CURRENT RESEARCH
In December of 1990, a medical research team at the University of Calgary School of Medicine reported an animal study tracing mercury from dental amalgam fillings rapidly into body tissues. The study was published in the highly prestigious FASEB medical journal. Since that time, there has been a great increase in reported studies of adverse health effects to chronic low dose mercury exposure.

In stark contrast, the dental profession continues to focus on the estimated daily dose of mercury from dental amalgam with comparison to various standards, even though expert medical toxicologists clearly state that no exposure to mercury vapor can be considered harmless (i.e.- there is no toxic threshold for exposure to mercury vapor).

In this issue of the Bio-Probe Newsletter, we present abstracts of current research demonstrating the widening gap between the scientific documentation being presented by the medical scientific community and the rigid position of the dental profession in direct contradiction to the scientific documentation.

The first abstract is the latest dental community attempt to discredit formal science by estimating daily intake of mercury from dental amalgam. It is followed by the abstract of the April 1992 FASEB article (reported in the May BPNL) that provides hard scientific data contradicting the dental profession estimates. The abstracts that follow are examples of the current medical research defining the direction of medical scientists on the issue, all of which cast serious doubt on the safety of mercury exposure from dental amalgam.

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REVIEWS/ABSTRACTS

ABSTRACT: Measurements of intra-oral mercury vapor from amalgam fillings are discussed. It was shown that the only quantity which it is possible to measure is the mercury release rate, and that the concentrations of mercury vapor in the oral cavity published in most earlier studies are the mercury concentrations in the measuring cell of the measuring apparatus and not the concentrations in the oral cavity. The consequences for the daily dose equations of the facts that the mercury source is present inside the oral cavity and that the amount of mercury released during a certain time is limited are
discussed. It was found that most daily dose equations used have a questionable mercury distribution on inspiration, expiration, and swallowing.

Re-calculations of almost all the available daily dose data showed a mean daily dose value of about 1.3 micrograms Hg/day (range, 0.3-2.2 micrograms Hg/day). The mean swallowed amount of mercury from intra-oral mercury vapor was calculated as being in the order of 10 micrograms Hg/day (range, 2.4-17 micrograms Hg/day), resulting in an estimated absorption of about 1 microgram Hg/day from the gastro-intestinal tract.

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Aposhian, HV; Bruce, DC; Alter, W; Dart, DC; Hurlbut, KM; Aposhian, MM.: Urinary Mercury after Administration of 2,3-Dimercaptopropane-1-sulfonic Acid: Correlation with Dental Amalgam Score. FASEB J. 6(7):2472-6. Apr 1992.

ABSTRACT: There is considerable controversy as to whether dental amalgams may cause systemic health effects in humans because they liberate elemental mercury. Most such amalgams contain as much as 50% metallic mercury. To determine the influence of dental amalgams on the mercury body burden of humans, we have given volunteers, with and without amalgams in their mouth, the sodium salt of 2,3-dimercaptopropane-1-sulfonic acid (DMPS), a chelating agent safely used in the Soviet Union and West Germany for a number of years. The diameters of dental amalgams of the subjects were determined to obtain the amalgam score.

Administration of 300 mg DMPS by mouth increased the mean urinary mercury excretion of the amalgam group from 0.70 to 17.2 micrograms and that of the non-amalgam group from 0.27 to 5.1 micrograms over a 9-h period. Two thirds of the mercury excreted in the urine of those with dental amalgams appears to be derived originally from the mercury vapor released from their amalgams. Linear regression analysis indicated a highly significant positive correlation between the mercury excreted in the urine 2 h after DMPS administration and the dental amalgam scores.

DMPS can be used to increase the urinary excretion of mercury and thus increase the significance and reliability of this measure of mercury exposure or burden, especially in cases of micromercurialism.

BIO-PROBE COMMENT: The Aposhian study, supported by the study by Zander and associates reported in the May 1992 BNPL, clearly contradicts the Olsson and Bergman estimates. The estimates by these dentists are also contradicted by the calculations of noted mercury toxicologists such as the World Health Organization expert mercury committee (WHO. Environmental Health Criteria 118: Inorganic Mercury. Geneva. 1991) and Drs. Thomas W. Clarkson and Lars Friberg and associates (Biological Monitoring of Toxic Metals. Plenum Press, New York. 1988).

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Östlin, L et al. AMALGAM REMOVAL - A ROAD TO BETTER HEALTH? Health Insurance Bureau, Stockholm County, 1991

ABSTRACT: We have examined the effect of removing amalgam from a group of patients where the patients themselves or their dentists have suspected a reaction to mercury in amalgam.

The aim of the present study was to define the patient group with respect to sex, age, reason for asking for amalgam removal and their health situation after replacing their amalgam fillings with other materials. They were also asked if all fillings were exchanged and the costs. The number of days off work for health reasons (paid by the insurance) was compared the year before removing amalgam, and one and two years after. The aim of the investigation was to evaluate if a removal of amalgam fillings will rehabilitate the patients with a subsequent reduction in sick leave and reduced total costs for society.

The study comprised 383 patients who between February 1987 and December 1989 asked the insurance system for cost coverage for replacing amalgam with other materials. These patients were asked to answer a questionnaire. 308 patients replied. These were then divided into three groups: suspected mercury poisoning, mercury allergy and lichenoid reactions. Two thirds of the respondents were between 40 and 74 years old and 74% were women.

Most of the patients have a polysymptomatic clinical picture. On average, the patients reported 8.6 symptoms. Nearly a quarter of the group reported all 16 symptoms in the questionnaire and a third had 0-4 symptoms. More than two-thirds reported mouth eruptions, unpleasant feeling in the mouth like smarting pains, metal taste and tiredness.

Nearly 73% reported that they had exchanged all amalgam fillings. Those who had exchanged all reported an improvement of all health symptoms. The local, oral symptoms improved most. More
than 80% reported that they had become well or better. Also generalized symptoms like tiredness, headache and muscle pains improved considerably after the exchange. The patients who had only partially removed amalgam or not at all, reported much less improvement. Comparing the two groups, we found significant differences (95%-level) regarding all symptoms.

Nearly 75% of the patients gave their own comments in the questionnaire. Among those who had removed amalgam, the majority expressed happiness for having been relieved of their previous health problems.

About a quarter of the whole group and more than half of those who had suspected mercury poisoning reported that they had been on sick leave for problems they connected to their amalgam dental fillings. On average there were 65 days sick leave the year before amalgam removal. At the end of two years this was reduced 30% to a mean of 44 days per year. The total costs for dental treatments varied from 704 kroner to 44,336 kroner with a mean of 17,938 kroner (ABOUT $3220.00) for each patient.

With this background we recommend a simplified procedure in the dental insurance system in order to make it easier for patients who suspect that they are affected by amalgam, are worried about future problems or for other reasons want to remove their amalgam fillings. This should lead to an improved health status with fewer days for sick leave and reduced administrative costs for the insurance system and reduced total costs for society. (Translation from Swedish to English by Mats Hanson Ph.D.)

BIO-PROBE COMMENT: Sweden’s socialized insurance system did not previously pay for the complete exchange of amalgam dental fillings. We consider it extremely significant that the motivating force for the study was the possible reduction of TOTAL HEALTH CARE COSTS. Not only the cost of the dental work, but the ultimate cost savings resulting from a healthier population. The reduction in expenditures for sick days per year is but the tip of the iceberg when it comes to evaluating the total effect of amalgam replacement. For example, increased productivity, reduced corporate personnel costs, reduced insurance expenditures for medical treatment of mercury implant related illnesses, etc.


ABSTRACT: The purpose of the present study was to examine the mercury content in the tissues of pregnant rats receiving dental amalgam restoration. Sixteen pregnant rats were equally divided into the control and experimental groups. At one day pregnancy, high copper amalgams were placed in the maxillary molars of experimental animals. At 20 days the animals were killed, and then brain, liver, kidney and spleen were removed, freeze-dried and pulverized. The tissues of fetus, except the spleen, were also treated in a same manner. The amount of mercury in these tissues was measured with a mercury analyzer.

The mercury contents in the brain, liver, kidney and spleen of the experimental group were significantly higher (p<0.01) than those in the control group. In the experimental animals, the amount of mercury was highest in the kidney, intermediate in the liver and spleen, and lowest in the brain. In the case of the fetus, mercury contents in the brain and kidney of the experimental group were almost the same with those in the control group. Mercury content in the liver of the experimental group was higher than that in the control group, but the difference was not significant.

The results of the present study suggested that there was a possibility of mercury release from dental amalgam restorations in humans. However, the transport of mercury from the pregnant rats into their fetal tissues was not clear in the present study.

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ABSTRACT: The purpose of the present study was to examine the relation between number of amalgam fillings in pregnant rats and mercury concentrations in the tissues of their fetuses. Thirty-two pregnant rats were divided equally into 4 groups (Groups A, B, C, and D). On day one of pregnancy, Groups B, C and D received an occlusal amalgam filling in 1, 2 and 4 maxillary molars, respectively. Group A received no treatment. On day 20, the brains, livers and kidneys of the fetuses of all groups were removed.

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The concentration of mercury in the fetal tissues was measured. In brains, livers and kidneys, Groups B, C and D showed a significantly higher mercury concentration than Group A (p<0.01). In brains, Group D showed a significantly higher mercury concentration than Group B (p<0.01). In brains and livers, Group C showed a higher mercury concentration than Group B, but there was no significant difference. Moreover, Group C showed almost the same mercury concentration as Group D. In livers, Group D showed a higher mercury concentration than Group B, but there was no significant difference. In kidneys, Group C showed a higher mercury concentration than Group B, but there was no significant difference. Group D showed a significantly higher mercury concentration than Groups B (p<0.01) and C (p<0.05).

The present results suggested that the mercury concentration in the fetal tissues tended to increase with the increasing number of amalgam fillings in their mother rats.

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ABSTRACT: The effect of mercury (HgCl₂) on placental amino acid and glucose transfer as determined by the use of their non-metabolizable radioactive analogues, aminoisobutyric acid (AIB) and 3-O-methyl glucose (3MG), respectively, was studied in an in vitro perfusion model of a term human placenta. Hg²⁺ was found to decrease the transfer and accumulation of AIB without affecting 3MG transfer. It was also found to decrease the placental oxygen consumption rate. Placental circulation and tissue morphology remained intact, as demonstrated by the antipyrine transfer rate, and by electron microscopy, respectively. The mechanism by which Hg²⁺ may interfere with placental amino acid transfer and accumulation is discussed.

Although much higher concentrations than those found in the ordinary polluted environment were used, this is the first report showing that Hg²⁺ interferes with an essential human placental function in a system employing a whole human placental cotyledon. This finding may indicate the possible involvement of Hg²⁺ in impaired organogenesis in early pregnancy or deranged fetal growth during the last trimester.

BIO-PROBE COMMENT: In 1991, the University of Calgary research team published findings in the American Journal of Physiology tracing a rapid transfer of mercury from amalgam fillings in pregnant sheep into maternal and fetal tissues. The two studies by Takahashi and associates, utilizing an entirely different experimental protocol, confirm the Calgary findings. The study by Urbach et al. demonstrates the potential human fetal hazards that can be caused by mercury, whatever the source. This provides further justification for the elimination of mercury dental implants in women of child bearing age.

One other interesting note, researchers at the California Institute of Technology have found a 67-70 percent similarity between human and mouse DNA coding for a component of the immune system.

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ABSTRACT: We have previously demonstrated that low level mercury exposure modulates human lymphocyte function. While the mechanism(s) for these immunotoxic effects have not been determined, alterations in cell viability may be involved. We examined the cytotoxic properties of both HgCl₂ and MeHgCl, in terms of their ability to alter human T-cell and monocyte viability.

Following treatment with HgCl₂ (0-20 mcg/ml) or MeHgCl (0-2 mcg/ml), there was minimal reduction in lymphocyte viability at 1-4 hr. However, after exposure to mercury for 24 hr, cell death was apparent. In comparison, monocytes exhibited significant loss of viability during the early exposure periods. MeHgCl was approximately 5-10 times more potent than HgCl₂.

Other indicators of cell death were also determined. Measurement of the energy charge ratio indicated profound changes in cellular energy conservation. Electron microscopic analysis of cells treated with mercury revealed early nuclear alterations characterized by hyperchromaticity, nuclear fragmentation and condensation of nucleoplasm. In concert with these nuclear changes, there was destruction of cytoplasmic organelles with loss of
membrane integrity. Studies of phospholipid synthesis by mercury treated cells confirmed that there were alterations in membrane structure. Thus, there was a decrease in total phosphatide synthesis by treated cells. Moreover, monocyte phospholipid synthesis appeared to be more sensitive to the presence of mercury than lymphocytes. Finally, both forms of mercury caused a rapid and sustained elevation in the intracellular levels of Ca++. These morphological and biochemical changes are consistent with the notion that mercury initiates cytotoxic changes associated with programmed cell death.

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ABSTRACT: Considerable attention has been directed at defining the health deficits associated with exposure to mercurial compounds. The major goal of our study was to assess whether low level mercury exposure modulates human lymphocyte function. Following treatment of human T-cells and B-cells with HgCl$_2$ (0-1000 ng) and MeHgCl (0-100 ng), their activation by mitogens was evaluated.

Both forms of mercury caused a dose dependent reduction in T and B cell proliferation, however, the effect on T-cells was dependent upon the presence of monocytes. B-cells were more sensitive to the effects of mercury than were T-cells, and MeHgCl was approximately 5-10 times more potent than HgCl$_2$. Mercury inhibited the ability of these cells to synthesize and secrete both cytokines and immunoglobulins. Analysis of the expression of activation markers on the cell surface indicated that one of the earliest markers of lymphocyte activation, CD69, was not affected by mercury. In comparison, T-cell expression of IL-2R and the transferrin receptor on T-cells was impaired. Similarly, B-cell expression of CD23 and the transferrin receptor were also inhibited. Of particular interest, cells activated by mitogen for 24 hr became refractory to the immunotoxic effects of mercury.

The results of the investigation clearly show that mercury-containing compounds are immunomodulatory; moreover, the decrease in lymphocyte function following exposure to mercury indicates that this metal is immunotoxic at very low exposure levels.
A significant suppression in acetylcholinesterase activity was recorded in all the organs from both mercury and zinc intoxicated fish at all the exposure periods. Concurrently, a significant increase in the content of acetylcholine in the organs was observed. These changes observed in the organs of mercury treated fish in different exposure periods were in the order 1 greater than 15 less than 30 days and in zinc treated fish 1 greater than 15 greater than 30 days. Further, these changes were greater in magnitude in the brain, liver and muscle (non-osmoregulatory organs) than in the gill, kidney and intestine (osmoregulatory organs) in both metal media.

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ABSTRACT: This article describes a study of the toxic, reproductive, and developmental effects of chronic methylmercury (MeHg) exposure in Macaca fascicularis monkeys. Adult and infant monkeys were studied using procedures to assess maternal and newborn blood Hg concentrations, menstrual cyclicity, conception rate, reproductive outcome, maternal toxicity, and offspring size at birth. Maternal intakes of 0, 50, 70, or 90 micrograms/kg/d MeHg hydroxide were studied. Maternal blood concentrations reached equilibrium by 10 weeks of exposure. The half-life of blood Hg for adult females ranged from 15 to 40 days (mean = 27 d) and did not vary with dose.

Maternal MeHg exposure did not affect the length of the menstrual cycle or the conception rate. Maternal MeHg exposure did significantly reduce the number of viable deliveries at blood Hg concentrations above 1.5 ppm. Maternal blood Hg concentrations at delivery were significantly lower than newborn concentrations. No effect of maternal MeHg exposure on offspring size at birth was observed. Maternal toxicity was related to blood Hg concentrations above 2.0 ppm following approximately one year of exposure.

Results indicate that MeHg exposure can affect reproductive outcome at levels that do not cause overt toxicity.

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Nuyts, GD; Roels, HA; Verpooten, GF; Fernand, AM; Lauwerys, RR; De Broe, ME.: Intestinal-Type Alkaline Phosphatase in Urine as an Indicator of Mercury Induced Effects on the S3 Segment of the proximal Tubule. Nephrol Dial Transplant. 7(3):225-9. 1992.

ABSTRACT: Intestinal-type alkaline phosphatase (IAP) is a specific and sensitive marker for alterations of the S3 segment of the human proximal tubule, the preferred part for several nephrotoxins. We studied IAP and other renal parameters in mercury-exposed workers and their controls. IAP excretion is clearly increased in the exposed workers, compared to other parameters, indicating that the determination of this enzyme can be a useful screening test of renal effects in occupational mercury exposure.

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ABSTRACT: Adverse environmental stimuli increase the synthesis of a class of proteins referred to as stress proteins. The effect of mercuric chloride, a model nephrotoxin, on protein synthesis in male rat kidney has been evaluated. Renal slices from exposed rats were incubated with [35S]methionine for 1 hr and subjected to SDS-PAGE, after which 35S-labeled proteins were detected by autoradiography.

Enhanced de novo synthesis of 70- and 90-kDa relative molecular mass (Mr)) proteins were detected 2 hr after exposure to 1 mg Hg/kg, with maximum activity occurring at 4-8 hr. By 16 hr post-injection, synthesis of these two proteins had decreased. Dose-related increases in synthesis of these proteins, and of a 110-kDa protein, were observed 4 hr after i.v. injection of 0.25, 0.5, and 1.0 mg Hg/kg, with concomitant inhibition of synthesis of proteins of Mr) 38 and 68 kDa. At a dose of 1 mg/kg, kidney proximal tubules exhibited progressive degenerative changes from 4 to 24 hr. A functional deficit, decreased uptake of [para-3H]aminoglutamate into renal slices, was not observed until 16 hr after i.v. injection of 1 mg/kg.

No significant histopathologic changes were observed in kidneys 4 hr after treatment with 0.25 or 0.5 mg Hg/kg, i.v. No changes in liver protein synthesis were apparent until 16-24 hr, where an increase in the 70- and 90-kDa proteins was observed. A concomitant increase in plasma sorbitol dehydrogenase activity occurred at 16-24 hr; however, there was no histopathological evidence of liver injury. The 72-kDa inducible
member of the 70-kDa stress protein family and the 88-kDa member of the 90-kDa protein family were detected by immunoblotting techniques using monoclonal antibodies.

The data demonstrate that Hg induces alterations in the expression of renal gene products in vivo as evidenced by enhanced stress protein synthesis and inhibition of synthesis of constitutive proteins. These changes in renal protein synthesis preceded overt renal injury, occurring in the early stages of nephropathy. Altered patterns of stress protein synthesis appeared to be target organ specific. The data suggest that altered protein synthesis patterns may serve as biomarkers of renal injury.

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ABSTRACT: Mercury has been detected by atomic absorption spectrophotometry in unfilled human deciduous teeth and also in rat molars after mercury vapor exposure. Mercury has furthermore been demonstrated on Vervet monkeys beneath silver amalgam fillings by autometallography. The aim of this study was to determine if mercury could be located in rat molars by autometallographic means after mercury vapor exposure. Male, grown-up Wistar rats were exposed to 500 µg/m3 Hg-vapor six hours per day, five days a week for four weeks. They were subsequently killed by transcardial perfusion. The molars were extracted, decalcified and embedded in resin (Agar Aids) before sectioning. The autometallographic development was performed according to the method of Danscher & Moller-Madsen (1985). Mercury deposits were found in small amounts in several areas of the pulp, but with larger accumulations in relation to odontoblasts. Mercury could also be seen in the predentin, and in parts of the dentin near the pulp. Our conclusion is therefore that systemic uptake of mercury vapor leads to inclusion of mercury in the dentin. Furthermore, it seems that mercury accumulates in the odontoblasts before being deposited in the dentin.

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ABSTRACT: LAMMS (Laser Microprobe Mass spectrometric) and ICP-MS (Inductively Coupled Plasma-Mass spectrometric) analyses were performed on 26 necropsies or biopsies taken from human gingiva featuring a direct contact with dental alloys (amalgam casting alloys). Although the selected samples did not show any tissue discoloration, it appeared that about 73% of the whole collective of samples were contaminated with metallic compounds, either as particles or as not-particulated material. In 42.3% of the samples one could unequivocally identify metallic particles. All samples taken near amalgam fillings demonstrated particles featuring (Cu), Ag, Sn and
Hg, i.e. most probably freshly implanted amalgam. Samples in direct contact with casting alloys revealed elements like Au, Pt, Ga, In or a combination thereof and, in some cases, even amalgam related elements (Cu, Ag, Sn, Hg). In about 29% of these samples metallic particles could be identified. Thus, most of the metallic inclusions apparently relate to partly deteriorated materials which have released some of their constituents. Moreover, active degradation of the incorporated material is further indicated by the simultaneous detection of endogenous elements (P, S, Ca, Fe, Se). Such observations agree with the results of earlier LAMMS studies of typically discolorated human oral mucosa (e.g. amalgam tattoos) which have convincingly demonstrated selective leaching out of elements (Hg, Cu). Also, selective associations between exogenous elements and most probably detoxifying endogenous elements have been observed (Innov Tech Biol Med 11, Spe 1:51-60, 1990). In conclusion, since even not-discolored gingiva may be contaminated by metallic compounds, it appears that the burden of gingiva and subsequent tissue reactions are more frequent than usually considered.

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**ABSTRACT:** The anti-carcinogenesis ability of garlic has been speculated for years. Based on our in vivo animal studies, crude garlic extract, better than the garlic fractions, possessed remarkable inhibitory efficacy on carcinogenesis was confirmed. The in vitro experiments were then designed for determining the cancer prevention potential of garlic, and the mechanism of that function as well. To the human oral cancer cell line, crude garlic extract over 3% (w/v) exhibited potent direct cytotoxicity. To the NIH 3T3 fibroblasts, 0.1 and 0.3% garlic extracts efficiently prevented the initial carcinogenesis--cell transformation, and this ability could be kept in the cells up to the third generation of passage. The memory of cancer prevention ability was thus postulated to be on cellular DNA level. The NIH 3T3 fibroblasts were then further treated with the garlic extract for 48 hours, and thereafter cultured with the medium containing 0.5% potent carcinogen DMBA for another 2 days. After foci of cells formed, both the foci and non-foci cells were isolated, massive cultured, and collected separately for DNA, RNA proteins and DNA adduct analyses. The results indicate that crude garlic can be a valuable agent for cancer chemoprevention, and its mechanism must be related to the DNA replication system.

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**FORUM**

The IAOMT Annual Scientific Meeting on Oral Medicine and Toxicology will be held in Pleasanton California at the Pleasanton Hilton on September 25-27, 1992.

Registration Information:
Meeting: (510) 352-5017, Dr. Eccles
Rooms: (510) 463-8000 Pleasanton Hilton You must mention IAOMT for special rate of $90.00/day

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Thursday Night: Welcome Hospitality Suite
Saturday Night: Installation Dinner and Awards
Sunday Morning: IAOMT Board Meeting

**Symposium Topics**

New research into the health of sheep and monkeys with amalgam fillings. Dr. Murray J. Vimy, D.D.S., FIAOMT

The politics of health; FDA & ADA. Michael Ziff, D.D.S., FIAOMT. Exec Dir IAOMT


Academy protocols for safe amalgam removal and personnel protection. David C. Kennedy, D.D.S., FIAOMT, Exec VP IAOMT

Fluoride, Science, and abuse of Science. Royal Lee, M.D.

Clinicians Panel and Legal Questions. Robert Reeves, JD & Aaron Rynd, Ph.D.

The three closest airports are San Francisco, San Jose and Oakland. An airport shuttle is available from all airports. Cost varies from $15.00-20.00.