based on the combination of strong support from the cross-sectional studies and the additional support from the three prospective studies that evaluate blood Pb levels in children on subsequent growth rather than current height.

Several prospective studies support an inverse association between maternal Pb <10 µg/dL and postnatal growth as indicated by head circumference, but the relationship is less clear for height. Higher maternal blood Pb at 36 weeks of gestation was associated with smaller head circumference up to 4 years of age in children in the Mexico City Prospective Study (Rothenberg *et al.* 1993, Rothenberg *et al.* 1999). Schell *et al.* (2009) also report an effect of maternal blood Pb on head circumference, but not on height or weight, in 6- to 12-month-olds in Albany, NY. Maternal blood Pb was not related to height or weight in children from 1 to 10 years of age in the Yugoslavia Prospective Study (Lamb *et al.* 2008). Data from the Cincinnati Pb study support a combined effect of maternal blood Pb as well as concurrent blood Pb levels in the children; height at 15 months was decreased only in children with blood Pb >3.4 µg/dL that also experienced maternal blood

Table 8.6: NTP conclusions on reproductive and developmental effects of low-level Pb

Health Effect	Population or Exposure Window	NTP Conclusion	Blood Pb Evidence	Bone Pb Evidence
Delayed puberty	Prenatal	*Inadequate*	No data	No data
	Children	*Sufficient*	Yes, <10 µg/dL	No data
		Limited	Yes, <5 µg/dL	
Postnatal growth	Prenatal	*Limited*	Yes, <10 µg/dL	One study
	Children	*Sufficient*	Yes, <10 µg/dL	One study available, no evidence of an association
Sperm parameters	Children	*Inadequate*	No data	No data
	Men	*Sufficient*	Yes, ≥15 µg/dL	No data
Fertility / delayed conception time	Men: time to conception	*Sufficient*	Yes, ≥20 µg/dL	No data
	Men: fertility	*Limited*	Yes, ≥10 µg/dL (one study)	No data
	Women	*Inadequate*	Unclear	No data
Spontaneous abortion	Men	*Limited*	Yes, >31 µg/dL	No data
	Women	*Limited*	Yes, <10 µg/dL	No data
Stillbirth	Adults	*Inadequate*	Unclear	No data
Reduced fetal growth and lower birth weight	Men	*Inadequate*	Unclear	No data
	Women	*Sufficient*	Yes, <5 µg/dL	Yes, tibia
Preterm birth and gestational age	Men	*Inadequate*	Unclear	No data
	Women	*Limited*	Yes, <10 µg/dL	No data
Endocrine effects	Adults	*Inadequate*	Unclear	One study
Birth defects	Adults	*Inadequate*	Unclear	No data