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## Abstract

Atmospheric testing of nuclear weapons during the 1950s and early 1960s doubled the level of radiocarbon ($^{14}C$) in the atmosphere. From the peak in 1963, the level of $^{14}CO_2$ has decreased exponentially with a mean life of about 16 years, not due to radioactive decay, but due to mixing with large marine and terrestrial carbon reservoirs. Since radiocarbon is incorporated into all living things, the bomb-pulse is an isotopic chronometer of the past half century. The absence of bomb radiocarbon in skeletonized human remains generally indicates a date of death before 1950. Comparison of the radiocarbon values with the post 1950 bomb-curve may also help elucidate when in the post 1950 era, the individual was still alive. Such interpretation however, must consider the age at death of the individual and the type of tissue sampled.


## Introduction

Estimation of time since death of skeletonized human remains represents an important yet difficult issue in forensic anthropology. Research and casework have demonstrated that many variables potentially affect post-mortem alteration of both soft and hard tissues. Usually, it is difficult to determine from morphological information alone, if skeletonized remains represent a postmortem interval of several or many years. This is unfortunate, because it is advantageous to the investigation and identification processes to determine if the remains are ancient and thus not of medico legal interest, or if they are more recent and perhaps can be linked to a missing person.

Traditionally, radiocarbon dating has been considered to be an archeological tool rather than a forensic one. With a half-life of 5730 years, the radiodecay of carbon-14 ($^{14}C$) is minimal within the time periods of interest in forensic cases and applicable for samples over 300 years of age. Radiocarbon analyses of bone traditionally use extracted collagen as the carbon source. Most of these analyses are of samples from archeological sites, often thousands of years old. Collagen is the most common protein in the body, and the largest source of carbon in bone. Procedures to extract and purify collagen are well developed. The mineral part of bone has not traditionally been used in radiocarbon dating due to the low carbon content and potential for mineral exchange of carbonate during long periods of burial.

Radiocarbon levels can be used to estimate time since death in modern forensic cases, utilizing the modern “bomb-curve” (Figure 1). Atmospheric testing of thermonuclear devices between 1950 and 1963 significantly increased the level of $^{14}C$ in the atmosphere and food chain. The testing events nearly doubled levels of $^{14}C$ in terrestrial organisms. In 1963, Great Britain, the United States and the Soviet Union agreed to stop atmospheric testing, although France and China continued until 1980 (Nydal et al. 1979, NRDC, 2002). Despite this variation in testing cessation, laboratory research reveals that artificial radiocarbon in the stratosphere within the northern hemisphere reached maximum concentration in 1963 and subsequently has declined steadily (Berger 1979; Levin and Kromer, 2004). Hua and Barbetti (2004) split the earth into four separate zones to account for mixing of the $^{14}CO_2$ from just a few anthropogenic sources to essentially uniform global distribution by the late 1960s. Through the food chain and related factors, these temporal fluctuations of artificially high levels of radiocarbon have been captured in organic material world-wide and thus offer an opportunity to elucidate time since death issues in recent forensic samples.

The adage “You are what you eat“ holds true. The isotopic content of new plant growth reflects the atmospheric radiocarbon concentration. Herbivore values lag those of the atmosphere slightly because their primary carbon source is on the order of months old. Values of omnivores and carnivores lag the atmosphere further because their carbon source is another step removed. Within organisms, tissues turn over at different rates so $^{14}C$ levels vary between tissues. The date of formation of a tissue or specific biomolecule can be estimated from the bomb-curve by considering these lags in incorporation and relating the $^{14}C$ concentration (y-axis)

with the date (x-axis). Of course, individual human diets vary considerably in the relative proportion of plant and animal foods. Using an annual average of the carbon intake over a growing season can account for much food chain lag while also producing a simpler curve (Figure 2). Caution must be exercised when dating an elevated sample since the pulse is double valued. Placing a sample on the ascending or descending side of the pulse can often be accomplished if an additional tissue (e.g., hair, different bone, tooth) or other information is available. Routine radiocarbon analyses using accelerator mass spectrometry (AMS) are performed on samples containing as little as 200 μg carbon.

## Non-Forensic Applications

The radiocarbon bomb-pulse is most widely used in studies of ocean circulation (Key, 2001) and the carbon cycle. In humans, the bomb-pulse has been used to date pathogenic structures and birth date cells through isotopic analysis of DNA. Stenhouse (1979) used bomb curve radiocarbon analysis to examine the formation time of coronary atherosclerotic lesions and gallstones, documenting that gallstones in one patient had formed at least 20 years before surgical removal. The progressive growth of gallstones has been examined through analysis of their layered morphology (Druffel and Mok, 1983). Senile plaques and neurofibrillary tangles in Alzheimer's disease brain have been dated to examine the progression of the disease (Lovell et al, 2002). Because DNA is synthesized only at the time of cell division, $^{14}C$ analysis of purified DNA can place cells' birthdates on the bomb-curve. Spalding et al (2005) used this approach to show that postnatal neurogensis does not occur in the cortex of the adult human brain.

## Bomb-Curve Radiocarbon Concentrations in Human Tissues

In 1959, Broecker et al. noted that bomb-curve radiocarbon concentrations in human tissues lag behind those of the atmosphere, relating to dietary issues and tissue turnover. They documented a lag time of about 1.1 years for blood and 1.8 years for lung tissue. Libby et al. (1964) further noted that concentrations in human brain tissue can lag atmospheric levels by only a few months, but levels in the collagen within cartilage were delayed considerably. Autopsy study of the cartilage collagen of 70-year-old adults showed little increase of artificial radiocarbon despite living throughout the entire rise of the bomb-pulse from 1955 to 1964. Harkness and Walton (1969) noted the important role that individual diet can play in establishing radiocarbon levels in humans. Nydal et al. (1971) found good agreement in radiocarbon levels between samples of human blood and hair.

## Data from Individuals with Known Birth and Death Dates

In 1972, Harkness and Walton reported radiocarbon levels in various tissues of a 37-year-old female who died of coronary artery disease in 1969. Highest concentrations of $^{14}C$ were found in the brain, followed by muscle, ovaries, kidney, liver, fat, uterus, bone marrow, bone collagen and bone mineral. Bone collagen and mineral contained substantially lower amounts of radiocarbon than the other tissues, apparently reflecting their lower rate of tissue turnover.

Additional comparative data were added in 1977 with a study by Stenhouse and Baxter reporting analyses of different tissues originating from a 72-year-old male with a birth date of 1899 and death date of 1971. Soft tissue levels were all much higher than that of bone collagen.

Wild et al. (2000) published additional data on tissues from four individuals with ages ranging from newborn to 85 years. As with the previous studies, they found $^{14}C$ concentrations consistent with atmospheric levels in tissues with rapid turnover such as lipids or hair while bone collagen exhibited much slower turnover.

Shin et al. (2004) examined radiocarbon levels within six human forensic and surgical bone samples from individuals ranging in age from 19 to 84 years. They estimated cortical bone

turnover at two to eight percent per year and examined variability of radiocarbon within “light” and “heavy” density fractions, which they interpreted to represent recent and longer term bone formations respectively. For individuals below the age of 50 years, the relative values of the two types of bone correlated with placement on the bomb curve. This important study suggests that carbon turnover and recycling varies with bone tissue type and is manifested in radiocarbon concentrations.

Taylor et al. (1989), Ubelaker (2001), Ubelaker and Houck (2002), and Wild et al. (2000) all have called attention to the potential applications of bomb-curve radiocarbon interpretations to determining time since death in modern medico legal cases. Basically, if human tissues are analyzed and the elevated bomb-curve levels of radiocarbon are not detected, those tissues date to time periods prior to the nuclear testing (about 1950). Similarly, elevated levels suggest that the tissues post-date 1950 and the magnitude of the levels provides information regarding placement on the bomb curve. Such analysis has proven useful in applications to forensic cases involving skeletonized human remains in determining ancient (pre-1950) or modern (post-1950) status (Taylor et al. 1989; Ubelaker 2001; Ubelaker and Houck 2002).

As noted by Geyh (2001) and studies cited above, interpretation in forensic contexts must consider the type of tissue as well as patterns of growth and aging, diet, and even medical treatment of pathological conditions. For example, Thompson and Ballou’s 1956 study of rats given doses of a tritium oxide marker suggested that collagen was relatively inert and was not replaced during the lifetime of the animal. Thus analysis of collagen in an elderly individual might reflect the formation time at an earlier age, rather than the age at death.

As noted above, four studies present radiocarbon values derived from tissues of 12 individuals of known birth dates and known death dates or dates of tissue death (surgical samples). These data are summarized in Table 1. The “lab numbers” (if available) are those designated in the individual publications. The “age at tissue death” represents the age of the individual, either at death or at the time the tissue was surgically removed. Similarly, the “tissue death date” either represents the date of death of the individual or the date of surgical removal of the specimen. Carbon-14 levels are given in F$^{14}$C where 1 is the atmospheric $^{14}$C level prior to the industrial revolution (Reimer et al, 2004; Stuiver and Polach, 1977). “Average age of formation” represents the possible ages of the individual when the level of $^{14}$C in the tissue examined corresponds to the appropriate value on the bomb curve. The slow, continuous turnover of bone means this “average age of formation” is accrued over several to many years, depending on the tissue sampled. Note that two possible values for “age at formation” are provided for NOC2, NOC11, NOCX24 and VERA-0024 due to their more recent tissue death dates. The radiocarbon values could correspond to either the earlier, upward side of the bomb curve or its later, downward side. Given the values presented in VERA-0027, the former, earlier dates of formation are much more likely.

Careful examination of Table 1 reveals the powerful role of individual age in age at death/radiocarbon level comparisons. With the obvious exception of the newborn, all other examples present a significant lag time between age of tissue formation and age at death. Furthermore, this lag time increases with increasing age at death. For example, in the 19 year old of FOR2, radiocarbon values of the cortical femur sample suggest an average tissue formation age of 15 years. In contrast, the radiocarbon level of the 85-year-old long bone collagen sample of VERA-0027 suggests an average formation age of 54 years, which in turn implies minimal bone turnover at that site in the final 30 years of the life of that individual. Note however, that the radiocarbon value for the bone collagen sample of the 72-year-old reported by Stenhouse and Baxter (1977) suggests a more rapid bone turnover, at least for that individual in that particular tissue. Surprisingly, the mineral fraction of bone from the 72-year-old exhibited faster turnover than the collagen.

Bone is continuously remodeled during adulthood. In a closely coupled process, osteoclasts resorb old bone and osteoblasts form new bone. Total annual bone turnover is often

estimated at 10%. No forensic investigation samples a whole skeleton, however, and remodeling varies with the type of bone. Approximately 25% of trabecular bone and 3% of cortical bone is believed to be resorbed annually in adults (Manolagas and Jilka, 1995). The extent to which resorbed material is recycled by osteoblasts reduces the carbon turnover in bone. The onset of rapid bone loss (osteoporosis) can discontinue the incorporation of new carbon into bone as the inhibited osteblasts merely recycle material. The onset of rapid bone loss varies with age and sex. It starts to occur in postmenopausal women (~ age 40-50) and 20-30 years later in men. Looking again at Table 1, it is entirely possible that an 85-year-old woman with osteoporosis ceased to add new carbon to her bone the last 30 years of her life. A 72-year-old man is much more likely to have continued to turn over bone carbon, and perhaps show no signs of osteoporosis.

## Conclusions

The information presented above suggests that radiocarbon dating, with special attention to the bomb-curve values, provides a powerful new tool in the important forensic problem of estimating time since death of skeletonized human remains. Artificially high values clearly indicate that the tissue, and thus the individual, was alive in the post-1950 era. Considerable potential exists to fine-tune the estimates of time since death in such analyses with a fuller understanding of the factors that influence the timing of bone turnover in particular tissues and different anatomical areas. Modern AMS procedures require minimal sampling and Lanting et al. (2001) have reported successful dating of the mineral (carbonate) component even of cremated bones. Investigators applying this procedure should remain aware however, that due to possible significant temporal differences between the time of bone formation and levels of atmospheric radiocarbon at the time of death, estimated ages or dates of individual tissues may lag behind actual age at death. This relationship appears to be influenced by diet, medical treatment, growth and remodeling patterns, age at death of the individual, types of tissue sampled regional variation of $^{14}C$ concentrations and other factors. Forensic scientists incorporating radiocarbon analysis into their protocols should have at least a rudimentary understanding of the mechanisms involved and the complex factors that potentially impact interpretation.

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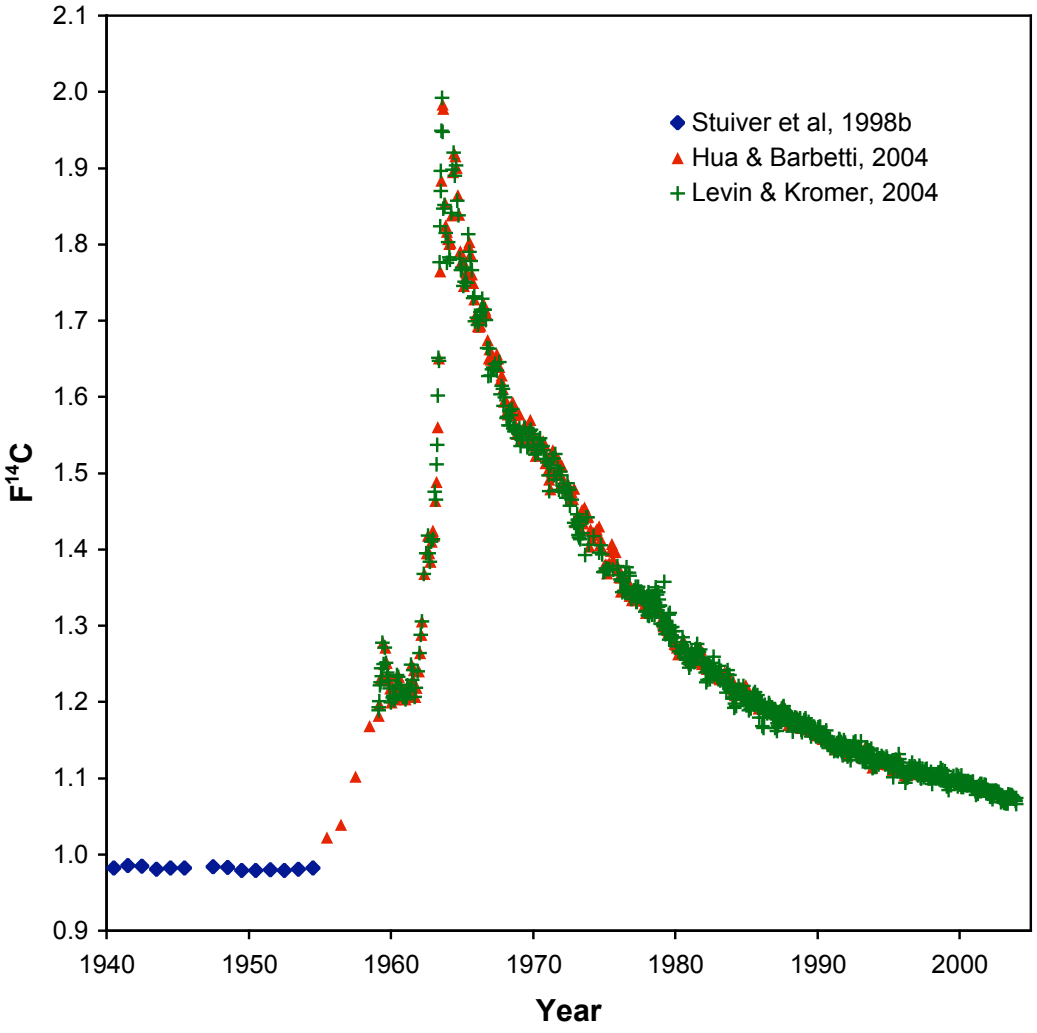


Figure 1. Atmospheric $^{14}CO_2$ concentrations expressed in $F^{14}C$. The graph includes annual averages from 1940-1954 harvested from a Douglas Fir on the Olympic Peninsula (Stuiver et al, 1998b), monthly averages for the mid latitudes of the Northern Hemisphere (Hua and Barbetti, 2004), and approximately biweekly sampling of sites in Europe Levin and Kromer, 2004).

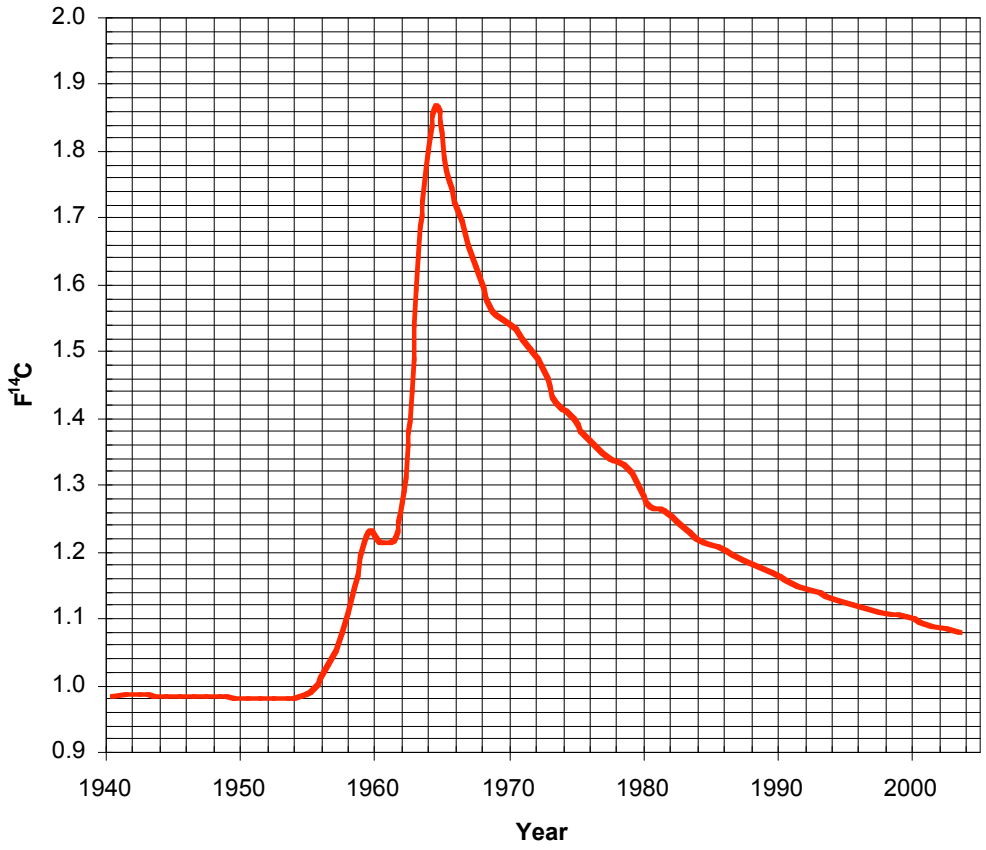


Figure 2. Average annual atmospheric $^{14}CO_2$ record for the Northern Hemisphere. Annual averaging smoothes the curve and reduces the 1963 peak. Adapted from Levin and Kromer (2004) and Stuiver et al, (1998a).

Table 1: $F^{14}C$ values of bone samples reported in the literature. Most samples ae extracted collagen, but the region of the bone (cortical or trabecular is seldom reported).

| Lab Number | Age at Tissue Death | Birth Date | Tissue Death Date | Bone Sampled | $F^{14}C$ | Average Date of Formation | Age at Formation | Reference |
|---|---|---|---|---|---|---|---|---|
| VERA-0021 | Newborn | 1994/95 | 1994/95 | long bone | 1.12 | 1994/95 | Newborn | Wild et al, 2000 |
| FOR2 | 19 | 1947 | 1966 | cortical femur | 1.26 | 1962 | 15 | Shin et al, 2004 |
| VERA-0020 | 30 | 1965 | 1995 | long bone | 1.41 | 1974 | 11 | Wild et al, 2000 |
| NOCX23 | 35 | 1964 | 1999 | tibia/femur compact | 1.20 | 1986 | 22 | Shin et al, 2004 |
| — | 37 | 1932 | 1969 | bone collagen<br>bone mineral | 1.27<br>1.21 | 1962<br>1959 | 30<br>27 | Harkness and Walton, 1972 |
| FOR1 | 42 | 1935 | 1977 | cortical femur | 1.10 | 1958 | 23 | Shin et al, 2004 |
| — | 72 | 1899 | 1971 | bone collagen<br>bone mineral | 1.11<br>1.49 | 1958<br>1963 | 59<br>64 | Stenhouse and Baxter, 1977 |
| NOC11 | 79 | 1920 | 1999 | femoral head | 1.10 | 1958 or 1998 | 38 or 78 | Shin et al, 2004 |
| NOC2 | 83 | 1915 | 1998 | proximal tibia | 1.14 | 1958 or 1992 | 43 or 77 | Shin et al, 2004 |
| NOCX24 | 84 | 1915 | 1999 | proximal tibia | 1.12 | 1958 or 1994 | 43 or 79 | Shin et al, 2004 |
| VERA-0024 | 85 | 1903 | 1988 | long bone collagen | 1.18 | 1959 or 1988 | 56 or 85 | Wild et al, 2000 |
| VERA-0027 | 85 | 1904 | 1989 | long bone collagen | 1.12 | 1958 | 54 | Wild et al, 2000 |

Average Annual Tropospheric 14CO2 in Mid-Latitudes of the Northern Hemishere (1940-2003)

Table 2

| Year | Delta14C | pMC | F14C |
|---|---|---|---|
| 1940.5 | -20.2 | 97.98 | 0.9849 |
| 1941.5 | -19.4 | 98.06 | 0.9857 |
| 1942.5 | -19.6 | 98.04 | 0.9855 |
| 1943.5 | -22.5 | 97.75 | 0.9825 |
| 1944.5 | -21.7 | 97.83 | 0.9834 |
| 1945.5 | -22.1 | 97.79 | 0.9829 |
| 1946.5 | -21.6 | 97.84 | 0.9835 |
| 1947.5 | -21.1 | 97.89 | 0.984 |
| 1948.5 | -22.3 | 97.77 | 0.9827 |
| 1949.5 | -24.6 | 97.54 | 0.9804 |
| 1950.5 | -24.8 | 97.52 | 0.9802 |
| 1951.5 | -24.8 | 97.52 | 0.9802 |
| 1952.5 | -24.9 | 97.51 | 0.9801 |
| 1953.5 | -23.9 | 97.61 | 0.9811 |
| 1954.5 | -21.1 | 97.89 | 0.984 |
| 1955.5 | -8.2 | 99.18 | 0.9969 |
| 1956.5 | 26.5 | 102.65 | 1.0318 |
| 1957.5 | 73 | 107.3 | 1.0785 |
| 1958.5 | 140.2 | 114.02 | 1.1461 |
| 1959.5 | 228 | 122.8 | 1.2294 |
| 1960.5 | 212.3 | 121.23 | 1.2139 |
| 1961.5 | 221.6 | 122.16 | 1.2233 |
| 1962.5 | 358.5 | 135.85 | 1.3606 |
| 1963.5 | 718.3 | 171.83 | 1.7212 |
| 1964.5 | 835.7 | 183.57 | 1.869 |
| 1965.5 | 756.3 | 175.63 | 1.7597 |
| 1966.5 | 691.9 | 169.19 | 1.6953 |
| 1967.5 | 623.6 | 162.36 | 1.6271 |
| 1968.5 | 564.5 | 156.45 | 1.568 |
| 1969.5 | 545.4 | 154.54 | 1.5492 |
| 1970.5 | 529.1 | 152.91 | 1.5329 |
| 1971.5 | 499.4 | 149.94 | 1.5033 |
| 1972.5 | 465.6 | 146.56 | 1.4696 |
| 1973.5 | 418.6 | 141.86 | 1.4227 |
| 1974.5 | 400.8 | 140.08 | 1.405 |
| 1975.5 | 369.8 | 136.98 | 1.3741 |
| 1976.5 | 352.5 | 135.25 | 1.3569 |
| 1977.5 | 333.9 | 133.39 | 1.3384 |
| 1978.5 | 325.8 | 132.58 | 1.3302 |
| 1979.5 | 295.8 | 129.58 | 1.3001 |
| 1980.5 | 264.5 | 126.45 | 1.2689 |
| 1981.5 | 256.7 | 125.67 | 1.2615 |
| 1982.5 | 238.3 | 123.83 | 1.2432 |
| 1983.5 | 224.2 | 122.42 | 1.2292 |
| 1984.5 | 209.3 | 120.93 | 1.2144 |
| 1985.5 | 201.3 | 120.13 | 1.2065 |
| 1986.5 | 191.1 | 119.11 | 1.1964 |
| 1987.5 | 182.6 | 118.26 | 1.188 |
| 1988.5 | 173.4 | 117.34 | 1.1789 |
| 1989.5 | 163.5 | 116.35 | 1.1691 |
| 1990.5 | 152.5 | 115.25 | 1.1582 |
| 1991.5 | 142.9 | 114.29 | 1.1487 |
| 1992.5 | 136.4 | 113.64 | 1.1423 |
| 1993.5 | 128.4 | 112.84 | 1.1344 |
| 1994.5 | 122.1 | 112.21 | 1.1282 |
| 1995.5 | 115.5 | 111.55 | 1.1217 |
| 1996.5 | 109.9 | 110.99 | 1.1162 |
| 1997.5 | 104.3 | 110.43 | 1.1107 |
| 1998.5 | 100.3 | 110.03 | 1.1068 |
| 1999.5 | 96.3 | 109.63 | 1.1029 |
| 2000.5 | 88.6 | 108.86 | 1.0953 |
| 2001.5 | 81.6 | 108.16 | 1.0884 |
| 2002.5 | 77.8 | 107.78 | 1.0847 |
| 2003.5 | 71.5 | 107.15 | 1.0785 |