1981

Cadmium and zinc levels in the hair of smokers and nonsmokers

Neal R. Simonsen

Portland State University

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AN ABSTRACT OF THE THESIS OF Neal R Simonsen for the Master of Science in Biology presented July 31, 1981.

Title: Cadmium and Zinc Levels in the Hair of Smokers and Nonsmokers.

APPROVED BY MEMBERS OF THE THESIS COMMITTEE:

Philip C. Withers, Chairman
Richard R. Petersen
William E. Morton

To determine the relationship of tobacco and marijuana smoking to levels of cadmium and zinc manifested in hair samples, a study was conducted at Portland State University using atomic absorption spectrophotometry. 97 adult student volunteers participated in the main study.

Tobacco smokers had significantly higher hair cadmium concentrations and marijuana smokers had significantly higher zinc concentrations than nonsmokers, confirming tobacco smoking as a significant source of cadmium and implicating marijuana as a significant source of zinc. Higher average concentrations of cadmium among marijuana smokers and zinc among tobacco smokers were also observed but lacked statistical significance. Since cadmium is a toxic metal
with a long biological half-life and a tendency to accumulate in certain target organs, particularly the kidney, the effects of smoking upon the level of long-term cadmium intake may have important consequences for health. In addition, comparative studies of hair metal levels neglecting to take smoking habits into account are suspect at least insofar as cadmium and probably zinc are concerned.

Trends toward increasing cadmium with age and observations of decreased cadmium among individuals with black hair and increased cadmium among individuals doing soldering are in general agreement with previous findings but the lack of significantly higher cadmium levels for males than for females contradicts previous work on Americans. Different proportions of smokers in the subject populations may be involved in this disagreement.

Former smokers had cadmium and zinc concentrations that did not exceed the average concentrations for nonsmokers, indicating that hair cadmium and zinc levels reflect exposure or intake rather than cumulative body burden.
CADMIUM AND ZINC LEVELS IN THE HAIR OF
SMOKERS AND NONSMOKERS

by
Neal R Simonsen

A thesis submitted in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE
in
BIOLOGY

Portland State University
1981
TO THE OFFICE OF GRADUATE STUDIES AND RESEARCH:

The members of the Committee approve the thesis of Neal R Simonsen presented July 31, 1981.

Philip C. Withers, Chairman

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To the members of my thesis committee, for combining their expertise to aid and abet a project that didn't fall neatly into any one individual's field . . .

To Marilyn Petersen and Barbara Stewart, for sharing their experience in questionnaire design and statistical analysis, respectively . . .

And to everyone who volunteered a piece of themselves to make this study possible . . .

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BACKGROUND

In 1817 Friedrich Strohmeyer isolated cadmium, a metallic element with an abundance in the earth's crust of less than one part per million. Although rare in nature, cadmium is usually closely associated with zinc, another member of group II B of the periodic table with which it shares many properties. Unlike zinc, however, cadmium is highly toxic, a fact which was recognized about a century ago and has become more important as increasing refining of zinc and other metal ores and the discovery of an increasing variety of uses for cadmium has led to increased exposure to cadmium in the general as well as the industrial environment (Fasset and Irish 1978). The potential hazards of cadmium exposure to the general population began to draw wider interest after the implication of cadmium in a debilitating syndrome in Japan dubbed Itai-Itai or "ouch-ouch" in recognition of the agony associated with its characteristic bone deformation (osteomalacia) and greatly increased proneness to bone fracture. Consumption of contaminated rice grown in cadmium-rich waste water from a mining operation was cited as the primary cause for this disease (Flick et al. 1969; Nomiyama 1980). Since that time, much progress has been made in the investigation of potential health hazards of acute and chronic cadmium exposure.

The general effects of acute exposure to cadmium are well established: renal damage and dysfunction, osteoporosis, enteropathy and anemia, plus lung damage and dysfunction (i.e. pulmonary edema
and interstitial pneumonitis) if exposure is via inhalation (DHEW 1977; Friberg et al. 1974). The effects of chronic cadmium exposure are likely to be of much more importance to both industrial workers and the general population but are less well established. They may be characterized both by specific effects on normal metabolic pathway functions and more generalized health effects, as will be detailed.

Cadmium was tentatively linked to hypertension when Schroeder and Vinton (1962) described the development of hypertension in rats given small doses of cadmium in drinking water. Hypertension in response to cadmium was substantiated by two later studies (Schroeder 1964a; Schroeder and Buckman 1967a). A potential role of cadmium as a human hypertensive agent was indicated by Schroeder's (1964b) analysis of autopsy data collected by Tipton and Cook (1963) which indicated a presence of high cadmium-to-zinc ratios in cadavers of hypertensive subjects. Direct vasoconstriction and increased cardiac output were suggested as mechanisms for the induction of hypertension by cadmium, based again on experiments with rats (Perry et al. 1967).

But in 1969 Morgan found no elevation of cadmium levels in kidneys or livers of hypertensive subjects and in an epidemiological study (Holden 1969) no increase in the incidence of hypertension was noted for subjects industrially exposed to cadmium. A link between cadmium and cardiovascular disease death rates in the general population was reported by Carroll (1966) with a marked correlation between those death rates and cadmium levels in the air of 28 U.S. cities, and reinforced by Hickey, Schoff and Clelland (1967) with similar findings for cadmium and vanadium levels in the air of 26 cities. This link
was weakened in 1971 when Hunt et al. found no association between cadmium in dustfall and cardiovascular disease rate in 77 cities, and criticized Carroll's study on the grounds that population density correlated more strongly than cadmium level with the death rate. More recently, cadmium in water supplies has been linked to coronary heart disease rates (Bierenbaum 1975), and in turn this link too has been disputed (Sharrett 1977). Animal experiments continue to demonstrate the induction of hypertension by cadmium (Perry et al. 1973; Doyle et al. 1975; Revis 1977; Perry et al. 1980), but the potential role of cadmium in human cardiovascular disease remains unsubstantiated.

The relationship of cadmium to emphysema also is unclear. Although a number of studies have found evidence for an association between chronic cadmium exposure and emphysema (Bonnell 1955; Holden 1965; Lewis et al. 1969), other studies have found no evidence for such an association (Suzuki et al. 1965; Lauwerys et al. 1974).

There is good experimental evidence for a teratogenic role of cadmium (Fern et al. 1969; Schroeder and Mitchener 1971; Chernoff 1973; Earl and Vish 1979) and mixed evidence for carcinogenic (Heath 1962; Gunn et al. 1963; Schroeder et al. 1964c; Gunn et al. 1967; Shimkin et al. 1978) and mutagenic (Epstein et al. 1972; Sissoeff et al. 1976; Mitra and Bernstein 1978) roles in animals, and some epidemiological evidence for the involvement of cadmium in human cancer (Potts 1965; Lemen et al. 1976). The importance of cadmium for the general population in regard to birth defects, mutations and cancer, however, remains to be established.
Cadmium has also been shown to alter the immune response in experimental animals, particularly inhibiting the cellular component (Cook et al. 1975; Loose et al. 1977; Muller et al. 1979); whether this effect has significance for human health is unknown.

Associations of cadmium with some other disease conditions are more straightforward. Changes in renal function of chronically exposed workers have been observed repeatedly since Friberg (1948) found renal damage and proteinuria in workers exposed to cadmium dust. Studies verifying the association of cadmium with renal tubule damage and excretion of low molecular weight proteins (particularly B2 microglobulins) have been widespread (e.g. Clarkson and Kench 1956; Nogawa 1980). Effects of cadmium on bone structure have been found in a number of studies upon chronically exposed workers and experimentally exposed animals. These studies have been extensively reviewed by Friberg et al. (1974) and Nomiyama (1980).

Cadmium metabolism is highly complex. The route of intake is of crucial importance to the impact of cadmium on the body. Cadmium retention is variable but potentially is of a very long-term nature and interactions with other metals and associated systems are myriad and marked (Prasad 1976).

Intraperitoneal and intravenous intake of cadmium are obviously of little significance under most circumstances despite the very high retention rates (over 90%) reported for these routes (Moore et al. 1973). Oral intake of cadmium generally results in absorption of 3-5% (Thomas 1980), although retention rate decreases with increasingly large doses (Moore et al. 1973); retention rates of from 1.5% to 29%
have been observed in humans (Nomiyama 1980). Intake of cadmium via inhalation, on the other hand, usually results in absorption of between 10% and 50% (Friberg et al. 1974). Retention of inhaled cadmium is greatly influenced by the size of particles or form of chemical in which cadmium is inhaled (Nomiyama 1980). Retention of orally ingested cadmium is apparently not influenced by chemical form but is sensitive to dietary and physiological factors, e.g. calcium, protein, Vitamin D and zinc levels (Moore et al. 1973; Nomiyama 1980).

Experiments on animals suggest that cadmium, once absorbed, has a half-life of from 200 days for mice (Durbin et al. 1957) to 2 years for squirrel monkeys (Nordberg et al. 1971), with a tendency toward decreasing rates of decline with increasing time from exposure. Mathematical models based on excretion studies with humans project 10 to 30 year half-lives for whole body cadmium (Friberg et al. 1974). Recently, Travis and Haddock (1980) have suggested that the biological half-life of cadmium is age-dependent, declining from around 34 years at birth to 11 years at age 80 as a result of progressive renal change.

Regardless of the exact biological half-life, cadmium interacts widely with other metal systems. The best known of these interactions involves zinc. Zinc is an essential mineral involved in many vital functions (Underwood 1971; Ochiai 1977). Cadmium has been characterized as an antimetabolite of zinc due to the inverse effect it exerts on such factors as growth, lymphocyte count and hemoglobin levels compared to zinc (Petering et al. 1971a). While cadmium seems to block many of the actions of zinc, the reverse is also true: thus, zinc has been shown to have a prophylactic effect against acute
cadmium toxicity (Gunn et al. 1968). Competition for protein binding sites in mucosal cells and tissues and zinc metalloenzymes is the accepted explanation for this behavior (Underwood 1979).

Cadmium also seems to strongly affect calcium metabolism. It is not clear, however, whether cadmium affects calcium metabolism directly or indirectly: inhibition of 1-25 dihydrocholecalciferol synthesis (Feldman and Cousins 1973) or alkaline phosphatase activity (Ribas-Ozonas 1971) could affect bone by blocking intestinal calcium uptake or bone deposition, for example. Recent studies confirm a decrease in alkaline phosphatase activity in vivo, apparently induced by degeneration of renal cells containing alkaline phosphatase (Peereboom-Stegeman et al. 1979) and indicate a reduction in calcium binding protein in the intestinal mucosa with dietary exposure to cadmium (Fullmer et al. 1980), thus suggesting that cadmium interferes with calcium metabolism at both the intestinal and the kidney level. Cadmium may interfere with nerve transmission by binding to $\text{Ca}^{+2}$ receptor sites and preventing $\text{Ca}^{+2}$ influx from enhancing neurotransmitter release at the presynaptic nerve terminals (Griffiths 1980).

Cadmium appears to also affect copper metabolism by displacing copper from sulfhydryl binding sites on metallothionein, which serves as the major copper-binding protein of the duodenum and liver (Evans et al. 1970), producing decreased plasma, liver and bone copper levels. Cadmium also appears to compete for iron binding sites in the intestinal mucosa, thus bringing about iron-deficiency anemia, while iron in turn produces amelioration of some effects of chronic cadmium
toxicity (Bremner 1974). Finally, selenium, like zinc, has a protective effect against acute cadmium poisoning (Gunn et al. 1968).

One of the keys to the ultimate fate of cadmium in the body is metallothionein, a protein of molecular weight 6,000 to 10,000. Metallothionein appears to be important in zinc and copper storage and regulation. Binding of cadmium to metallothionein, however, forms an exceptionally stable complex and hence metallothionein combines with cadmium preferentially over these metals (Ochiai 1977). Synthesis of metallothionein by kidney, liver and spleen is induced by exposure to cadmium (Cherian and Goyer 1978; Suzuki et al. 1981) and cadmium-thionein complex is stably sequestered after resorption from the glomerular filtrate primarily in the kidney parenchyma (Nordberg 1978). This is thought to be a detoxification mechanism. When levels of cadmium in the cortex of the kidney reach 100 µg/g, however, renal tubular dysfunction may occur, and irreversible renal failure is associated with accumulations of 200 µg/g (Friberg et al. 1974; Cherian and Goyer 1978).

In light of the potential adverse health effects of cadmium, an understanding of the environmental sources of exposure to cadmium is important. The most obvious—and to date the most investigated—sources of cadmium exposure are industrial. In fact, nearly all cadmium exposure is essentially industrial, since cadmium concentrations characteristic of undisturbed nature are very low (except in the rare cadmium sulfide-based mineral greenockite) and relatively immobile, but mining, refining and manufacturing processes provide greatly improved opportunities for accumulation by animals and plants.
alike. Nevertheless, industrial settings offer potentially heavy exposure through welding, soldering, electroplating, pigment-manufacturing, and other processes which may involve the use of cadmium (Hunter 1969). Exposure to air or water-borne cadmium produced as a by-product of the refining of metals, particularly zinc, may affect the general population as well as industrial workers.

The most obvious source of cadmium exposure which is not directly industrial is diet. For the most part measured levels of cadmium in food are quite low—below 0.05 µg/g (Friberg et al. 1974). Conspicuous exceptions are shellfish in general and oysters in particular (.1-7.8 µg/g; Pringle et al. 1968), anchovies and tea (5.39 and 1.38-2.50 µg/g respectively; Schroeder et al. 1967b).

Levels of cadmium reported in drinking water vary greatly. Although "unpolluted" water has cadmium concentrations of less than 1 µg/L (Friberg et al. 1974), the APHA (1976) reports concentrations ranging from 0.4 to 60 µg/L in U.S. drinking water. Altogether, from both indirect calculations and based on analysis of foodstuffs and average consumption (Duggan and Corneliusen 1972) and direct studies of fecal excretion (Tipton and Stewart 1970), it is estimated that total intake of cadmium in areas free of serious pollution will generally be in the range of 40-50 µg/day in the U.S. The World Health Organization has provisionally established tolerable weekly intake of cadmium as 400-500 µg (57-71 µg/day) for a "standard 60 kg man" (Cheftel et al. 1972).

A less obvious non-industrial source of cadmium is tobacco smoking. A 1969 study of 6 brands of cigarettes found that an average
pack of 20 cigarettes contained nearly 23 µg of cadmium with only minor variation between brands (Nandi et al. 1969). Menden et al. (1972) measured the cadmium concentrations in whole cigarettes and in mainstream smoke. They found that of 1.56 to 1.96 µg of cadmium per whole cigarette, 0.10 to 0.12 µg was in particulate form in the fraction of smoke normally inhaled. Based on this data it can be estimated that smoking one pack of cigarettes per day will contribute at least 2 µg to daily cadmium intake. This is not a large contribution to average raw intake, but much higher retention is to be expected because the cadmium is inhaled rather than taken orally. The potential contribution to daily cadmium retention of tobacco smoking is thus quite substantial, being estimated at 1 µg/day for a pack-a-day smoker versus 2.25 µg/day average retention via food (Friberg et al. 1974).

Studies confirming the potential contribution of tobacco smoking to retained cadmium and hence body burden have been conducted on cadavers and, on a small scale, in vivo. In an autopsy study Lewis et al. (1972) found a total burden of 14.2 mg of cadmium for kidney, liver and lung combined in smokers and 6.8 mg in nonsmokers. Ostergaard (1978) reported that renal cadmium concentrations in autopsied samples from cigarette smokers were nearly twice as high as in nonsmokers. Finally, Ellis et al. (1979) determined cadmium levels in the left kidney and liver of 20 male volunteers using partial body neutron activation and found tobacco smokers to have nearly double the levels of nonsmokers in both organs.
INTRODUCTION

A more convenient analytical technique for cadmium other than autopsy or partial body neutron activation would be of obvious value in the investigation of aspects of cadmium metabolism, such as its relationship with smoking, for studies on living subjects. Blood analysis is a fairly common technique for investigating metals, but levels of cadmium in the blood are unfortunately low. The median normal concentration of cadmium is about 0.6 ng/g (Ediger and Coleman 1973) yet there can be enormous fluctuation. Values of less than .5 to 14.2 µg/100 ml have been reported for whole blood in a single study (Kubota et al. 1968), for example. This fluctuation may derive in part from cadmium's rapid turnover rate upon entering the bloodstream. For example, Shaikh and Lucis (1972b) reported that cadmium levels in the plasma of rats declined within 48 hours after subcutaneous administration of $^{109}$Cd to the lower limit of detection. The other common technique for determining metal levels in vivo, urinalysis, also involves low sample concentrations: excretion of cadmium appears to be mainly via the intestinal tract (Shaikh and Lucis 1972a), which accounts for the relatively low urinary levels of cadmium, generally in the range of 1-2 µg/L (Friberg et al. 1974). A less common analytical technique, however, potentially offers specimens with higher normal metal levels and lower concentration transience than either blood or urine analysis. This technique is hair analysis.
Initial interest in analysis of hair for trace metals was primarily for forensic applications. However, Kopito et al. (1967) reported good correlation between hair lead content and clinical findings of lead poisoning in children, and recommended the use of hair analysis as a simple screening procedure. The use of hair analysis in medical and biological applications has since grown (Maugh 1978; Klevay 1978), while frustration over the inability to control for intra-individual variations in the distribution of elements has dulled interest in forensic applications to the point that one worker was moved to subtitle a report evaluating the forensic analysis of hair the "Failure of a mission" (Cornelius 1973).

Hair is a cystine-rich keratinized tissue. The disulfide bonds formed between cystine residues of the helically-coiled polypeptide chains which make up the keratin molecule give it most of its stability (Baden et al. 1973; Hopps 1977). Sulfur groups in general and cystine's in particular are also preferred binding sites for "heavy" trace metals like cadmium (Ochiai 1977); thus the same chemical characteristics which stabilize the keratin of hair result in its having high concentrations of many metals. Another valuable characteristic of hair is that it provides a relatively nonperishable specimen. And, as a constantly forming tissue, it potentially acts as a continual monitor of metal levels over time. A lock of hair thus avoids the transience problems associated with blood or urine specimens, for example, by providing an average reading of levels over a long time period.
The question of the extent to which metal levels in hair reflect actual body metal burdens is debated (e.g. Prasad et al. 1963; Oleru 1975; Maugh 1978; Chittleborough 1980). A fundamental problem is determining just what "body burden" indicators should be compared to hair metal concentrations since concentrations found in the body normally vary from tissue to tissue and hair may be considered a tissue itself. Numerous studies have demonstrated associations between increased or decreased intake, or tissue levels in general, and hair levels, from which many investigators have concluded, as did Hopps (1977), that hair is suitable for evaluating body stores.

Schroeder and Nason (1969) apparently published the first survey of cadmium concentrations in human hair. In a study of 117 males and 47 females using atomic absorption spectrophotometry they found average levels of 2.76 µg/g for men and 1.77 µg/g for women, with lower levels of cadmium for older than for younger women. They also studied zinc, and found essentially identical average levels of 167 and 172 µg/g for men and women, respectively, with no significant difference with age. Another 1969 study, using spark source mass spectrometry (Yurachek et al.), found cadmium concentrations in hair ranging from 0.34 to 1.8 µg/g but apparently involved only a few subjects; values of 143 to 246 µg/g were found for zinc. Values of cadmium and zinc levels published in these and subsequent studies are summarized in Appendix A.

Studies of hair cadmium directed to more specific purposes followed these initial works. Mean hair levels of cadmium, as well as lead, accurately reflected community exposure in investigations by
Hammer et al. (1971), results which were mirrored by investigations of cadmium, mercury and lead by Chattopadhyay et al. (1977). Zinc levels did not reflect exposure and this was attributed to relatively small differences in total zinc exposure between groups, due in part to the large normal dietary zinc component, and the possession of more effective homeostatic mechanisms for zinc than for cadmium. Eads and Lambdin (1973) report a decline in cadmium and zinc in the hair of females in the age range from 32 to 72 years and higher zinc levels in darker colored hair for both sexes which, they concluded, implicated zinc in the production of melanin involved in hair pigmentation. Oleru (1976) compared cadmium levels in kidney, liver, hair and lungs for 50 autopsied New Yorkers and found significant positive correlations between all 4 tissues, with a particularly strong correlation between kidney and hair (0.52). Oleru also reported a general increase in hair and kidney cadmium levels with age. Finally, in experimental studies on laboratory animals, Kollmer and Berg (1979) found the amount of cadmium detected in hair as well as total body, various tissues, and blood samples to be proportional to doses of cadmium administered intravenously.

Hair studies of only zinc had preceded those including only cadmium or both elements. Bate and Dyer (1965), Perkons and Jervis (1966), and Harrison et al. (1969) surveyed hair for zinc and other metals. In 1966 Strain et al. found good correlations between low zinc levels in hair and zinc deficiency syndromes. Petering et al. (1971b) found a close relationship between decreasing hair zinc concentration and age for males over 15 and for females, with no significant
difference in levels between the sexes. A study by Obrusnik et al. (1973) revealed a slight decrease in concentration of zinc with distance from the scalp, contrasting with a previous study (1972) by the same group which had found no such decrease, but another study (Rendic et al. 1976) found zinc concentrations essentially constant along the length of hair from 0 to 30 cms distal. Experimental studies by Deeming and Weber (1977) found good correlations between dietary zinc intake and hair zinc levels in rats. Finally, McKenzie (1979) reported no significant differences between hair zinc levels in oyster openers or industrial workers and unexposed individuals with the exception of galvanizers, but there was a significant difference between levels in the sexes (195 µg/g for females vs 180 µg/g for males).

Despite the discovery of substantial amounts of cadmium in tobacco and its potential contribution to cadmium exposure when smoked, no previous studies have examined the possible contribution of marijuana smoking to cadmium exposure. Nor were there any studies exploring the relationship between cadmium and zinc levels in human smokers and nonsmokers or even the impact of smoking on hair metal levels in evidence in the literature. Thus, this study investigates the relationship of cadmium and zinc levels in hair to smoking habits, including both tobacco and marijuana use.
MATERIALS AND METHODS

The research project entailed two separate studies, an initial small scale pilot study being carried out six months prior to the main study. Particulars in which the pilot study differed from the main study are indicated following the general descriptions.

Subjects and Sample Collection

Volunteer subjects were recruited from students enrolled in biology courses at Portland State University. The volunteers completed an informed consent form and a separate anonymous questionnaire (identified only by a code number) covering tobacco and marijuana smoking habits, and a variety of other factors which potentially affect hair trace metal levels. The questionnaire is reprinted in Appendix B.

Hair samples were taken from the occipital region of the scalp within 24 hours after the hair had last been washed and were stored in clean plastic bags. Only the portion of the hair strand within 6 cm of the scalp was used in the subsequent analysis.

In the pilot study no restriction was placed on the distance of analyzed hair sections from the scalp.

Laboratory Reagents and Cleansing Protocol

All chemicals used were of A.C.S. reagent grade. All distilled water used was deionized by passage through an ion exchange column. Stock standards for atomic absorption spectroscopy were prepared in the lab by dissolving cadmium (Cd) and zinc (Zn) metal in acid; fresh
working standards were made prior to spectroscopic analysis of samples. Standards were made in 0.4 M HNO₃ to minimize loss of cadmium from solution through adsorption onto the container walls.

All glassware (pyrex) used in sample pretreatment and digestion and plasticware (linear polyethylene) used in sample storage was subjected to a stringent cleansing procedure entailing four major steps, each step followed by rinsing with deionized distilled water. These steps consisted of: 1) Washing and scrubbing with hot tap water; 2) Soaking at least 12 hours in nonionic detergent solution prepared with distilled water; 3) Soaking in 2 M HCl; and 4) Letting stand for 2 hours filled with 3 M HNO₃.

Sample Pre-treatment

Samples of 0.2 to 0.6 gram of hair were placed in 50ml beakers in which they were rinsed twice with distilled water and then, after addition of about 35ml of distilled water, placed on a mechanical agitator for 40 minutes. Following agitation the washwater was poured off and the samples rinsed three times with distilled water and placed in a drying oven at 110°C overnight. Samples were stored in a desiccator after drying until ready for use. Duplicates were prepared for hair samples of one gram or more.

In the pilot study a 30 minute nonionic detergent wash was used instead of the 40 minute water wash.

Sample Digestion and Analysis

Washed, dried hair samples of from 0.2 to 0.6 g were weighed to ± 1/10 mg into 25ml Erlenmeyer flasks. After addition of 6ml
5:1 HNO₃:H₂O samples were covered with watchglasses and allowed to sit overnight. The flasks were then placed on hot plates where after 50 minutes of gentle heating they were cooled for 10 minutes, the watchglasses were removed, 4ml of conc. HNO₃ was added, and heating was continued for about 3 hours. At this point the samples were allowed to cool for 15 minutes, 2ml of 1:2 HClO₄:HNO₃ was added and gradual heating to a gentle boil (c. 110°C) continued for another hour and 40 minutes, when they were again allowed to cool for 15 minutes and then transferred to 10ml beakers. The samples were then heated until a clear solution was obtained and just enough solution remained to cover the bottom of the beakers, HNO₃ being added dropwise whenever a solution darkened, and then allowed to cool. When cool, samples were transferred to 5ml volumetric flasks and 1:20 HNO₃:H₂O was added to bring the solutions to 5ml. These solutions were then stored in 5ml polyethylene bottles prior to analysis.

Reagent blanks were treated with the same procedure for each batch of hair samples.

Digested hair sample solutions and standards were analyzed for Cd by direct aspiration into an IL 551 atomic absorption spectrophotometer in flame mode under standard conditions (as per IL 551 Operator's Manual). Replicate determinations of Cd were made for each sample, and the order in which the samples were analyzed was reversed for the second determination. Accurate determination of Zn required dilution of the original sample solution. Thus, remaining volume permitting, samples were then diluted ten-fold by pipetting 1.00ml of sample into a 10ml volumetric flask, diluting to volume with
deionized water and transferring the diluted solution to a 20ml polyethylene bottle. These solutions were then analyzed for Zn using the same general methodology as for Cd.

In the pilot study hair samples were weighed out onto weighing paper which was then folded into packets and samples were gradually added to the digestion medium as digestion proceeded. No HClO₄ was used in the pilot study, nor was the initial digestion carried out in a 25ml Erlenmeyer. Instead all digestion was carried out in 10ml beakers, using HNO₃ exclusively.
RESULTS

The pilot study included a total of 37 subjects, all of whom were tested for hair cadmium. Table I summarizes the data obtained for cadmium levels in the hair of the group as a whole and of subdivisions based on sex and smoking habits. The main study included a further 97 subjects, all of whom were tested for cadmium and 72 of whom were tested for zinc. On the average, 40-45\% of the individuals in classes recruited volunteered in the main study. Concentrations ranged from 0.28 to 3.58 µg/g for cadmium and from 99 to 300 µg/g for zinc. Tables II and III summarize the data obtained for cadmium and zinc, respectively. Individual values for cadmium and zinc by smoking habits and sex are presented in Appendices C and D.

In both studies the average cadmium level in the hair of current tobacco smokers (1.35 and 0.97 µg/g for the pilot and main study, respectively) exceeded that of nonsmokers (0.69 and 0.71 µg/g for pilot and main study). This difference was statistically significant in both studies (P=.031 for main and .048 for pilot study). Since Bartlett's Test for homogeneity of variance indicated that the cadmium data summarized in Tables I and II deviated significantly from homogeneity unless it was logarithmically transformed, an additional ANOVA was performed on log transformed data. This analysis, too, yielded statistically significant results for the main study (P=.036), but results for the pilot study exceeded the limit of statistical significance after transformation (P=.080). In addition, the average
### TABLE I

**COMPARISON OF HAIR CADMIUM LEVELS AND CURRENT SMOKING HABITS--PILOT STUDY**

(Values are Concentrations in µg/g: $\bar{x} \pm SE (n)$)

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Males</th>
<th>Females</th>
<th>Significant inter-column differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Current tobacco smokers</td>
<td>1.35 ± .22 (8)</td>
<td>1.60 ± .28 (5)</td>
<td>0.94 ± .36 (3)</td>
<td></td>
</tr>
<tr>
<td>2 Current marijuana (but not tobacco) smokers</td>
<td>0.85 ± .22 (8)</td>
<td>0.74 ± .44 (2)</td>
<td>0.89 ± .25 (6)</td>
<td></td>
</tr>
<tr>
<td>3 Not current smokers</td>
<td>0.69 ± .14 (21)</td>
<td>0.64 ± .24 (7)</td>
<td>0.72 ± .17 (14)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.86 (37)</td>
<td>0.99 ± .17 (14)</td>
<td>0.79 ± .13 (23)</td>
<td></td>
</tr>
</tbody>
</table>

Significant inter-row differences:

- 1 vs. 3 $P=0.048$ $P=0.021$
- (.080) (.079)

---

\(^{a}\) $\bar{x}$ = average; SE = standard error of the mean; n = number of subjects

\(^{b}\) Significance levels in parentheses are for log transformed data
# TABLE II

COMPARISON OF HAIR CADMIUM LEVELS AND CURRENT SMOKING HABITS--MAIN STUDY
(Values are Concentrations in µg/g; \( \bar{x} \pm SE (n) \))

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Males</th>
<th>Females</th>
<th>Significant inter-column differences</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \bar{x} \pm SE (n) )</td>
<td>( \bar{x} \pm SE (n) )</td>
<td>( \bar{x} \pm SE (n) )</td>
<td></td>
</tr>
<tr>
<td>1 Current tobacco smokers</td>
<td>0.97 ± .11 (16)</td>
<td>1.12 ± .15 (8)</td>
<td>0.82 ± .15 (8)</td>
<td></td>
</tr>
<tr>
<td>2 Current marijuana (but not tobacco) smokers</td>
<td>0.78 ± .13 (10)</td>
<td>0.83 ± .21 (4)</td>
<td>0.75 ± .17 (6)</td>
<td></td>
</tr>
<tr>
<td>3 Not current smokers</td>
<td>0.71 ± .05 (71)</td>
<td>0.67 ± .08 (30)</td>
<td>0.74 ± .07 (41)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.76 (97)</td>
<td>0.77 ± .06 (42)</td>
<td>0.75 ± .06 (55)</td>
<td></td>
</tr>
</tbody>
</table>

Significant inter-row differences:
1 vs. 3 \( P=.031 \) \( (.036) \)
1 vs. 3 \( P=.018 \) \( (.045) \)

---

\( \bar{x} \) = average; \( SE \) = standard error of the mean; \( n \) = number of subjects

\( b \) Significance levels in parentheses are for log transformed data
TABLE III

COMPARISON OF HAIR ZINC LEVELS AND CURRENT SMOKING HABITS
(Values are Concentrations in µg/g: X ± SE (n))a

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Males</th>
<th>Females</th>
<th>Significant inter-column differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>1   Current tobacco smokers</td>
<td>204 ± 15 (14)</td>
<td>195 ± 21 (7)</td>
<td>212 ± 21 (7)</td>
<td></td>
</tr>
<tr>
<td>2   Current marijuana (but not tobacco) smokers</td>
<td>237 ± 32 (6)</td>
<td>230 ± 32 (3)</td>
<td>245 ± 32 (3)</td>
<td></td>
</tr>
<tr>
<td>3   Not current smokers</td>
<td>180 ± 8 (52)</td>
<td>164 ± 12 (22)</td>
<td>192 ± 10 (30)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>189 (72)</td>
<td>177 ± 10 (32)</td>
<td>199 ± 9 (40)</td>
<td>P = .080</td>
</tr>
</tbody>
</table>

Significant inter-row differences

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2 vs. 3</td>
<td>P = .019</td>
</tr>
</tbody>
</table>

a x = average; SE = standard error of the mean; n = number of subjects
cadmium level observed for current marijuana smokers who were not also current tobacco smokers (0.79 and 0.78 µg/g for pilot and main study, respectively) exceeded that of nonsmokers but not significantly so. These relationships held for the group as a whole and for each sex individually. (Tables I and II)

Average hair zinc levels for current tobacco smokers (204 µg/g) also exceeded those for nonsmokers (180 µg/g) but not significantly so. However, average zinc levels for current marijuana-but-not-tobacco smokers (237 µg/g) did significantly exceed (P=.019) those for nonsmokers and were also observed to exceed those for current tobacco smokers. This relationship, too, held for the group as a whole and for both sexes individually. (Table III)

In the pilot study, average cadmium levels for males exceeded those for females but average levels for nonsmoking males were lower than those for nonsmoking females; neither difference was, however, significant. In the main study observed average cadmium levels for males were essentially the same as those for females for both smokers and nonsmokers. Zinc levels, on the other hand, appeared higher for females than for males regardless of smoking habits (199 vs. 177 µg/g, P=.080). (Table III)

An alternative analysis of the data on smoking habits and hair cadmium concentration is summarized in Table IV. The average hair Cd level observed for heavy (tobacco) smokers (1.06 µg/g) exceeded that for nonsmokers (P=.014), as did the average level for light (tobacco and/or marijuana) smokers (0.79 µg/g; P=.430). In addition,
## TABLE IV

COMPARISON OF HAIR CADMIUM LEVELS AND CURRENT SMOKING STATUS--
ALTERNATE GROUPING, MAIN STUDY
(Values are Concentrations in µg/g: $\bar{x} \pm SE (n)$)

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Males</th>
<th>Females</th>
<th>Significant inter-column differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Heavy tobacco smokers&lt;sup&gt;b&lt;/sup&gt;</td>
<td>$1.06 \pm .13 (10)$</td>
<td>$1.40 \pm .18 (5)$</td>
<td>$0.72 \pm .18 (5)$</td>
<td></td>
</tr>
<tr>
<td>2 Light tobacco and/or marijuana smokers&lt;sup&gt;c&lt;/sup&gt;</td>
<td>$0.79 \pm .10 (16)$</td>
<td>$0.75 \pm .16 (7)$</td>
<td>$0.83 \pm .14 (9)$</td>
<td></td>
</tr>
<tr>
<td>3 Nonsmokers</td>
<td>$0.71 \pm .05 (71)$</td>
<td>$0.67 \pm .08 (30)$</td>
<td>$0.74 \pm .06 (41)$</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> $\bar{x}$ = average; SE = standard error of the mean; $n$ = number of subjects

<sup>b</sup> Over 1 pack per day

<sup>c</sup> 1/3 to 1 pack per day for tobacco or at least 4 days per month for marijuana
the average Cd level observed for heavy smokers exceeded that for light smokers (P=.110).

A comparison of current and former tobacco smokers is presented in Table V. Both average hair cadmium and average hair zinc were lower but not significantly so for former tobacco smokers (0.61 and 161 µg/g) than for current tobacco smokers or for nonsmokers (0.73 and 183 µg/g).

The results for relationship between age and hair cadmium are summarized in Table VI and Figure 1. Cadmium concentration tended to increase with age, although for females the relationship was relatively inconsistent and broke down after age 30. Zinc concentration showed no discernible correlation with age (see Table VI).

The relationship between body weight and hair cadmium concentration was also examined but no apparent correlation was found. Figure 2 summarizes these data.

Possible correlation of hair color, soldering, recent flu and oral contraceptive use with hair metal levels was examined using the cadmium and zinc measurements and the questionnaire responses. The results are summarized in Table VII. Black hair had a marginally significant elevation in zinc level (P=.050). Observed but not statistically significant associations were noted between black hair, recent flu, and oral contraceptive use and lower than average Cd levels whereas soldering was associated with higher than average levels; recent flu and oral contraceptive use were also associated with lower than average zinc levels.
TABLE V

COMPARISON OF HAIR CADMIUM AND ZINC LEVELS FOR CURRENT, FORMER AND NON-SMOKERS
(Values are Concentrations in µg/g: \( \bar{x} \pm SE \) (n))

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \bar{x} \pm SE ) (n)</td>
<td>( \bar{x} \pm SE ) (n)</td>
<td>( \bar{x} \pm SE ) (n)</td>
</tr>
<tr>
<td>Cd</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Current tobacco smokers</td>
<td>0.97 ± .11 (16)</td>
<td>1.12 ± .16 (8)</td>
<td>0.82 ± .16 (8)</td>
</tr>
<tr>
<td>2 Former tobacco smokers</td>
<td>0.61 ± .13 (11)</td>
<td>0.53 ± .18 (5)</td>
<td>0.70 ± .20 (5)</td>
</tr>
<tr>
<td>3 Not current tobacco or marijuana smokers</td>
<td>0.73 ± .06 (60)</td>
<td>0.71 ± .09 (24)</td>
<td>0.74 ± .07 (36)</td>
</tr>
<tr>
<td></td>
<td>0.76 (97)</td>
<td>0.77 ± .07 (38)</td>
<td>0.75 ± .06 (49)</td>
</tr>
<tr>
<td>Significant inter-row differences</td>
<td>1 vs. 2</td>
<td>P=.042</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 vs. 3</td>
<td>P=.059</td>
</tr>
<tr>
<td>Zn</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Current tobacco smokers</td>
<td>204 ± 14 (14)</td>
<td>195 ± 20 (7)</td>
<td>212 ± 20 (7)</td>
</tr>
<tr>
<td>2 Former tobacco smokers</td>
<td>161 ± 20 (7)</td>
<td>158 ± 27 (4)</td>
<td>166 ± 31 (3)</td>
</tr>
<tr>
<td>3 Not current tobacco or marijuana smokers</td>
<td>183 ± 8 (45)</td>
<td>165 ± 13 (18)</td>
<td>194 ± 10 (27)</td>
</tr>
<tr>
<td></td>
<td>185 (66)</td>
<td>171 ± 10 (29)</td>
<td>195 ± 9 (37)</td>
</tr>
<tr>
<td>Significant inter-row differences</td>
<td>1 vs. 2</td>
<td>P=.09</td>
<td></td>
</tr>
</tbody>
</table>

\( \bar{x} \) = average; SE = standard error of the mean; n = number of subjects
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17-20</td>
<td>0.66(6)³</td>
<td>0.73(13)</td>
<td>1.52(1)</td>
<td>0.49(5)</td>
<td>1.38(1)</td>
<td>0.68(12)</td>
</tr>
<tr>
<td>21-25</td>
<td>0.68(15)</td>
<td>.71(18)</td>
<td>.69(4)</td>
<td>.68(11)</td>
<td>.75(4)</td>
<td>.70(14)</td>
</tr>
<tr>
<td>26-30</td>
<td>.74(9)</td>
<td>.83(14)</td>
<td>—</td>
<td>.74(9)</td>
<td>.65(3)</td>
<td>.88(11)</td>
</tr>
<tr>
<td>31-39</td>
<td>.79(5)</td>
<td>.61(6)</td>
<td>.52(1)</td>
<td>.85(4)</td>
<td>.33(1)</td>
<td>.67(5)</td>
</tr>
<tr>
<td>40-49</td>
<td>.99(1)</td>
<td>—</td>
<td>—</td>
<td>.99(1)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>50+</td>
<td>—</td>
<td>1.02(2)</td>
<td>—</td>
<td>—</td>
<td>.80(1)</td>
<td>1.25(1)</td>
</tr>
<tr>
<td>Zn</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17-20</td>
<td>217(5)</td>
<td>193(11)</td>
<td>300(1)</td>
<td>197(4)</td>
<td>242(1)</td>
<td>188(10)</td>
</tr>
<tr>
<td>21-25</td>
<td>182(11)</td>
<td>209(11)</td>
<td>161(4)</td>
<td>194(7)</td>
<td>215(3)</td>
<td>207(8)</td>
</tr>
<tr>
<td>26-30</td>
<td>158(7)</td>
<td>199(11)</td>
<td>—</td>
<td>158(7)</td>
<td>230(3)</td>
<td>188(8)</td>
</tr>
<tr>
<td>31-39</td>
<td>173(4)</td>
<td>188(3)</td>
<td>184(1)</td>
<td>169(3)</td>
<td>115(1)</td>
<td>224(2)</td>
</tr>
<tr>
<td>40-49</td>
<td>166(1)</td>
<td>—</td>
<td>—</td>
<td>166(1)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>50+</td>
<td>—</td>
<td>234(2)</td>
<td>—</td>
<td>—</td>
<td>279(1)</td>
<td>190(1)</td>
</tr>
</tbody>
</table>

³ Numbers in parentheses are n values for corresponding concentrations.
Figure 1. Cadmium concentration in hair (µg/g) as a function of age (years) for male and female nonsmokers.
Figure 2. Cadmium concentration in hair (µg/g) as a function of body weight (lbs) for male and female nonsmokers.
TABLE VII
DIFFERENCES IN AVERAGE HAIR CADMIUM AND ZINC LEVELS (µg/g) ASSOCIATED WITH MISCELLANEOUS FACTORS

<table>
<thead>
<tr>
<th>Factor</th>
<th>Subjects</th>
<th>X Cd</th>
<th>(n)</th>
<th>X Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black hair</td>
<td>7 black-haired male smokers</td>
<td>.542 ± .15</td>
<td>(4)</td>
<td>200 ± 19</td>
</tr>
<tr>
<td></td>
<td>17 male nonsmokers</td>
<td>.777 ± .09</td>
<td>(14)</td>
<td>155 ± 10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soldering</td>
<td>5 solderers</td>
<td>.833 ± .19</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>92 non-solderers</td>
<td>.744 ± .05</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral contraceptives</td>
<td>6 oral. contra. users</td>
<td>.578 ± .13</td>
<td>(5)</td>
<td>163 ± 26</td>
</tr>
<tr>
<td></td>
<td>35 nonusers</td>
<td>.763 ± .05</td>
<td>(25)</td>
<td>197 ± 12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recent flu</td>
<td>3 males w/rec. flu</td>
<td>.465 ± .32</td>
<td>(3)</td>
<td>139 ± 28</td>
</tr>
<tr>
<td></td>
<td>39 males w/o rec. flu</td>
<td>.795 ± .09</td>
<td>(29)</td>
<td>181 ± 9</td>
</tr>
</tbody>
</table>

Note: solderers include 2 individuals with black hair and 1 with recent flu.

Note: all users/nonusers compared for Zn are nonsmokers

Note: Males w/recent flu include 1 solderer, 1 with black hair, and 1 curr. tob. and mar. smoker

\(^a\) P value in parenthesis is for log transformed data
DISCUSSION

The results of this study indicate that current tobacco smoking is associated with elevated hair cadmium levels compared to nonsmoking (ANOVA, P=.031). This observed elevation in hair cadmium levels is consistent with the observation of higher cadmium body burdens for tobacco smokers after autopsy (Lewis et al. 1972; Ostergaard 1978) and in vivo (Ellis et al. 1979) studies of cadmium load, and with the significant cadmium content of tobacco (1.56-1.96 µg/cigarette; Menden et al. 1972). These data support the hypothesis that intake of cadmium through tobacco smoking is reflected by higher cadmium levels in hair as well as higher total body cadmium burden.

Although elevation of mean hair cadmium concentration in current marijuana smokers was observed in both the main and pilot studies and for both sexes, the relationship was not statistically significant. Thus, although the data is consistent with the hypothesis that marijuana smoking, too, contributes to cadmium exposure, the results are inconclusive.

The observation of a smaller elevation of concentrations of cadmium in the hair of marijuana smokers than of tobacco smokers relative to nonsmokers is, however, consistent with the idea that a smoker's average total consumption of marijuana is likely to be much lower than that of tobacco. This is prompted by differences in styles of usage, price, ease of obtainment and frequency of circumstances where use is unacceptable (due to legal and/or social
pressures), all of which would presumably favor consumption of lower quantities of marijuana than tobacco. This idea is borne out by the observation of higher average hair cadmium concentrations in current heavy tobacco smokers (1.06 µg/g) than in marijuana-light tobacco smokers pooled (0.79 µg/g), while the average for this pooled group is essentially the same as that for current regular marijuana-but-not-tobacco smokers alone (0.78 µg/g), and is also higher than that for nonsmokers (0.71 µg/g) but not significantly so. (Table IV)

The average hair cadmium values obtained in this study are lower than those obtained by Schroeder (1969), Eads and Lambdin (1973), Petering et al. (1973), and Chattopadhyay et al. (1977) for urban adults. The subjects of the present study were, however, relatively young (average age under 26 years) and included a relatively small proportion of smokers, both factors which, as previous and ensuing discussion indicates, lower average cadmium concentrations should be associated with. This study's values are in general agreement with the work of Yurachek et al. (1969), and the values of Chattopadhyay et al. (1977) for adults in nonindustrial settings. (Appendix A)

The elevation of hair zinc levels in tobacco smokers was initially somewhat surprising. The competition for binding sites on proteins that is associated with the metabolic antagonism of cadmium and zinc suggested that zinc levels and overall zinc-cadmium ratios might be lower in smokers. However, zinc normally was present in hair at concentrations approximately 200-fold that of cadmium. Thus, indications of any simple displacement or replacement phenomenon
of zinc by cadmium would be obscured by variation in zinc levels. Furthermore, it is known that cadmium and zinc tend to be found in close association in nature and that zinc is an essential nutrient for plants in general (Epstein 1972; Gauch 1972) and tobacco in particular (Hoagland et al. 1936), so it would not be surprising if tobacco contained substantial amounts of zinc as well as containing cadmium. The results, then, indicate that tobacco smoking may be a source of exposure to zinc as well as cadmium, but again lack sufficient statistical significance (P=.16) to be conclusive.

On the other hand, the results for zinc levels in current marijuana smokers and nonsmokers are statistically significant (P=.019) in their indication of an association between marijuana smoking and elevated hair zinc levels. The explanation for this result may be generally the same as that cited in the case of tobacco smokers. Metabolic alteration by some inhaled component of the smoking material is another possibility, but there are no data pertaining to trace metal constituents of marijuana smoke and its effects on metal metabolism.

The overall results of this study also reflect upon the usefulness of hair analysis as an indicator of body metal burden. If tobacco smoking does serve as a source of cadmium, as previous studies and the elevation of hair cadmium associated with current tobacco smoking habits observed in this study suggest, and if hair metal levels do reflect body metal burden, then former tobacco smokers as well as current smokers should show higher hair cadmium concentrations than nonsmokers. In fact, former smokers do not show a higher average
hair cadmium concentration than nonsmokers. (Only two of the twelve former heavy tobacco smokers showed levels above the average for nonsmokers.) This suggests that hair cadmium levels reflect recent exposure rather than total body burden.

Average cadmium (and zinc) levels observed for former heavy tobacco smokers are actually lower than those observed for nonsmokers, but are not significantly different statistically (P=.43 for males and .48 for females for Cd., .52 and .21 for Zn). The possibility of an increase in metallothionein activity in response to cadmium exposure via tobacco smoking which carries over after smoking ceases, resulting in more effective sequestration of cadmium in internal organs at the expense of hair, is an interesting but purely speculative interpretation of these observations.

Since zinc is an essential metal for humans and has effective homeostatic regulation (Underwood 1971), body burden of zinc is not expected to increase significantly unless intake is substantially elevated. Thus the appearance of elevated zinc levels in the hair of tobacco smokers appears anomalous unless the hair is viewed as serving in the role of an excretory pathway to some extent. This suggests that in the case of zinc, too, hair may reflect exposure (an increase in which, results in increased excretion) rather than body burden.

Average cadmium levels were higher for all males than for all females in the pilot study but were essentially the same for all individuals of both sexes in the main study. Although this at first appears inconsistent, it is notable that in the pilot study the
fraction of males and females who were current tobacco smokers was 5/14 and 3/23, respectively, whereas in the main study the fraction was 8/42 and 8/55. Thus the proportion of male current tobacco smokers decreased by almost half between the two studies while the proportion of female current tobacco smokers remained virtually unchanged. Also notable is the fact that observed average levels for male nonsmokers were actually lower--though not statistically so--than those for female nonsmokers in both studies. The results of this study regarding difference in hair cadmium between the sexes are more in agreement with Petering et al. (1973) than the classical results compiled by Schroeder and Nason (1969) or those reported by Eads and Lambdin (1973). Part of the discrepancy may arise from the smoking variable, e.g. the proportion of the general U.S. population who smoke is around 40% for ages 15-50 (DHEW 1979) while the proportion of this project's main study population who smoked was under 20%.

In addition, Schroeder and Nason's and/or Eads and Lambdin's study population may have contained an abnormally high proportion of male smokers--and in any case more U.S. males smoked relative to females at the time of the Schroeder study.

Hair zinc levels were lower for males than for females. This may indicate a characteristic sex difference, for although not statistically significant (P=.080) the average levels of 177 µg/g and 199 µg/g for males and females, respectively, are in excellent agreement with the work of McKenzie (1979), who found mean concentrations of 180 for males and 195 for females in a group of New Zealand students ranging from 18 to 27 years of age. Klevay (1970) also found
significantly higher hair levels in females than males, and it is notable that the 4 other studies which have compared concentrations for both sexes, while reporting no significant difference between the sexes, all observed higher average concentrations in females than in males (Reinhold et al. 1966; Schroeder and Nason 1969; Petering et al. 1971b; Eads and Lambdin 1973; also see Appendix A).

The trend toward increasing cadmium with increasing age is also in general agreement with previous studies such as Eads and Lambdin's (1973) and Oleru's (1976). For the women, however, the not-very-marked increase in the under 30 year age range and the irregularity of results for the post-30 year range contradicts Petering et al.'s 1973 study's findings of a steady increase up to a peak between ages 40 and 50 and, for the men, the general increase with age also contradicts that study's findings of a levelling off around age 20. The larger sample size of the Petering study (95 males and 83 females) and its much broader age distribution caution against acceptance of this project's indications regarding age and male hair cadmium levels.

Although it seemed probable that a general increase in food intake would also serve to increase cadmium intake and thereby increase hair levels, the lack of apparent correlation between weight and hair cadmium level does not support this hypothesis. Perhaps a more direct estimate of food intake such as daily calorie intake or body fat test would be more successful in this regard.

Finally, the findings for the influence of miscellaneous factors on hair metal level, although in most cases involving too few samples to be of statistical significance, are nonetheless worthy of comment.
The lower average cadmium concentration for black haired males is in agreement with previous findings (Schroeder and Nason 1969); the exact cause for this relationship is unknown but apparently involves a difference(s) in structural makeup related to hair pigmentation. The significantly higher zinc concentration for black haired males is likewise in agreement with previous findings (Eads and Lambdin 1973). The higher cadmium among individuals who solder is consistent with the high cadmium content of most solder (Fasset and Irish 1978) and the observation of elevated blood and urine cadmium concentrations in solderers (Welinder et al. 1977). No previous studies dealing with the effect of oral contraceptive use or recent flu on hair cadmium and zinc are known. Thus the finding that both seem to depress hair cadmium and zinc levels cannot be compared to any previous findings and must await verification before being considered significant. Still, oral contraceptives are known to alter trace metal metabolism, including lowering serum and plasma zinc concentrations (Smith and Brown 1976). And it is not unreasonable that a bout of flu might put a heavy demand on zinc since zinc is involved in the immune system, thus resulting in a decrease of zinc levels in hair formed during the course of illness, although this does not explain the concurrent depression in cadmium level.
LITERATURE CITED


Obrusnik, I., et al. 1972. The variation of trace element concentra­

________. 1973. The variation of trace element concen­
trations in single human head hairs. J. Radioanal. Chem. 15: 115-133.


APPENDICES
### APPENDIX A

#### PREVIOUS STUDIES ON Cd AND Zn IN HUMAN HAIR

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Concentration&lt;sup&gt;1&lt;/sup&gt; (µg/g)</th>
<th>Analysis Technique&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd ? Americans</td>
<td>1.30&lt;sup&gt;1&lt;/sup&gt; (0.25--2.7)</td>
<td>PAA</td>
<td>Yurachek et al. 1969</td>
</tr>
<tr>
<td>66 rural Canadians</td>
<td>2.00&lt;sup&gt;1&lt;/sup&gt; (0.32--3.4)</td>
<td>&quot;</td>
<td>Chattopadhyay et al. 1977</td>
</tr>
<tr>
<td>45 urban Canadians</td>
<td>2.2 ± 0.2</td>
<td>AAS</td>
<td>Petering et al. 1973</td>
</tr>
<tr>
<td>82 U.S. males</td>
<td>2.76 ± 0.483</td>
<td>&quot;</td>
<td>Schroeder 1969</td>
</tr>
<tr>
<td>18 Port Arthur, TX males</td>
<td>2.2 (0.1--9.3)</td>
<td>&quot;</td>
<td>Eads et al. 1973</td>
</tr>
<tr>
<td>95 Cincinnati, OH males</td>
<td>2.2 ± 2</td>
<td>&quot;</td>
<td>Petering et al. 1973</td>
</tr>
<tr>
<td>47 U.S. females</td>
<td>1.77 ± 0.239</td>
<td>&quot;</td>
<td>Schroeder 1969</td>
</tr>
<tr>
<td>21 Port Arthur, TX females</td>
<td>1.0 (0.2--3.6)</td>
<td>&quot;</td>
<td>Eads et al. 1973</td>
</tr>
<tr>
<td>83 Cincinnati, OH females</td>
<td>2.43 ± 0.26</td>
<td>&quot;</td>
<td>Petering et al. 1973</td>
</tr>
<tr>
<td>Zn ? Oak Ridge, Tennesseeans</td>
<td>177 ± 73.2&lt;sup&gt;3&lt;/sup&gt;</td>
<td>NAA</td>
<td>Bate &amp; Dyer 1975</td>
</tr>
<tr>
<td>Canadian city</td>
<td>300--1000</td>
<td>&quot;</td>
<td>Perkins &amp; Jervis 1966</td>
</tr>
<tr>
<td>12 Egyptians</td>
<td>103 ± 4.9</td>
<td>AES</td>
<td>Strain et al. 1966</td>
</tr>
<tr>
<td>31 U.S. subjects</td>
<td>143--246</td>
<td>SSMS</td>
<td>Yurachek et al. 1969</td>
</tr>
<tr>
<td>75 Iranian children aged 6-12</td>
<td>199 ± 22&lt;sup&gt;4&lt;/sup&gt;</td>
<td>AAS</td>
<td>McBean et al. 1971</td>
</tr>
<tr>
<td>4 Egyptian males</td>
<td>105--136</td>
<td>&quot;</td>
<td>Presad et al. 1963</td>
</tr>
<tr>
<td>33 Hastings, N.Z. schoolboys</td>
<td>126 ± 14&lt;sup&gt;4&lt;/sup&gt;</td>
<td>&quot;</td>
<td>Bate &amp; Dyer 1965</td>
</tr>
<tr>
<td>33 Napier, N.Z. schoolboys</td>
<td>132 ± 15&lt;sup&gt;4&lt;/sup&gt;</td>
<td>&quot;</td>
<td>Bate &amp; Dyer 1965</td>
</tr>
<tr>
<td>20 Iranian male students</td>
<td>181 ± 36&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Z/D</td>
<td>Reinhold et al. 1966</td>
</tr>
<tr>
<td>6 New York male adults</td>
<td>119.6 ± 4.6</td>
<td>AES</td>
<td>Strain et al. 1966</td>
</tr>
<tr>
<td>18 U.S. male adults</td>
<td>178.7 (149--206)</td>
<td>AAS</td>
<td>Harrison et al. 1969</td>
</tr>
<tr>
<td>82 U.S. males</td>
<td>167.0 ± 5.09</td>
<td>&quot;</td>
<td>Schroeder 1969</td>
</tr>
<tr>
<td>64 Panamanian male adults</td>
<td>142 ± 43.2&lt;sup&gt;3&lt;/sup&gt;</td>
<td>&quot;</td>
<td>Kleavy 1970</td>
</tr>
<tr>
<td>14 Washington, D.C. male adults</td>
<td>176 ± 37&lt;sup&gt;3&lt;/sup&gt;</td>
<td>&quot;</td>
<td>McBean et al. 1971</td>
</tr>
<tr>
<td>29 c. 100 Cincinnati, OH males</td>
<td>147</td>
<td>&quot;</td>
<td>Petering et al. 1971</td>
</tr>
<tr>
<td>18 Port Arthur, TX males</td>
<td>156 (75--454)</td>
<td>&quot;</td>
<td>Eads et al. 1973</td>
</tr>
<tr>
<td>42 New Zealand males aged 18-27</td>
<td>180 ± 25</td>
<td>&quot;</td>
<td>McKenzie 1979</td>
</tr>
<tr>
<td>16 Iranian female adults</td>
<td>268 ± 59&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Z/D</td>
<td>Reinhold et al. 1966</td>
</tr>
<tr>
<td>47 U.S. females</td>
<td>172.1 ± 9.32</td>
<td>AAS</td>
<td>Schroeder 1969</td>
</tr>
<tr>
<td>70 Panamanian female adults</td>
<td>167 ± 129&lt;sup&gt;3&lt;/sup&gt;</td>
<td>&quot;</td>
<td>Kleavy 1970</td>
</tr>
<tr>
<td>c. 100 Cincinnati, OH females</td>
<td>153</td>
<td>&quot;</td>
<td>Petering et al. 1971</td>
</tr>
<tr>
<td>21 Port Arthur, TX females</td>
<td>173 (65--308)</td>
<td>&quot;</td>
<td>Eads et al. 1973</td>
</tr>
<tr>
<td>54 New Zealand females</td>
<td>196 ± 23</td>
<td>&quot;</td>
<td>McKenzie 1979</td>
</tr>
</tbody>
</table>

<sup>1</sup> Concentrations are average ± standard error unless noted (D) as standard deviation; if means were not reported medians (M) and/or ranges are given.

<sup>2</sup> AAS = Atomic absorption spectroscopy
AES = Atomic emission spectroscopy
NAA = Neutron activation analysis
PAA = Photon activation analysis
SSMS = Spark source mass spectroscopy
Z/D = Zincon/Dithizone analysis
APPENDIX B

INFORMED CONSENT

I, ____________________________________________, hereby agree to serve as a subject in the research project on cadmium conducted by Neal Simonsen.

I understand that the study involves the donation of a sample of scalp hair for trace metal analysis and candid completion of a background questionnaire by each subject.

I understand the possible risks and discomforts to me associated with this study, including a demand on my time and effort involved in the filling out of the study questionnaire as well as the inconvenience of collecting and tagging a sample of scalp hair.

It has been explained to me that the purpose of this study is to explore the differences in levels of cadmium (and zinc) in the hair of individuals in the population as they relate to smoking habits. I may not receive any direct benefit from participating in this study, but my participation may help to increase knowledge which may benefit others in the future. Neal Simonsen has offered to answer any questions I may have about the study and what is required of me in the study.

I understand that I am free to withdraw from participation in this study at any time without jeopardizing my relationship with Portland State University.

I have read and understand the foregoing information.

Date ______________________ Signature ___________________________
1. Age (in years at last birthday): _______  Weight: _______  Height: _______

2. Sex:
   a  Male
   b  Female

3. Race:
   a  White (non-Hispanic)
   b  Black
   c  Asian or Pacific Islander
   d  Hispanic
   e  Other (please specify) _______

4. Natural predominant hair color:
   a  Brown
   b  Black
   c  Blond
   d  Red
   e  Grey or white

5. Do you or have you smoked tobacco? (Check Yes or No; if Yes please also answer i, ii, iii, iv and v below)
   a  Yes
      i  Do you or did you smoke? (Check all which apply)
         a  Cigarettes
         b  Cigars
         c  A pipe
      ii  Are you a current or only a former smoker?
         a  Current
         b  Former
      iii  How many years have you or did you smoke(d)?:
      iv  If you are or were a cigarette smoker, how many packs per day do you or did you smoke, on the average?:
      v  If you are or were a cigarette smoker and smoke(d) one brand most of the time, which brand is or was it?: _______
   b  No

6. Do you or did you smoke marijuana? (Check Yes or No; if Yes please answer i, ii and iii)
   a  Yes
      i  Are you a current or only a former smoker?
         a  Current
         b  Former
      ii  How many years have you or did you smoke(d)?:
      iii  If you are or were a marijuana smoker, about how many days per month do you or did you smoke it, on the average?:
   b  No

7. What is the zip code of your place of residence?: _______

8. Are there any major refineries, smelters or other metal or chemical industrial complexes within a mile of the place? (If Yes, please list in space provided)
   a  Yes: __________________________________________
   b  No __________________________________________
   c  Don't know
9. If there are any other areas you regularly spend 30 hours per week or more in, please locate them by listing their zip codes:

1. ________
2. ________
3. ________

10. Are there any major refineries, smelters or other industrial complexes in these areas? (If unsure, answer No)
   a. Yes, in area 1.
   b. Yes, in area 2.
   c. Yes, in area 3.
   d. No

11. Are you regularly exposed to other people’s tobacco smoke at home, work or otherwise?
   a. Yes, for more than 5 hours per week
   b. Yes, for 5 hours or less per week
   c. No

12. Do you do any soldering work at home or at your job?
   a. Yes
   b. No

13. Are you a vegetarian?
   a. Yes
   b. No

14. Which of the following items are a part of your diet at least once a week, on the average? (Check all which apply)
   a. Fish
   b. Anchovies
   c. Shellfish
   d. Oysters
   e. Rice

15. Do you drink at least two cups of tea a week, on the average?
   a. Yes
   b. No

16. What hair care products do you use? (Please list)

17. How often do you wash your hair, on the average?
   a. 4 or more times per week
   b. 3 times or less per week

18. Do you use oral contraceptives?
   a. Yes
   b. No

19. Do you have any chronic conditions (such as asthma or hay fever) or current illnesses (such as the flu or strep throat)?
   a. Yes
   b. No

If Yes, please list: ____________________________

______________________________

______________________________
### APPENDIX C

**TABLE VIII**

HAIR CADMIUM CONCENTRATIONS (µg/g) FOR INDIVIDUALS, GROUPED ACCORDING TO SMOKING HABITS

<table>
<thead>
<tr>
<th></th>
<th>Not current regular marijuana smoker</th>
<th>Current regular marijuana smoker&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current tobacco smoker&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.751, .559, .822</td>
<td>.518, .588, .594, 1.62, 3.58</td>
</tr>
<tr>
<td>Former tobacco smoker&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.460, .435, .500, .423, .680 .693</td>
<td>None</td>
</tr>
<tr>
<td>Neither current nor former tobacco smoker</td>
<td>.368, .446, .444, .284, .426, 1.27, .638, 1.78, .760, .564, .596, .796, .524, .507, .367, .592, .737, .440, .786, .573, .432, 1.08, .991</td>
<td>.522, .770, .894, 1.02</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current tobacco smoker&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.800, .572, .750, .657, .960, 1.38</td>
<td>.616, .819</td>
</tr>
<tr>
<td>Former tobacco smoker&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.618, .958, .621, .733, .575</td>
<td>.423</td>
</tr>
<tr>
<td>Neither current nor former tobacco smoker</td>
<td>.899, .430, .961, .546, .496, .810, .384, 1.25, .282, .778, .776, .735, 1.46, .974, .653, .886, .915, 1.23, .673, .886, .773, .597, .342, .655, 1.74, .683, .606, .532, .415, .599, .467, .433, .599, 1.43, .330, .591</td>
<td>.686, .718, .745, .887, 1.02</td>
</tr>
</tbody>
</table>

<sup>a</sup> At least 4 days per month  
<sup>b</sup> At least 1/3 pack per day  
<sup>c</sup> At least 5 pack-years
### APPENDIX D

**TABLE IX**

HAIR ZINC CONCENTRATIONS (µg/g) FOR INDIVIDUALS, GROUPED ACCORDING TO SMOKING HABITS

<table>
<thead>
<tr>
<th></th>
<th>Not current regular marijuana smoker</th>
<th>Current regular marijuana smokera</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current tobacco smokerb</td>
<td>107, 131, 238</td>
<td>184, 185, 221, 300</td>
</tr>
<tr>
<td>Former tobacco smokerc</td>
<td>118, 130, 188, 196</td>
<td>None</td>
</tr>
<tr>
<td>Neither current nor former tobacco smoker</td>
<td>99, 104, 129, 134, 139, 143, 141, 156, 166, 174, 176, 180</td>
<td>178, 242, 269</td>
</tr>
</tbody>
</table>

|                |                                      |                                  |
| **Females**    |                                      |                                  |
| Current tobacco smokerb | 132, 181, 242, 247, 279 | 186, 218 |
| Former tobacco smokerc | 158, 165, 175 | 143 |
| Neither current nor former tobacco smoker | 110, 115, 127, 129, 148, 148, 156, 157, 158, 162, 166, 168, 182, 184, 188, 190, 201, 203, 224, 228, 236, 252, 278, 291, 293, 371 | 224, 368 |

---

a At least 4 days per month

b At least 1/3 pack per day

c At least 5 pack-years
APPENDIX E

SUMMARY OF SELECTED QUESTIONNAIRE RESPONSES - MAIN STUDY

Average age: 25.8 years

Sex: Male 43%  Female 57%

Race: White 93%  96%
       Black --  --
       Asian or Pacific Islander 5  4
       Hispanic 2  --

Natural predominant hair color:
       Brown 57  67
       Black 17  2
       Blonde 21  24
       Red 5  5
       Grey or white --  2

Do or did smoke tobacco 36  35
Do or did smoke marijuana 52  49

Regularly exposed to other people’s tobacco smoke:
       5 or more hours per week 15  37
       Less than 5 hours per week 28  20
       Not regularly exposed 57  43

Vegetarian 5  20

Eat at least once per week:
       Fish 33  51
       Anchovies 3  --
       Shellfish --  39
       Oysters --  --
       Rice 38  35

Drink 2+ cups tea/week 23  44

Wash hair more than 3 times per week 85  61

Use oral contraceptives 16

Chronic conditions, current illness:
       Hay fever or "allergy" 13  18
       Recent flu or cold 13  4
       Asthma --  2
       Strep throat 3  --
       Ulcers --  2
       Kidney infection --  2