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Cadmium and Zinc Levels in the Hair of Smokers and Nonsmokers

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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 concen-
trations and marijuana smokers had significantly higher zinc
concentrations than nonsmokers, confirming tobacco smoking as a
significant source of cadmium and implicating marijuana as a signi-
ficant 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.

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TABLE OF CONTENTS

PAGE

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

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 associ-
ated with zinc, another member of group IIB 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 (osteo-
malacia) 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 rein-forced 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 (Ferm 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 B_2 micro-globulins) 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 Ca^{+2} receptor sites and preventing 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 partic-
ular (.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 Corneliussen 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. Oster-gaard (1978) reported that renal cadmium concentrations in autopsied samples from cigarette smokers were nearly twice as high as in non-smokers. 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 charac- teristic 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, impli-
cated 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 correla-
tions 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 concen-
tration 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 mari-juana 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 (iden-
tified 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 HNO_3 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 HNO_3.

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 HNO_3:H_2O 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. HNO_3 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 $HClO_4$:HNO_3 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, HNO_3 being added dropwise
whenever a solution darkened, and then allowed to cool. When cool,
samples were transferred to 5ml volumetric flasks and 1:20 HNO_3:H_2O
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 spectrophoto-
meter 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 $HClO_4$ 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 HNO_3 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: $\overline{x}$ ± SE (n))[a]

	All	Males	Females	Significant inter-column differences
1 Current tobacco smokers	1.35 ± .22 (8)	1.60 ± .28 (5)	0.94 ± .36 (3)	—
2 Current marijuana (but not tobacco) smokers	0.85 ± .22 (8)	0.74 ± .44 (2)	0.89 ± .25 (6)	—
3 Not current smokers	0.69 ± .14 (21)	0.64 ± .24 (7)	0.72 ± .17 (14)	—
	0.86 (37)	0.99 ± .17 (14)	0.79 ± .13 (23)	
Significant inter-row differences[b]	1 vs. 3 P=.048 (.080)	1 vs. 3 P=.021 (.079)		

[a] $\overline{x}$ = 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: $\overline{x} \pm$ SE (n))[a]

	All	Males	Females	Significant inter-column differences
1 Current tobacco smokers	0.97 ± .11 (16)	1.12 ± .15 (8)	0.82 ± .15 (8)	—
2 Current marijuana (but not tobacco) smokers	0.78 ± .13 (10)	0.83 ± .21 (4)	0.75 ± .17 (6)	—
3 Not current smokers	0.71 ± .05 (71)	0.67 ± .08 (30)	0.74 ± .07 (41)	—
	0.76 (97)	0.77 ± .06 (42)	0.75 ± .06 (55)	
Significant inter-row differences[b]	1 vs. 3 P=.031 (.036)	1 vs. 3 P=.018 (.045)		

[a] $\overline{x}$ = 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 $\mu g/g$: $\bar{x} \pm SE$ (n))[a]

	All	Males	Females	Significant inter-column differences
1 Current tobacco smokers	204 ± 15 (14)	195 ± 21 (7)	212 ± 21 (7)	——
2 Current marijuana (but not tobacco) smokers	237 ± 32 (6)	230 ± 32 (3)	245 ± 32 (3)	——
3 Not current smokers	180 ± 8 (52)	164 ± 12 (22)	192 ± 10 (30)	——
	189 (72)	177 ± 10 (32)	199 ± 9 (40)	P=.080
Significant inter-row differences	2 vs. 3 P=.019			

[a] $\bar{x}$ = average; SE = standard error of the mean; n = number of subjects

23

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: $\overline{x}$ ± SE (n))[a]

	All	Males	Females	Significant inter-column differences
1 Heavy tobacco smokers[b]	1.06 ± .13 (10)	1.40 ± .18 (5)	0.72 ± .18 (5)	—
2 Light tobacco and/or marijuana smokers[c]	0.79 ± .10 (16)	0.75 ± .16 (7)	0.83 ± .14 (9)	—
3 Nonsmokers	0.71 ± .05 (71)	0.67 ± .08 (30)	0.74 ± .06 (41)	—
Significant inter-row differences	1 vs. 3 P=.014	1 vs. 3 P=.015	—	

a $\overline{x}$ = average; SE = standard error of the mean; n = number of subjects

b Over ½ pack per day

c 1/3 to ½ 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: $\overline{x} \pm$ SE (n))[a]

	All	Males	Females
Cd			
1 Current tobacco smokers	0.97 ± .11 (16)	1.12 ± .16 (8)	0.82 ± .16 (8)
2 Former tobacco smokers	0.61 ± .13 (11)	0.53 ± .18 (6)	0.70 ± .20 (5)
3 Not current tobacco or marijuana smokers	0.73 ± .06 (60)	0.71 ± .09 (24)	0.74 ± .07 (36)
	0.76 (97)	0.77 ± .07 (38)	0.75 ± .06 (49)
Significant inter-row differences	1 vs. 2 P=.042 1 vs. 3 P=.059		
Zn			
1 Current tobacco smokers	204 ± 14 (14)	195 ± 20 (7)	212 ± 20 (7)
2 Former tobacco smokers	161 ± 20 (7)	158 ± 27 (4)	166 ± 31 (3)
3 Not current tobacco or marijuana smokers	183 ± 8 (45)	165 ± 13 (18)	194 ± 10 (27)
	185 (66)	171 ± 10 (29)	195 ± 9 (37)
Significant inter-row differences	1 vs. 2 P=.09		

[a] $\overline{x}$ = average; SE = standard error of the mean; n = number of subjects

TABLE VI

CADMIUM AND ZINC CONCENTRATIONS ($\mu g/g$) VS. AGE (years)

Age	All Males	All Females	Males		Females	
			Tob. Smok.	Non Smok.	Tob. Smok.	Non Smok.
			Cd			
17-20	0.66(6)[a]	0.73(13)	1.52(1)	0.49(5)	1.38(1)	0.68(12)
21-25	0.68(15)	.71(18)	.69(4)	.68(11)	.75(4)	.70(14)
26-30	.74(9)	.83(14)	——	.74(9)	.65(3)	.88(11)
31-39	.79(5)	.61(6)	.52(1)	.85(4)	.33(1)	.67(5)
40-49	.99(1)	——	——	.99(1)	——	——
50+	——	1.02(2)	——	——	.80(1)	1.25(1)
			Zn			
17-20	217(5)	193(11)	300(1)	197(4)	242(1)	188(10)
21-25	182(11)	209(11)	161(4)	194(7)	215(3)	207(8)
26-30	158(7)	199(11)	——	158(7)	230(3)	188(8)
31-39	173(4)	188(3)	184(1)	169(3)	115(1)	224(2)
40-49	166(1)	——	——	166(1)	——	——
50+	——	234(2)	——	——	279(1)	190(1)

[a] Numbers in parentheses are n values for corresponding concentrations

28
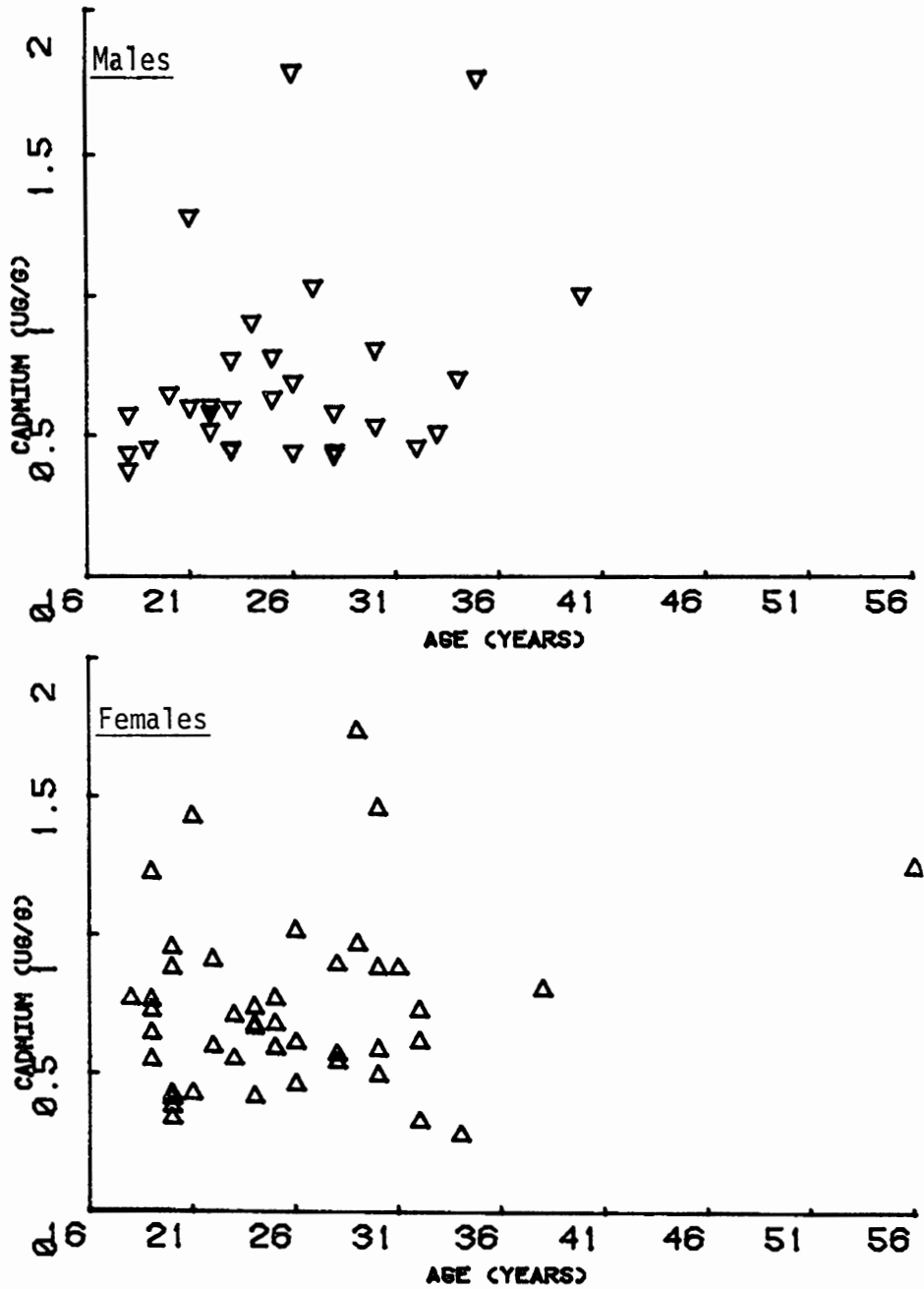

Figure 1. Cadmium concentration in hair (µg/g) as a function of age (years) for male and female nonsmokers.
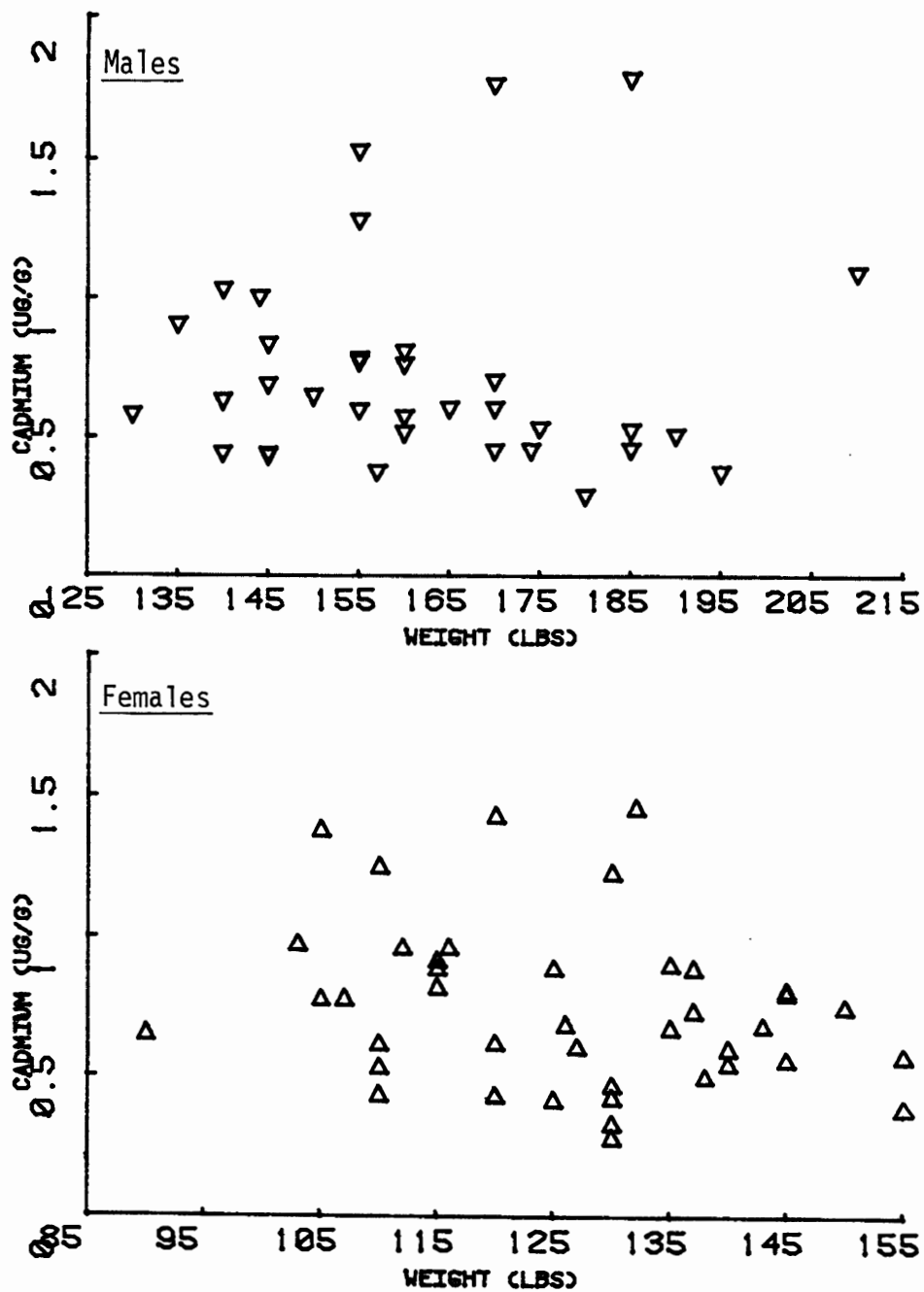
Figure 2. Cadmium concentration in hair (µg/g) as a function of body weight (lbs) for male and female nonsmokers.

TABLE VII

DIFFERENCES IN AVERAGE HAIR CADMIUM AND ZINC LEVELS (μg/g)
ASSOCIATED WITH MISCELLANEOUS FACTORS

Factor	Subjects	$\overline{x}$ Cd	(n)	$\overline{x}$ Zn
Black hair	7 black-haired male smokers	.542 ± .15	(4)	200 ± 19
	17 male nonsmokers	.777 ± .09	(14)	155 ± 10
		P=.20(.18)[a]		P=.05
Soldering	5 solderers	.833 ± .19		_____
	92 non-solderers	.744 ± .05		(Only 2 Zn values were obtained for solderers)
		P=.65(.52)		
	Note: solderers include 2 individuals with black hair and 1 with recent flu.			
Oral contraceptives	6 oral. contra. users	.578 ± .13	(5)	163 ± 26
	35 nonusers	.763 ± .05	(25)	197 ± 12
		P=.20(27)		P=.23
	Note: all users/nonusers compared for Zn are nonsmokers			
Recent flu	3 males w/rec. flu	.465 ± .32	(3)	139 ± 28
	39 males w/o rec. flu	.795 ± .09	(29)	181 ± 9
		P=.33(.17)		P=.18
	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 Chatto-padhyay 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 mari-
juana 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 meta-
bolism.

The overall results of this study also reflect upon the useful-
ness 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 inter-pretation 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 contra-
dicts 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.

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APPENDICES

PREVIOUS STUDIES ON Cd AND Zn IN HUMAN HAIR

	Subjects	Concentration[1] (µg/g)	Analysis Technique[2]	Reference
Cd	? Americans	0.34--1.8	SSMS	Yurachek et al. 1969
	66 rural Canadians	1.2^M(0.25--2.7)	PAA	Chattopadhyay et al. 1977
	45 urban Canadians	2.0^M(0.32--3.4)	"	Chattopadhyay et al. 1977
	82 U.S. males	2.76 ± .483	AAS	Schroeder 1969
	18 Port Arthur, TX males	2.2 (0.1--9.3)	"	Eads et al. 1973
	95 Cincinatti, OH males	2.2 ± .2	"	Petering et al. 1973
	47 U.S. females	1.77 ± .239	"	Schroeder 1969
	21 Port Arthur, TX females	1.0(0.2--3.6)	"	Eads et al. 1973
	83 Cincinatti, OH females	2.43 ± .26	"	Petering et al. 1973
Zn	33 Oak Ridge, Tennesseans	177 ± 73^D	NAA	Bate & Dyer 1975
	? Canadians	300--1000	"	Perkons & Jervis 1966
	12 Egyptians	103.3 ± 4.9	AES	Strain et al. 1966
	3? U.S. subjects	143--246	SSMS	Yurachek et al. 1969
	75 Iranian children aged 6-12	199 ± 22^D	AAS	McBean et al. 1971
	4 Egyptian males	105--136	"	Prasad et al. 1963
	33 Hastings, N.Z. schoolboys	126 ± 14^D	NAA	Bate & Dyer 1965
	33 Napier, N.Z. schoolboys	132 ± 15^D	"	Bate & Dyer 1965
	20 Iranian male students	181 ± 36.3^D	Z/D	Reinhold et al. 1966
	6 New York male adults	119.6 ± 4.6	AES	Strain et al. 1966
	18 U.S. male adults	178.7(143--206)	AAS	Harrison et al. 1969
	82 U.S. males	167.0 ± 5.09	"	Schroeder 1969
	64 Panamanian male adults	142 ± 43^D	"	Klevay 1970
	14 Washington, D.C. male adults	176 ± 37^D	"	McBean et al. 1971
	c. 100 Cincinatti, OH males	147	"	Petering et al. 1971
	18 Port Arthur, TX males	156(75--454)	"	Eads et al. 1973
	42 New Zealand males aged 18-27	180 ± 25	"	McKenzie 1979
	16 Iranian female adults	268 ± 59.3^D	Z/D	Reinhold et al. 1966
	47 U.S. females	172.1 ± 9.32	AAS	Schroeder 1969
	70 Panamanian female adults	167 ± 129^D	"	Klevay 1970
	c. 100 Cincinatti, OH females	153	"	Petering et al. 1971
	21 Port Arthur, TX females	173(65--308)	"	Eads et al. 1973
	54 New Zealand females	195 ± 23	"	McKenzie 1979

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

[2] 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 _____

Number _____

1. Age (in years at last birthday): _____ Weight: _____ Height: _____

2. Sex:
    a Male
    b Female

3. Race: Country of birth: _____
    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 smoke(d) 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

		Not current regular marijuana smoker	Current regular marijuana smoker[a]
Males	Current tobacco smoker[b]	.751, .559, .822	.518, .588, .594, 1.52, 3.58
	Former tobacco smoker[c]	.450, .435, .500, .423, .680, .693	None
	Neither current nor former tobacco smoker	.368, .446, .444, .284, .426, 1.27, .638, 1.78, .760, .564, .596, 1.76, .796, .524, .507, .367, .592, .573, .440, .786, .573, .432, 1.08, .991	.622, .770, .894, 1.02
Females	Current tobacco smoker[b]	.800, .572, .750, .657, .960, 1.38	.616, .819
	Former tobacco smoker[c]	.618, .958, .621, .733, .575	.423
	Neither current nor former tobacco smoker	.899, .430, .961, .546, .498, .810, .384, 1.25, .282, .778, .776, .735, 1.46, .974, .653, .886, .915, 1.23, .673, .886, .773, .597, .342, .555, 1.74, .683, .606, .532, .415, .599, .467, .433, .559, 1.43, .330, .591	.686, .718, .745, .887, 1.02

[a] At least 4 days per month

[b] At least 1/3 pack per day

[c] At least 5 pack-years

APPENDIX D

TABLE IX

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

		Not current regular marijuana smoker	Current regular marijuana smoker[a]
Males	Current tobacco smoker[b]	107, 131, 238	184, 185, 221, 300
	Former tobacco smoker[c]	118, 130, 188, 196	None
	Neither current nor former tobacco smoker	99, 104, 129, 134, 139, 143 141, 156, 166, 174, 176, 180 180, 182, 185, 194, 223, 269	178, 242, 269
Females	Current tobacco smoker[b]	132, 181, 242, 247, 279	186, 218
	Former tobacco smoker[c]	158, 165, 175	143
	Neither current nor former tobacco smoker	110, 115, 127, 129, 148, 148 156, 157, 158, 162, 166, 168, 182, 184, 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%

		Males	Females
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