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CARCINOGENIC, DEVELOPMENTAL TOXICITY, AND REPRODUCTIVE TOXICITY HAZARD DETERMINATIONS

LEAD AND LEAD COMPOUNDS

Lead and lead compounds have been reviewed according to DuPont Corporate Standard S18T (Hazard Determination Process) for assessment and control of carcinogenic, developmental toxicity, and reproductive toxicity hazards. This hazard determination supersedes the current classification letter dated July 10, 1989 for lead and lead compounds, and April 10, 1989 for lead naphthenate.

Lead and lead compounds have now been classified as *Probable Human Developmental Toxins* and *Probable Human Reproductive Toxins*. Lead naphthenate and lead acetate have now been classified as *Possible Human Carcinogens* and lead chromate has now been classified as a *Known Human Carcinogen* (see lead chromate HDL dated 2-17-2000). DuPont AELs of 50 µg of lead/dL of blood and 50 µg/m^3 (8-hour TWA with skin notation) have been established to protect against potential toxic effects of lead and lead compounds, and lead chromate and lead naphthenate, respectively. Based on the *Probable Human Developmental Toxin* and *Probable Human Reproductive Toxin* classifications, an annual review of lead and lead compound toxicology with potentially exposed employees will be required.

Some additional detail in support of the preceding hazard determination follows:

Carcinogenic Hazard (Lead Acetate and Lead Naphthenate only)

Ten rat bioassays and one mouse assay have shown statistically significant increases in renal tumors with dietary and subcutaneous exposure to several soluble lead salts (IRIS, 1994). Some specific studies on lead naphthenate and lead acetate are described below.

Renal tumors were observed in mice dermally administered a 20% solution of lead naphthenate in benzene. In a group of 59 mice treated twice a week for up to 569 days, one renal carcinoma, four renal adenomas, and seven benign skin tumors were observed. No control group was included in this study (Baldwin et al., 1964).

Male rats were fed 1% lead acetate in their diet for 1 year. Of 16/20 rats that survived for 320 days or more 15 developed kidney tumors, 14 of which were renal carcinomas. No untreated control group was studied (Boyland et al., 1962).

Groups of male and female rats received a diet containing lead acetate (3 mg/day for 2 months then 4 mg/day for 16 months). Renal adenomas were found in 55 rats and renal carcinomas in 17. Leydig-cell tumors of the testis were found in 23/94 male rats (the incidence of these tumors was unspecified in control animals). No renal tumors were seen in 32 control rats (Zawirska, 1968).

In a study in rats with lead acetate, administration of 500 ppm (as lead; blood lead levels of 77.8 µg/dL) of lead acetate for 2 years produced an increased incidence of kidney tumors. Another group administered 100 ppm (blood lead levels of 35.2 µg/dL) had no kidney tumors (Azar et al., 1972).

Groups of male and female rats were fed a diet of 3 mg/rat per day of lead acetate for 60-504 days. In treated rats, 12 renal adenomas, 10 cerebral gliomas, 17 pituitary adenomas, 11 thyroid adenomas, 5 parathyroid adenomas, 15 adrenal adenomas, 11 prostatic adenomas, and 8 mammary adenomas were found. No tumors were found in control animals (Zawirska et al., 1972).

In epidemiology studies, a statistically significant excess of cancers of the digestive system (21 observed, 12.6 expected) was found in a study of battery workers in the UK, spanning 1925-1976 (excesses were confined to 1963-1966) (Malcolm et al., 1982). Significant excesses of stomach cancer (34 observed, 20.2 expected) and of respiratory cancers (116 observed, 93.5 expected) were seen in a study of US battery plant workers, although there was a downward trend in standardized mortality ratio by number of years of employment. In the lead production facilities, the excesses noted for stomach and respiratory cancers were not significant (Cooper et al., 1985). A non-significant excess of respiratory cancer (41 observed, 35.9 expected) was reported in a study of smelters, with 28 observed and 25.7 expected in the group with high exposure to lead. Excesses were also noted for kidney cancer (6 observed, 2.9 expected) and bladder cancer (6 observed, 4.2 expected) (Selevan et al., 1985). A non-significant excess of lung cancer (8 observed, 5 expected) was reported in a small study of workers at Swedish smelters with long-term exposure to lead (Gerhardsson et al., 1986). Confounding factors such as smoking and exposure to arsenic, the lack of a clear trend associated with length or degree of exposure, and the relatively small study sizes in the above studies makes it difficult to draw any conclusions relative to the carcinogenic potential of lead in humans.

Twenty-six employees in a coating and resin plant containing a broad spectrum of organic and inorganic chemicals, including lead naphthenate (>100 and <1000 pounds per year) were examined. A review of the medical findings revealed no evidence of an unusual prevalence of diseases of the CNS, kidney, liver, the blood forming organs, or the skin (Tabershaw, 1982).

In genotoxicity studies lead naphthenate was not mutagenic in the Ames test either in the presence or absence of a S-9 activation system (Cameron, 1983). Lead acetate was also negative for mutagenicity in non-mammalian cells: *Salmonella*/microsome assay for point mutations and *Escherichia coli* for DNA-modifying effects (Rosenkranz et al., 1979), and *Saccharomyces cerevisiae* for mitotic recombination (Simmon, 1979). Lead acetate gave conflicting results in mammalian cells for chromosome aberrations (Bauchinger, 1972; Beek et al., 1974; Schmid et al., 1972; Stella et al., 1978). Lead acetate did not induce heritable dominant lethal effects (Kennedy et al., 1971).

ACGIH (1999) has classified elemental and inorganic lead compounds as A3 (Confirmed Animal Carcinogen With Unknown Relevance to Humans). IARC (1980, 1987) has classified lead and inorganic lead compounds as Group 2B (Possibly Carcinogenic to Humans), and organolead compounds as Group 3 (Not Classifiable as to its Carcinogenicity in Humans).

The following classification is not based on a comprehensive review of the toxicity of all lead compounds; however, the DuPont AEL Committee believes that the preceding information is sufficient to classify lead acetate and lead naphthenate. Although the one dermal study is not enough to classify lead naphthenate, kidney tumors found in this study were similar to those induced by lead acetate treatment. Therefore, it is likely that kidney tumors in the lead naphthenate study were treatment-related. Genotoxicity results suggests a non-genotoxic mechanism of carcinogenicity for these lead compounds. No firm conclusions for carcinogenic potential of lead in humans could be drawn from the above epidemiology studies.
The DuPont AEL Committee concludes that lead naphthenate and lead acetate should be classified as *Possible Human Carcinogens.*

Developmental Toxicity Hazard (Lead and Lead Compounds)

Animal Studies

In many animal studies where lead was administered by the oral and inhalation routes, embryo- and fetotoxicity, but not teratogenicity, were observed at non-maternally toxic concentrations. Two animal studies with more descriptive developmental effects are summarized below.

Rats were administered 1.0 mM lead acetate during the pre- and postnatal periods. Control rats received deionized water. Growth and neuromotor development were assessed by monitoring 20 litters daily for several parameters. Lead treatment hastened the day of appearance of the following parameters: eye opening, startle reflex, and negative geotaxis. Spontaneous alternation performance was hindered in lead-exposed animals. These results suggest that lead exposure without concomitant undernutrition alters rat development, affecting specific subsets of motor skills (Mello et al., 1998).

Pregnant squirrel monkeys were perorally exposed to lead during the latter two-thirds of pregnancy (mean blood lead 0.54 µg/ml), at a dosing regime producing no maternal toxic symptoms. Lesions similar to lead encephalopathy and growth retardation of the fetal cerebrum were seen in some of the offspring, as well as neurological and behavioral symptoms at adult age. Cerebral lead levels in offspring were between 0.1-0.7 µg/g. Pre- and perinatal mortality, and prematurity, was increased, and the size of the offspring at birth was reduced. The head circumference tended to be reduced postnatally (Logdberg et al., 1987).

Human Studies

In humans, prenatal exposure to lead produces toxic effects on the fetus including reductions in gestational age, birth weight, and mental development. No clear evidence of an association with congenital malformations was found (Ernhart et al., 1986; McMichael et al., 1986; Needleman et al., 1984). A more recent case-control study of drinking water and reproductive outcome showed that detectable lead levels (>0.001 mg/L) were associated with anomalies of the ear, face, and neck (adjusted odds ratio [AOR] of 1.7), and cardiovascular system (AOR 2.2). But the quality of this study was questioned (Aschengrau et al., 1993).

Many well-designed epidemiology studies in the 1980s confirmed that low-level subclinical lead exposures in early life are associated with decrements in children's intelligence. In a comprehensive review of 26 epidemiology studies since 1979, including a meta-analysis, a doubling of body lead burden (from 10-20 µg/dL) blood lead or (from 5-10 µg/dL) tooth lead is typically associated with a mean deficit in full-scale IQ of around 1-2 IQ points. All children with blood levels of 20 µg/L or greater should receive environmental evaluation and medical examinations, and may need pharmacological treatment (Koike, 1997).

Lead levels in blood from 133 live newborns (69 babies in high-level group and 64 in the low-level group; the geometric mean was 9.2 µg/dL) were determined in a prospective study to assess the effects of prenatal low-level lead exposure on the development of urban, inner-city children in Shanghai. At 3, 6, and 12 months, the Mental Development Index (MDI) scores, adjusted for confounders, were inversely related to the infants' cord blood lead levels. There were significant differences in MDI scores between the high- and low-level groups. No significant association between cord blood lead levels and the psychomotor Development Index (PDI) scores was detected. Postnatal lead levels were unrelated to concurrent developmental status (Shen et al., 1998).

Blood lead measurements, neurological examinations, and cognitive tests were conducted on 96 children living in Ecuadorian villages where lead is used extensively in the glazing of ceramics. Group I consisted of 55 children with a mean blood level of 48 µg/dL. Among the children who showed neurological deficits, higher lead levels were associated with abnormal tendon reflexes, finger tapping, visual pursuit, size discrimination, draw-a-person, and math calculation skills. Group II consisted of 41 children with a mean blood lead level of 47.4 µg/dL and who were administered Raven's Colored Progressive Matrixes (RCPM) non-verbal test. Performance was normal in 22 of these children. Children with abnormal RCPM scores had higher blood lead levels. There was a significant inverse correlation between RCPM scores and blood lead levels for children ages 9 years and older (Counter et al., 1998).

Literature on the developmental toxicity associated with lead, is voluminous. The studies described above provide evidence of the potential adverse effects lead can have on animal and human development. The database on lead also contains studies that do not consistently support a significant concern for lead developmental toxicity. Although the following classification is not based on a comprehensive review of the literature, the evidence of developmental effects across species, including humans, indicates that concern for developmental effects in humans is warranted. Therefore, the AEL Committee concludes that the preceding references adequately support the following classification, and lead and lead compounds should be classified as *Probable Human Developmental Toxins.*

Reproductive Toxicity Hazard (Lead and Lead Compounds)

A literature search on male reproductive toxicity of lead identified a total of 32 experimental studies in animals and 22 epidemiology studies, one case report on humans, and 5 review articles or documents. Several studies on rats and other rodents indicated that blood lead levels >30-40 µg/dL were associated with the impairment of spermatogenesis and reduced concentrations of androgens. However, other animal studies, mainly about histopathology, spermatozology, and hormonal endpoints, indicated that certain species and strains were quite resistant to the reproductive toxicity of lead and that different testicular lead concentrations could account for these differences. The human studies showed that exposure to concentrations of inorganic lead of >40 µg/dL in blood impaired male reproductive function by reducing sperm count, volume, and density; and, changing sperm motility and morphology. No relevant effects were detected for endocrine profiles (Apostoli et al., 1998). Some studies in different animal species and some human experiences with lead exposure are further described below.

Animal Studies

In a 3-generation study, rats and mice received 25 mg/L lead as a soluble salt in drinking water and a diet containing 0.2 mg/kg lead per wet weight of diet In the treated group of 72 offspring of the first generation, 69 runts were observed (0/209 control animals), and 2/8 litters were born dead. No maternal mortality was reported. In the F_2 generation, 23 offspring were obtained from treated animals (248 from controls), and the experiment was discontinued before the F_3 generation. In rats the average litter size was 9.1 (11.4 for controls) in the F_1 generation. In the F_1 generation, 2/19 litters were born dead (0/10 for controls), 40/173 runts were observed (0/114 for controls), and the dose was lethal to 2 mothers (0 for controls). In the F_2 generation, 1/32 litters was born dead, 26/311 runts were observed, 35 young animals died, and 3 animals failed to breed (1/113 runts for controls in 10 litters with no mortalities). In the F_3 generation 4/22 runts were observed (6 litters), one animal did not breed, and 1/6 litters was born dead. No maternal deaths occurred in the F_2 and F_3 generations (Schroeder et al., 1971).

Groups of mice received either 2, 4, or 8 mg/kg/day for 60 days (5days/week) of lead acetate by gavage, to study ovarian follicular development and maturation. Small and medium follicles were significantly affected even at the lowest dose; however, large follicles were affected mostly at the highest dose. Atresia even in the medium follicle reflected the extent of damage caused by lead. These findings correlated well with increased blood lead levels (Junaid et al., 1997).

Rats were administered 0.6% (w/v) lead acetate in drinking water *ad libitum*. Three series of experiments were conducted in which lead exposure was initiated beginning *in utero*, prepubertally, or postpubertally. In male rats, secondary sex organ weight were significantly decreased only in animals exposed prepubertally. Serum testosterone levels were significantly suppressed, most severely in animals exposed from *in utero*. Little effect was observed in adult female rats; however, those exposed prepubertally demonstrated delayed vaginal opening and disrupted estrous cycling. The effects of lead appear to involve multiple sites on the hypothalamic-pituitary-gonadal axis (Ronis et al., 1996). Further work with this rat model using lead acetate in drinking water confirmed that the reproductive axis is particularly sensitive during specific developmental periods and delayed sexual maturation was due to suppression of sex steroid biosynthesis (Ronis et al., 1998).

Mice were administered either 0.25 or 0.5% of lead in drinking water for 6 weeks. The low lead level significantly reduced the number of sperm within the epididymis, while the high dose reduced both sperm count and percentage of motile sperm, and increased the percentage of abnormal sperm within the epididymis. There was no significant effect on testis weight. Plasma sex steroid levels were not affected (Wadi et al., 1999).

Rabbits were administered lead acetate by s.c. injection in a dose range of 0 to 3.85 mg/kg, 3 times a week. In each 8 treatment groups the dosing regimen produced blood lead levels from 0 to 110 µg/dL. A 5-week pre-exposure period was followed by a 15-week exposure testing period allowing for response through 6 cycles of the seminiferous epithelium. Increased blood lead levels were associated with adverse changes in sperm count, ejaculate volume, percent motile sperm, swimming velocities, and morphology (Moorman et al., 1998).

Human Studies

The prostatic function of 30 men exposed to lead were studied and compared to 20 unexposed men. The exposed individuals included workers at a battery manufacturing plant. Acid and prostatic phosphatases were analyzed to show that extensive exposure to lead initially causes a direct prostatic toxicity likely to be reflected in alteration of seminal characteristics (Lerda, 1993).

A group of 150 workers with long-term lead exposure was categorized by clinical and toxicological data into 4 groups: 1) lead-poisoned (74.5 µg/dL), moderately-exposed (52.8 µg/dL), slightly-exposed (41 µg/dL), and physiologically-exposed (23 µg/dL). The lead-poisoned and moderately-exposed groups had significant decreases in fertility as measured by asthenospermia, hypospermia, and teratospermia (Lancranjan et al., 1975). Effects on sperm were also observed in another group of lead-exposed workers with mean blood-lead levels of 44.6-46.1 µg/dL (and had levels of 50 µg/dL or greater at least once prior to study) (Wildt et al., 1983).

There is evidence that lead and lead compounds can have adverse effects on animal and human reproductive performance. The database of literature is large and contains results that support and do not support the concern that lead and lead compounds are likely to pose a substantial risk to human health due to reproductive toxicity. Although a comprehensive review of the literature is not the basis for the following classification, the DuPont AEL Committee concludes that the studies described above are sufficient to classify lead and lead compounds as *Probable Human Reproductive Toxins*.

Contacts

For more detail on the studies used in the preceding hazard determination or to obtain a copy of the original classification letters dated July 10, 1989 and April 10, 1989; in which lead naphthenate was classified as "c" – Possible Human Carcinogen, and lead and lead compounds were classified as "D" – Potent Developmental Toxin, and "r" – Considered to be a Weak Reproductive Toxin, contact Richard C. Graham (366-5222) at Haskell Laboratory.

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