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## Biological Effect of Lead-212 Localized in the Nucleus of Mammalian Cells: Role of Recoil Energy in the Radiotoxicity of Internal Alpha-Particle Emitters<sup>1</sup>

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### Abstract

The radiochemical dipyrrolidinedithiocarbamate-<sup>212</sup>Pb(II) [<sup>212</sup>Pb(PDC)<sub>2</sub>] is synthesized and its effects on colony formation in cultured Chinese hamster V79 cells are investigated. The cellular uptake, biological retention, subcellular distribution and cytotoxicity of the radiocompound are determined. The <sup>212</sup>Pb is taken up quickly by the cells, reaching saturation levels in 1.25 h. When the cells are washed, the intracellular activity is retained with a biological half-life of 11.6 h. Gamma-ray spectroscopy indicates that the <sup>212</sup>Pb daughters (<sup>212</sup>Bi, <sup>212</sup>Po and <sup>208</sup>Tl) are in secular equilibrium within the cell. About 72% of the cellular activity localizes in the cell nucleus, of which 35% is bound specifically to nuclear DNA. The mean cellular uptake required to achieve 37% survival is 0.35 mBq of <sup>212</sup>Pb per cell, which delivers a dose of 1.0 Gy to the cell nucleus when the recoil energy of <sup>212</sup>Bi and <sup>212</sup>Po decays is ignored and 1.7 Gy when recoil is included. The corresponding RBE values compared to acute external <sup>137</sup>Cs  $\gamma$  rays at 37% survival are 4.0 and 2.3, respectively. The chemical Pb(PDC)<sub>2</sub> is not chemotoxic at the concentrations used in this study. Because the  $\beta$ -particle emitter <sup>212</sup>Pb decays to the  $\alpha$ -particle-emitting daughters <sup>212</sup>Bi and <sup>212</sup>Po, these studies provide information on the biological effects of  $\alpha$ -particle decays that occur in the cell nucleus. Our earlier studies with cells of the same cell line using <sup>210</sup>Po (emits 5.3 MeV  $\alpha$  particle) localized predominantly in the cytoplasm resulted in an RBE of 6. These earlier results for <sup>210</sup>Po, along with the present results for <sup>212</sup>Pb, suggest that the recoil energy associated with the <sup>212</sup>Bi and <sup>212</sup>Po daughter nuclei plays little or no role in imparting biological damage to critical targets in the cell nucleus.

### INTRODUCTION

The extreme radiotoxicity of radionuclides that emit  $\alpha$  particles has long been of concern in radiation protection and more recently has attracted considerable attention for therapeutic applications. The biological effects of external  $\alpha$ -particle beams have been well studied in terms of dose response and relative biological effectiveness (RBE) for a variety of end

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points (1–3). Comparatively less experimental data where RBE values are clearly delineated are available on the effects of tissue-incorporated  $\alpha$ -particle emitters (4). A number of studies have been published recently using *in vitro* techniques where the radionuclides were located in the culture medium, thereby irradiating the cells with a spectrum of  $\alpha$ -particle energies (5–10). Other studies have been performed both *in vitro* and *in vivo* to elucidate the RBE of  $\alpha$ -particle emitters specifically incorporated into cells (10–15). However, to the best of our knowledge no data are available on the RBE of  $\alpha$ -particle emitters when most of the cellular activity is incorporated into the nucleus, where the primary radiosensitive targets lie.

In the present work, the radiochemical dipyrrolidinedithiocarbamate- $^{212}\text{Pb}(\text{II})$  [ $^{212}\text{Pb}(\text{PDC})_2$ ] is synthesized and its biological effects in cultured Chinese hamster V79 cells are investigated. The  $\beta$ -particle emitter  $^{212}\text{Pb}$  decays with a 10.64-h half-life to two  $\alpha$ -particle-emitting daughters,  $^{212}\text{Bi}$  and  $^{212}\text{Po}$  (Fig. 1) (16). The cellular uptake, biological retention, subcellular distribution and cytotoxicity of the radiocompound are determined. The  $^{212}\text{Pb}(\text{PDC})_2$  localizes predominantly in the cell nucleus. Therefore, these studies provide information on the biological effects of  $\alpha$ -particle decays which occur in the cell nucleus and thereby shed light on the role of recoil energy associated with  $\alpha$ -particle decay in causing biological damage to the primary radiosensitive targets.

## MATERIALS AND METHODS

### Radiochemistry

Ammonium pyrrolidinedithiocarbamate (APDC) is an excellent chelating agent for heavy metals (17, 18). The structure of its anion ( $\text{PDC}^-$ ) is shown in Fig. 2A. The lead dipyrrolidinedithiocarbamate complex was synthesized with carrier-free  $^{212}\text{Pb}$  that was eluted from a  $^{224}\text{Ra}$  generator (Argonne National Laboratory, Argonne, IL). The generator, a shielded cation exchange column containing 925 MBq of  $^{224}\text{Ra}$ , was eluted with 3 ml of 2 M HCl to obtain the lead complex chloro ion  $\text{PbCl}_4^{2-}$  and subsequently flushed with 3 ml of 0.02 M HCl. The eluate containing the  $^{212}\text{Pb}$  was then passed through a cation exchange column (Dowex 50W hydrogen form, 1 % crosslinked, packed in 5 ml syringe) preconditioned with 0.02 M HCl to remove breakthrough impurities, if any. In addition, the eluent was passed through an anion exchange column (Dowex<sup>TM</sup> 1 $\times$  10–200 mesh, chloride form, 10% crosslinked, packed in 5 ml syringe) preconditioned with  $1 \times 10^{-5}$  M HCl. The resulting eluate was then allowed to stand until secular equilibrium was established with the  $^{212}\text{Pb}$  daughter products (Fig. 1). The  $^{212}\text{Pb}$ ,  $^{212}\text{Bi}$  and  $^{208}\text{Tl}$  activities in the eluate were determined by placing the sample 1.73 cm above an energy-calibrated HpGe detector (Canberra, Meriden, CT) and using the 238 [yield = 0.436 (16), efficiency = 0.0325], 727 [yield = 0.0665 (16), efficiency = 0.0113] and 583 keV [yield = 0.867 (16), efficiency = 0.0145]  $\gamma$ -ray photopeaks of  $^{212}\text{Pb}$ ,  $^{212}\text{Bi}$  and  $^{208}\text{Tl}$ , respectively. This solution (3 ml) was rotary-evaporated to dryness, dissolved in a minimum of absolute ethanol (2.5 ml) and transferred to a pear-shaped 5 ml flask containing a magnetic stir-bar; the activity was assayed, and the molarity of the  $^{212}\text{Pb}$  in solution was calculated.

The ligand solution was prepared by dissolving the ammonium salt of pyrrolidine dithiocarbamate (MW 164) (Aldrich Chemical Co., Milwaukee, WI) in absolute ethanol. A stoichiometric amount of the ligand was added to the reaction vessel and the solution maintained at 50°C for 1 h. The contents of the flask were transferred to a 12  $\times$  75-mm borosilicate glass tube and rotary-evaporated, and the sample was resuspended in sterile deionized water. Finally, the solution containing  $^{212}\text{Pb}(\text{PDC})_2$  was sterilized by filtration through a Gelman 0.22  $\mu\text{m}$  filter and the activity determined using the HpGe detector. It should be noted that bismuth also complexes with PDC to form  $^{212}\text{Bi}(\text{PDC})_3$ , although,

based on half-life considerations, there are about 10 times fewer  $^{212}\text{Bi}$  atoms than  $^{212}\text{Pb}$  atoms at secular equilibrium (18).

The purity of the radiochemical was ascertained by thin-layer chromatography on aluminum-backed silica gel with fluorescence indicator (0.2 mm DC-Karten SI F 254 nm obtained from Riedel-de Haën AG, D-3016 Seelze 1) with acetone:toluene:H<sub>2</sub>O serving as the solvent system (70:24:6). A nonradioactive standard of  $\text{Pb}(\text{PDC})_2$  was prepared with stable lead for which an  $R_f$  of 0.26 was obtained using UV visualization. In contrast,  $R_f$  values of 0.17 and 0.0 were obtained for PDC and  $\text{PbCl}_2$ , respectively. An autoradiograph of the TLC plate also yielded these  $R_f$  values for  $^{212}\text{Pb}(\text{PDC})_2$  and  $^{212}\text{PbCl}_2$ . No spots other than these were observed on the autoradiograph. Ultraviolet absorption spectra for  $\text{PDC}^-$ ,  $\text{Pb}(\text{PDC})_2$  and  $^{212}\text{Pb}(\text{PDC})_2$  are shown in Figs. 3A–C. Note that the UV spectra for  $\text{Pb}(\text{PDC})_2$  and  $^{212}\text{Pb}(\text{PDC})_2$  shown in Figs. 3B and 3C are identical. Elemental analysis of the synthesized nonradiolabeled compound is given in Table I. Finally, the proposed structure of  $\text{Pb}(\text{PDC})_2$  in aqueous solution is shown in Fig. 2B. The structure, based on the chemical analysis data in Table I and valence shell electron pair repulsion (VSEPR) theory, should be a pseudo-5-coordinate trigonal bipyramidal configuration with a non-bonding electron pair located in the trigonal plane.

### Cell Culture

Chinese hamster lung fibroblasts (V79-513 cells) were used in these studies with clonogenic survival serving as the biological end point. The experimental methods and protocols were the same as in our earlier report (11). The cells were cultured in minimum essential medium (MEM) supplemented with 2 mM L-glutamine, 10 mM HEPES, 10% fetal calf serum, 50 U/ml penicillin, 50 µg/ml streptomycin and 0.1 mM nonessential amino acids. The pH of the culture medium was adjusted to 7.0 with  $\text{NaHCO}_3$ . Media and supplements were from Gibco (Grand Island, NY).

### Cellular Uptake of Radioactivity

Determination of the kinetics of cellular uptake established the time required to achieve maximum incorporation of the radiochemical into the cells. Chinese hamster V79 cells, in logarithmic growth as monolayers in 75 cm<sup>2</sup> flasks, were washed two times with 10 ml of phosphate-buffered saline, trypsinized and suspended at  $4 \times 10^5$  cells/ml in calcium-free MEM. Aliquots of 1 ml were placed in sterile Falcon 17 × 100 mm polypropylene round-bottom culture tubes and conditioned on a rocker-roller for 4 h at 37°C in an atmosphere of 95% air and 5% CO<sub>2</sub>. After the conditioning period, 1 ml of calcium-free MEM containing various activities of  $^{212}\text{Pb}(\text{PDC})_2$  was added to the tubes (final concentrations 35.3, 16.8 and 9.67 kBq/ml) and the rolling continued. Aliquots of the cell suspension were taken at different times and the cellular uptake of  $^{212}\text{Pb}$ ,  $^{212}\text{Bi}$  and  $^{208}\text{Tl}$  was determined using the microfuge method of Kassis and Adelstein (19) and the HpGe detector described earlier. Figure 4 shows the fraction of cellular uptake of radioactivity as a function of incubation time. The cellular uptake of radioactivity saturates within 1.25 h. This pattern was independent of the extracellular concentration of  $^{212}\text{Pb}(\text{PDC})_2$  in the culture medium over the range studied.

### Cellular Retention of Radioactivity

Cells were exposed to various concentrations of  $^{212}\text{Pb}(\text{PDC})_2$  (35.3, 16.8 and 9.67 kBq/ml) as described above and the cellular uptake of radioactivity was determined using the microfuge method (19). After an exposure time  $T_1$  of 1.25 h, the cells were washed four times with MEM and resuspended in calcium-free MEM. Aliquots of the cell suspension were then assayed for cellular uptake of radioactivity (this was designated  $t = 0$ ). Approximately 8% of the intracellular activity was removed during the washing process.

The remaining cells containing  $^{212}\text{Pb}$  were distributed among 75  $\text{cm}^2$  flasks containing MEM ( $1\text{--}2 \times 10^6$  cells per flask) and the flasks were incubated under standard conditions. At various times a flask was removed from the incubator, the cells were washed with two 10-ml aliquots of sterile phosphate-buffered saline, and the cells were removed from the flask with trypsin. The cells were resuspended in MEM and the activity per cell of  $^{212}\text{Pb}$  and its daughters was determined.

### Clonogenic Survival of V79 Cells

Ten culture tubes of cells were exposed to various concentrations (0–25.0 kBq/ml) of  $^{212}\text{Pb}(\text{PDC})_2$  as described above. After an exposure time of  $T_1 = 1.25$  h, an 0.8-ml aliquot was extracted from each tube and the extracellular activity concentration and cellular uptake of radioactivity were determined using Ecoscint liquid scintillation cocktail (National Diagnostics, Manville, NJ) and an automatic liquid scintillation counter. The remaining cells were washed four times with 10 ml MEM, resuspended in 2 ml of MEM and seeded in triplicate into Falcon 25  $\text{cm}^2$  flasks for colony formation, and the flasks were incubated for 1 week ( $T_{\text{CF}} = 7$  days) under standard conditions. The resulting colonies were washed three times with normal saline, fixed with methanol and stained with crystal violet. The colonies were then counted; the criterion for cell survival was taken to be its ability to form a colony with 50 or more cells. Additional survival studies were carried out with equimolar concentrations of nonradioactive  $\text{Pb}(\text{PDC})_2$ , freshly prepared from stable lead, to determine whether chemotoxicity contributes to cell death at the concentrations used in the present studies.

### Subcellular Distribution of Activity

The procedures described by Kassis (20) were used to determine the subcellular distribution of activity in the cells. Cells were exposed to the radiochemical as described above. After a 1.25-h exposure to the radiochemical, the cells were washed two times with 10 ml of cold calcium-free salt solution composed of 0.4 mM  $\text{KH}_2\text{PO}_4$ , 0.4 mM  $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ , 0.74 mM  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 5 mM KCl and 0.12 M NaCl. The cells were then suspended in 2 ml of cold sucrose buffer (0.25 M sucrose, 3 mM  $\text{CaCl}_2$ , 50 mM Tris, pH 7.0) and placed on ice for 5 min, and aliquots were taken to determine the cellular activity. The cell membrane was ruptured by adding 2 ml of sucrose buffer containing 2% Triton X-100 and vortex mixing. The resulting cell nuclei were placed on ice for an additional 5 min and vortexed vigorously for 30 s, and aliquots of the suspension were assayed for radioactivity. The nuclei were centrifuged at 2000 rpm for 15 min at 4°C and aliquots of the supernatant (cytoplasmic fraction) assayed. The nuclei were washed once with cold sucrose buffer (40.0 ml) and suspended in 2 ml of the same, and the activity in the nucleus was determined. Activity in the nucleus that was associated with RNA, DNA and proteins was precipitated with cold 10% trichloroacetic acid. DNA-bound activity was precipitated with guanidine-HCl by adding 2 ml of cold 6 M guanidine-HCl to 1 ml of the suspension of cell nuclei and mixing gently with a glass rod. Three milliliters of cold 95% ethanol was added followed by further gentle mixing. The contents of the test tube were transferred to a Gelman Type A-E filter and then the test tube, stirring rod and filter were washed three times with 2 ml cold guanidine-HCl:ethanol solution (1:1). The filter paper was transferred to a test tube and the activity assayed.

## RESULTS

### Cellular Uptake and Retention of Radioactivity

Figure 4 shows the kinetics of cellular uptake of  $^{212}\text{Pb}$ . The same pattern was observed for the  $^{212}\text{Pb}$  daughters, indicating that the intracellular daughters were in equilibrium with the parent during the uptake period. The observation of secular equilibrium throughout the

uptake period may be due to the presence of  $^{212}\text{Bi}(\text{PDC})_3$  in the stock radiochemical solution which in turn is taken up by the cells upon addition to the culture medium. The cellular uptake of radioactivity saturated after about 1.25 h of incubation. A linear least-squares fit of the data in the range 0–1.25 h yields  $f(t) = 0.831t$ , where  $f(t) = A(t)/A(t = 1.25 \text{ h})$  and  $A(t)$  is the cellular activity at time  $t$  in hours. Integration of  $f(t)$  over the exposure time  $T_1 = 1.25 \text{ h}$  yields the residence time of the activity in the cells during the uptake period  $\tau_1 = 0.65 \text{ h}$ . The dependence of cellular uptake of  $^{212}\text{Pb}$  radioactivity  $A(\kappa)$  on the activity concentration of  $^{212}\text{Pb}(\text{PDC})_2$  in the culture medium  $\kappa$  is shown in Fig. 5. A least-squares fit to a second-order polynomial gives  $A(\kappa) = 0.0257\kappa + 0.00184\kappa^2$ . Finally, Fig. 6 shows the retention of  $^{212}\text{Pb}$  by the V79 cells. The radioactivity was retained with a biological half-life of 11.6 h, which is reasonable given that this was about the same as the cell doubling time for cells of the V79 cell line. The  $^{212}\text{Pb}$  daughters cleared with the same biological half-life, indicating that the intracellular daughters were in equilibrium with the parent during the colony-forming period. Using the physical half-life of  $^{212}\text{Pb}$ , the effective half-life was 5.5 h, and the residence time during the colony-forming period  $\tau_{\text{CF}}$  was 7.9 h.

### Clonogenic Survival of V79 Cells

Figure 7 shows the dependence of cell survival fraction on cellular uptake of radioactivity. No chemotoxicity associated with  $^{212}\text{Pb}(\text{PDC})_2$  was observed even at the highest concentrations used. The survival curve is exponential, indicative of a high-LET radiation type response. A least-squares fit to the data yields a mean lethal uptake of  $0.35 \pm 0.02 \text{ mBq/cell}$ .

### Subcellular Distribution

Subcellular distribution studies revealed that 72% of the  $^{212}\text{Pb}$  was in the nucleus and 28% in the cytoplasm. Approximately 77% of the activity in the nucleus was bound to the RNA, DNA and proteins, and 35% was specifically bound to DNA. Thus 25% of the intracellular activity was bound to DNA in the cell nucleus. The subcellular distribution was independent of the extracellular activity concentration to which the cells were exposed (35.3, 16.8 and 9.67 kBq/ml) and the distribution was the same for the  $^{212}\text{Pb}$  daughters.

### Cellular Dosimetry

As in our earlier work (11), the absorbed dose to the cell nucleus is calculated. There are four contributions to the mean absorbed dose to the cell nucleus: (1) nontarget-to-target dose from extracellular decays that occur in the culture medium during the exposure period  $T_1$ , (2) self-dose from intracellular decays during the exposure period  $T_1$  (3) self-dose from intracellular decays during the colony-forming period  $T_{\text{CF}}$ , and (4) cross-irradiation from decays occurring in neighboring cells of the colony. The contributions from extracellular decays and cross-irradiation from decays in neighboring cells of the colony (11) are small relative to the self-dose contributions and are ignored.

Calculation of contributions (2) and (3) requires the cumulated activity in the cells during the uptake ( $\tilde{A}_1$ ) and colony-forming ( $\tilde{A}_{\text{CF}}$ ) periods. The cumulated activity during the uptake period is simply  $\tilde{A}_1 = A_1\tau_1 = 2.34 \times 10^3 A_1 \text{ Bq s}$ , where  $A_1$  is the cellular uptake in becquerels at the end of the 1.25-h incubation period. Similarly, the cumulated activity during the colony-forming period is  $\tilde{A}_{\text{CF}} = \tau_{\text{CF}} A_1 = 2.84 \times 10^4 A_1 \text{ Bq s}$ . Hence the total cumulated  $^{212}\text{Pb}$  activity in the cell is  $3.07 \times 10^4 A_1 \text{ Bq s}$ , which corresponds to about 11 intracellular decays of  $^{212}\text{Pb}$  at 37% survival.

The absorbed dose to the cell nucleus  $D_{\text{N}}$  from intracellular decays of  $^{212}\text{Pb}$  in equilibrium with its daughters is given by

$$D_N = (\tilde{A}_I + \tilde{A}_{CF}) \cdot \left( f_N \sum_j b_j S_{j,N \leftarrow N} + f_{Cy} \sum_j b_j S_{j,N \leftarrow Cy} \right), \quad (1)$$

where  $b_j$  is the branching ratio for the  $j$ th radionuclide in the cell (see Table II), and  $f_N$  and  $f_{Cy}$  are the fraction of cellular activity in the nucleus and cytoplasm, respectively. The quantities  $S_{j,N \leftarrow N}$  and  $S_{j,N \leftarrow Cy}$  are the cellular  $S$  values (absorbed dose per unit cumulated activity) for the  $j$ th radionuclide localized in the nucleus and cytoplasm, respectively. The  $S$  values for  $^{212}\text{Pb}$ ,  $^{212}\text{Bi}$ ,  $^{212}\text{Po}$  and  $^{208}\text{Tl}$  are taken from Goddu *et al.* (21) for our V79 cells (cell diameter = 10  $\mu\text{m}$ , nuclear diameter = 8  $\mu\text{m}$ , ref. 11) and tabulated in Table II. Substituting  $f_N = 0.72$ ,  $f_{Cy} = 0.28$ , and the branching ratios and  $S$  values given in Table II into Eq. (1) yields the total absorbed dose to the cell nucleus  $D_N = 2.87 \times 10^3 A_I \text{ Gy/Bq}$ , where  $A_I$  is in becquerels. Accordingly, the survival data in Fig. 7 are replotted as a function of the total absorbed dose to the cell nucleus. The dose–response curve (Fig. 7) is what would be expected after exposure to high-LET radiation with the survival fraction given by  $\text{SF} = \exp(-\alpha D)$ , where  $\alpha = 1.0 \pm 0.1 \text{ Gy}^{-1}$ . The corresponding dose required to achieve 37% survival ( $D_{37}$ ) is  $1.0 \pm 0.1 \text{ Gy}$ .

## DISCUSSION

### RBE of Intracellular $^{212}\text{Pb}$ in Equilibrium with $^{212}\text{Bi}$ , $^{212}\text{Po}$ and $^{208}\text{Tl}$

Determination of the RBE of intracellular  $^{212}\text{Pb}(\text{PDC})_2$  in equilibrium with its daughters requires dose–response relationships for the radiocompound as well as the reference radiation ( $^{137}\text{Cs}$   $\gamma$  rays). The dose response for V79-513 cells irradiated with acute external  $^{137}\text{Cs}$   $\gamma$  rays was given in our earlier report as  $\text{SF} = \exp(-0.086D - 0.040D^2)$ , where  $\text{SF}$  is the survival fraction and  $D$  the absorbed dose (11). The corresponding  $D_{37}$  is  $4.0 \pm 0.5 \text{ Gy}$ . Hence the RBE of the intracellular mixed radiation field ( $^{212}\text{Pb}$ - $\beta$ ,  $^{212}\text{Bi}$ - $\alpha$ ,  $^{212}\text{Po}$ - $\alpha$ ,  $^{208}\text{Tl}$ - $\beta$ ) compared to acute external  $^{137}\text{Cs}$   $\gamma$  rays is  $4.0 \pm 0.7$  at  $D_{37}$ , which is similar to the RBE of  $4.7 \pm 0.5$  obtained previously *in vivo* for  $^{212}\text{Pb}$  in equilibrium with its daughters in the mouse testis model (13). The present RBE of 4.0 is substantially lower than the value of 6 obtained earlier for 5.3 MeV  $\alpha$  particles emitted by intracellularly localized  $^{210}\text{Po}$ -citrate using the same V79 cell culture model (11). Based on the LETs of the particles, this is expected because in the present case 57% of the dose to the cell nucleus is deposited by the 57 keV/ $\mu\text{m}$  (1) (8.8 MeV)  $\alpha$  particle emitted by  $^{212}\text{Po}$  and 40% of the dose is deposited by the 76 keV/ $\mu\text{m}$  (1) (6.0 MeV)  $\alpha$  particle emitted by  $^{212}\text{Bi}$  which have substantially lower LETs than the 83 keV/ $\mu\text{m}$  (1) of the 5.3 MeV  $\alpha$  particle of  $^{210}\text{Po}$ .

Given that less than 3% of the dose to the cell nucleus is deposited by the  $\beta$ -particle-emitting parent  $^{212}\text{Pb}$  in this model, it is appropriate to compare our RBE values with those in the literature for  $^{212}\text{Pb}$  as well as the RBE values for its daughter  $^{212}\text{Bi}$ . Charlton *et al.* (9) obtained a  $D_{37}$  of 1 Gy for V79 cells when  $^{212}\text{Bi}$  was distributed uniformly in the culture medium. This corresponds to an RBE of 5.8 when compared to their  $D_{37}$  for 250 kVp X rays (22). Shadley *et al.* (6) obtained an RBE of 3.8 compared to 250 kVp X rays at  $D_{10}$  for CHO K1 cells when  $^{212}\text{Bi}$  was distributed extracellularly in the culture medium. Kurtzman *et al.* (10) reported an RBE (at  $D_{10}$ ) compared to 15 MeV X rays of about 5 for SHAW human pancreatic carcinoma cells under essentially the same irradiation conditions. Our RBE at  $D_{10}$  for V79 cells containing  $^{212}\text{Pb}$  in equilibrium with its daughters is similar albeit lower with a value of  $2.9 \pm 0.4$ .

### RBE of the $^{212}\text{Bi}$ and $^{212}\text{Po}$ $\alpha$ Particles

The individual RBE values for the 6.0 MeV and 8.8 MeV  $\alpha$  particles of  $^{212}\text{Bi}$  and  $^{212}\text{Po}$  may be estimated using the expression provided in Section 6.3 of ICRP Publication 58 (23)

which relates the RBE of a mixture of high- and low-LET radiations to the RBE values of the individual contributions (23). The RBE of the mixed-field  $R$  is given by the ICRP (23) as

$$R = \sum_i q_i \cdot \text{RBE}_i, \quad (2)$$

where  $\text{RBE}_i$  and  $q_i$  are the RBE and fraction of total dose delivered by radiation component  $i$ , respectively. Hence the RBE of intracellular  $^{212}\text{Pb}$  in equilibrium with its daughters is

$$R = q_{6.0} \text{RBE}_{6.0} + q_{8.8} \text{RBE}_{8.8} + q_{\text{other}} \text{RBE}_{\text{other}}, \quad (3)$$

where the numerical subscripts denote the  $\alpha$ -particle energy and “other” denotes all radiations with  $\text{RBE} = 1$  (i.e. photons,  $\beta$  particles, etc.). The quantities  $q_{6.0}$ ,  $q_{8.8}$  and  $q_{\text{other}}$  are given in Table II as 0.40 (>98% of the dose from  $^{212}\text{Bi}$  is from its 6.0 MeV  $\alpha$  particle), 0.57 and 0.03, respectively. The quantity  $R$  is simply the experimental RBE value of 4.0. Only the RBE values of the 6.0 and 8.8 MeV  $\alpha$  particles remain unknown. The data of Barendsen *et al.* (1) for irradiation of cultured human cells with  $\alpha$ -particle beams indicate that the RBE of a 6.0 MeV  $\alpha$  particle is about 1.3 times greater than the RBE of an 8.8 MeV  $\alpha$  particle. It is not unreasonable to suggest that a similar value would be obtained for V79 cells; hence one may approximate  $\text{RBE}_{6.0} \sim 1.3 \text{RBE}_{8.8}$ . Using this ratio, one obtains  $\text{RBE}_{6.0} = 4.7$  and  $\text{RBE}_{8.8} = 3.6$  at 37% survival. As expected, based on the LETs of the  $\alpha$  particles, these values are lower than the RBE of 6 for monoenergetic 5.3 MeV  $\alpha$  particles emitted by intracellularly localized  $^{210}\text{Po}$  (11). It should also be noted that these RBE values are similar to those obtained for these radionuclides *in vivo* (13) and *in vitro* using external beams of  $\alpha$  particles (1–3).

### Recoil Energy

The contribution of recoil energy associated with the intracellular  $^{212}\text{Bi}$  and  $^{212}\text{Po}$  decays has not been considered in the dosimetry calculations above. The recoil energies are 169, 108–117 and 103 keV for  $^{212}\text{Po}$ ,  $^{212}\text{Bi}$  and  $^{210}\text{Po}$ , respectively (24). The ranges of these recoiling nuclei are on the order of 100 nm in water with an LET of about 1600–1800 keV/ $\mu\text{m}$  (25). Clearly, with such short ranges, recoil energy will have little or no biological impact when the decay occurs in the cytoplasm. However, recoil energy could in principle contribute to the overall biological effect of  $\alpha$ -particle emitters when the decay occurs in the cell nucleus, particularly on the DNA. The mean recoil energy released per decay of  $^{212}\text{Bi}$  and  $^{212}\text{Po}$  is  $6.71 \times 10^{-15}$  and  $2.43 \times 10^{-14}$  Gy kg/Bq s (24). Taking account of the branching ratios (Table II) and that 72% of the cellular activity is localized in the nucleus, the combined recoil energy deposited in the nucleus by the  $^{212}\text{Bi}$  and  $^{212}\text{Po}$  decays corresponds to an absorbed dose per unit cumulated activity of 0.065 Gy/Bq s. Using this quantity and the total cumulated activity in the cell, one obtains a recoil dose to the cell nucleus of 0.70 Gy at 37% survival. The total dose including recoil energy is then 1.70 Gy and the RBE for  $^{212}\text{Pb}(\text{PDC})_2$  in equilibrium with its daughters is 2.3. This translates to RBE values of 2.1 and 2.7 for the 8.8 and 6.0 MeV  $\alpha$  particles of  $^{212}\text{Po}$  and  $^{212}\text{Bi}$ , respectively. This is in contrast to the values 3.6 and 4.7 when recoil energy is ignored.

What are the correct RBE values? Technically, the RBE values which include recoil energy are more appropriate because the doses used to compute the RBE value should take into account all energy deposited in the cell nucleus. However, in the context of assessing the biological effects of the recoil energy, it may be more appropriate to compare both sets of RBE values with our data obtained previously for 5.3 MeV  $\alpha$  particles emitted by cytoplasmically localized  $^{210}\text{Po}$ -citrate using the same experimental model (11). In the  $^{210}\text{Po}$

experiments 28% of the intracellular activity was localized in the cell nucleus, and only about 10% of the nuclear activity was bound to DNA (11). Recoil energy was not included in our reported RBE value of 6 for  $^{210}\text{Po}$ -citrate (11). When recoil is included in the calculations, an RBE of about 5 is obtained for  $^{210}\text{Po}$ . Hence, when recoil is not included, RBE values of 3.6, 4.7 and 6 are obtained for the 8.8, 6.0 and 5.3 MeV  $\alpha$  particles of  $^{212}\text{Po}$ ,  $^{212}\text{Bi}$  and  $^{210}\text{Po}$ , respectively. When recoil is included, the corresponding RBE values are 2.1, 2.7 and 5, respectively. As discussed above, the higher RBE observed for the 5.3 MeV  $\alpha$  particles is expected based on the LETs of the radiations (13); however, in the case of recoil energy, the factor of nearly two between the RBE for the 6.0 and 5.3 MeV  $\alpha$  particles is unreasonably large compared to our earlier data obtained in the mouse testis model (13) and *in vitro* data in the literature (1). Furthermore, the RBE values of 2.1 and 2.7 at  $D_{37}$  for the 8.8 and 6.0 MeV  $\alpha$  particles are very low compared to values obtained for V79 cells under different experimental conditions (9) as well as the values obtained in the mouse testis model (13). In contrast, the RBE values calculated without including recoil energy are in line with expectations (5, 13, 22). These considerations suggest that the recoil energy plays little or no role in the biological damage from intranuclearly localized  $\alpha$ -particle emitters, particularly because if the recoil energy was effective and not included in the dose calculations, the RBE should be substantially higher than expected.

There are a number of plausible explanations for the apparent ineffectiveness of the recoil energy. One possible explanation is simply “overkill” owing to the extremely high LET (1600–1800 keV/ $\mu\text{m}$ , ref. 25) of the recoiling nucleus. This may be understood better by considering Barendsen’s (26) theoretical calculations which suggest that an  $\alpha$  particle will interact with 50 sites on the DNA molecule during its traversal across the cell nucleus. In the present case, about 11 intracellular decays of  $^{212}\text{Pb}$  are required to deliver the 37% survival dose of 1 Gy. This corresponds to about 1.7 MeV of energy deposited in the cell nucleus excluding recoil energy. About 40 (0.68 MeV) and 57% (1.02 MeV) of this energy is delivered by the 6.0 and 8.8 MeV  $\alpha$  particles, respectively. This is equivalent to about 1 complete traversal across the cell nucleus by the 6.0 MeV  $\alpha$  particles plus 2 traversals by the 8.8 MeV particle, for a total of 3 traversals. Thus 3 traversals at 50 interactions per traversal corresponds to about 150 interactions with the DNA. Meanwhile, the 11 disintegrations required to achieve 37% survival result in only about 8 recoil events in the cell nucleus. With 35% of the activity in the cell nucleus bound to DNA, only about 3 of these events occur on the DNA. Therefore, the local damage imparted by these few recoil events may be overshadowed by the damage caused by the 150 interactions between the  $\alpha$  particles and the DNA. Although this explanation appears to be most plausible, it is possible that the location of the decay relative to the DNA and mode of binding to the DNA could also be factors. That is, perhaps none of the  $^{212}\text{Bi}$  and  $^{212}\text{Po}$   $\alpha$ -particle decays occur on the DNA if the ligand is destroyed as a consequence of the parent  $^{212}\text{Pb}$   $\beta$ -particle decay. Under this scenario, the recoil energy may be wasted.

## CONCLUSIONS

The lethality of dipyrrolidinedithiocarbamate- $^{212}\text{Pb}$ (II) in Chinese hamster V79 cells is investigated. The  $^{212}\text{Pb}$  localizes predominantly in the cell nucleus where it is in secular equilibrium with its daughters  $^{212}\text{Bi}$ ,  $^{212}\text{Po}$  and  $^{208}\text{Tl}$ . Ninety-seven percent of the absorbed dose to the cell nucleus is delivered by the  $^{212}\text{Bi}$  and  $^{212}\text{Po}$   $\alpha$  particles when the recoil energy associated with the intracellular  $^{212}\text{Bi}$  and  $^{212}\text{Po}$  decays is ignored. The mean lethal absorbed dose ( $D_{37}$ ) to the cell nucleus is 1.0 Gy, and there is no chemotoxicity associated with the radiocompound. The RBE at  $D_{37}$  compared to  $^{137}\text{Cs}$   $\gamma$  rays is 4.0 when recoil energy is ignored and 2.3 when the recoil energy is included. These data, when compared to our data obtained previously for 5.3 MeV  $\alpha$  particles from cytoplasmically localized  $^{210}\text{Po}$ ,



suggest that recoil energy does not play a significant role in imparting biological damage when  $\alpha$ -particle emitters are localized in the cell nucleus.

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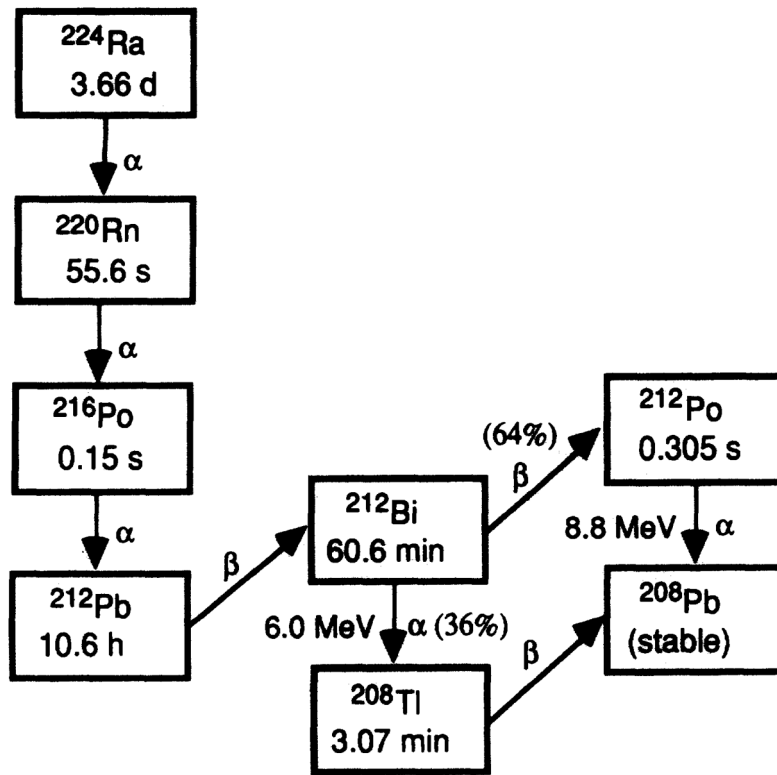
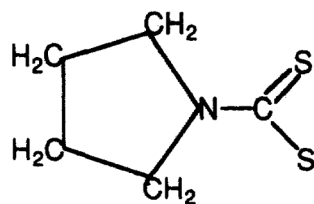
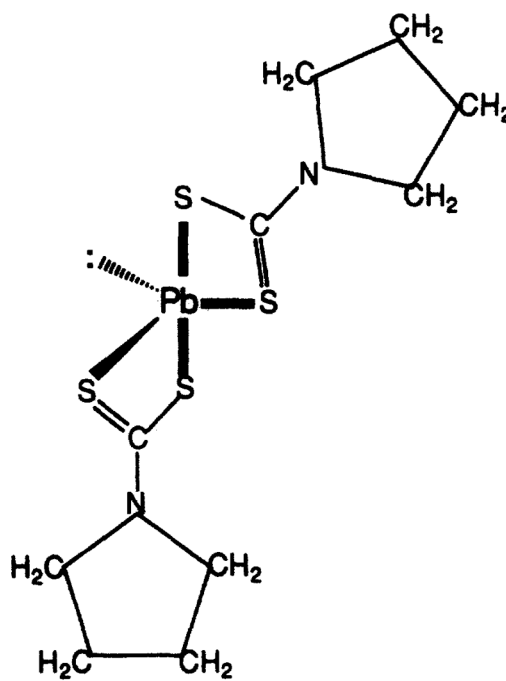
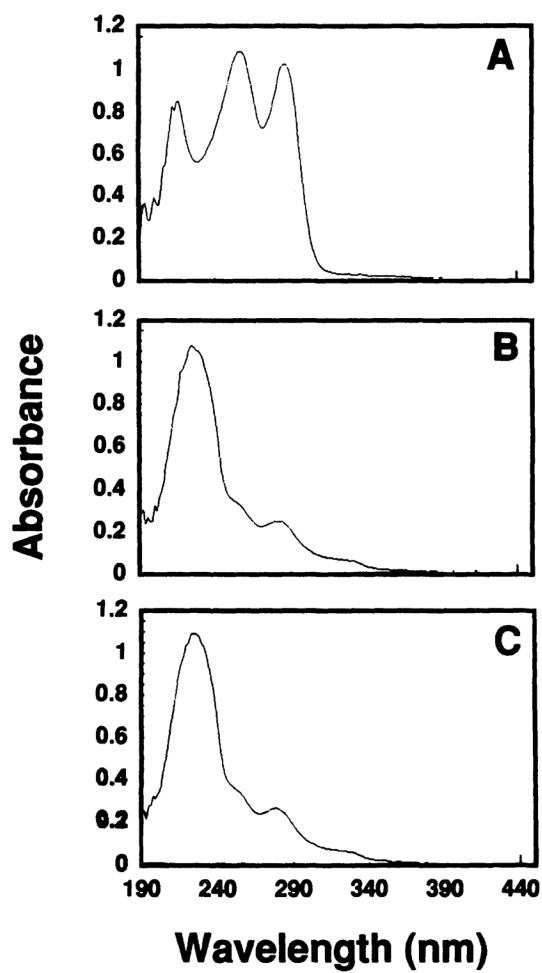


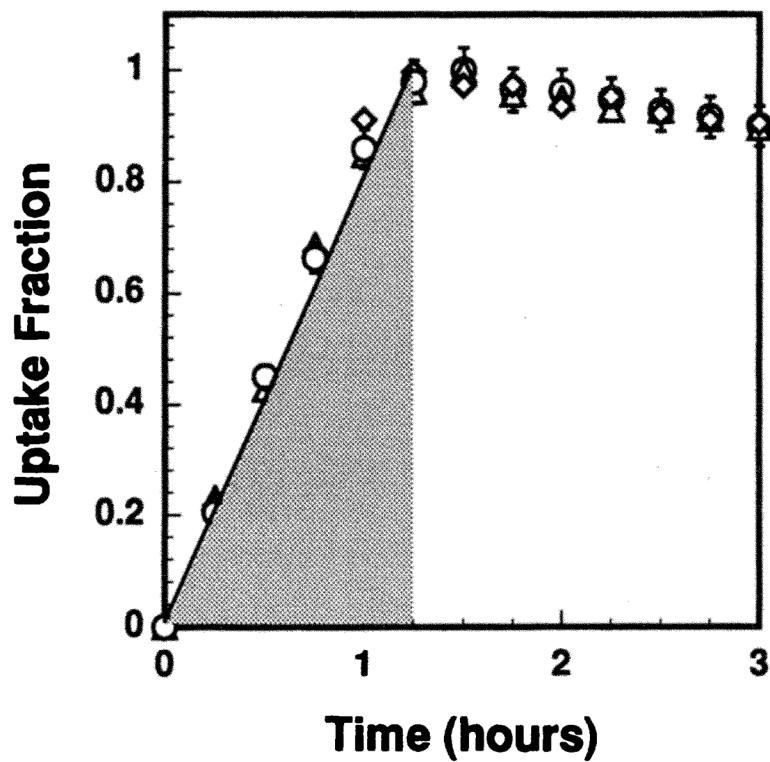
FIG. 1.  
Radium-224 decay series (16).

**A****B****FIG. 2.**

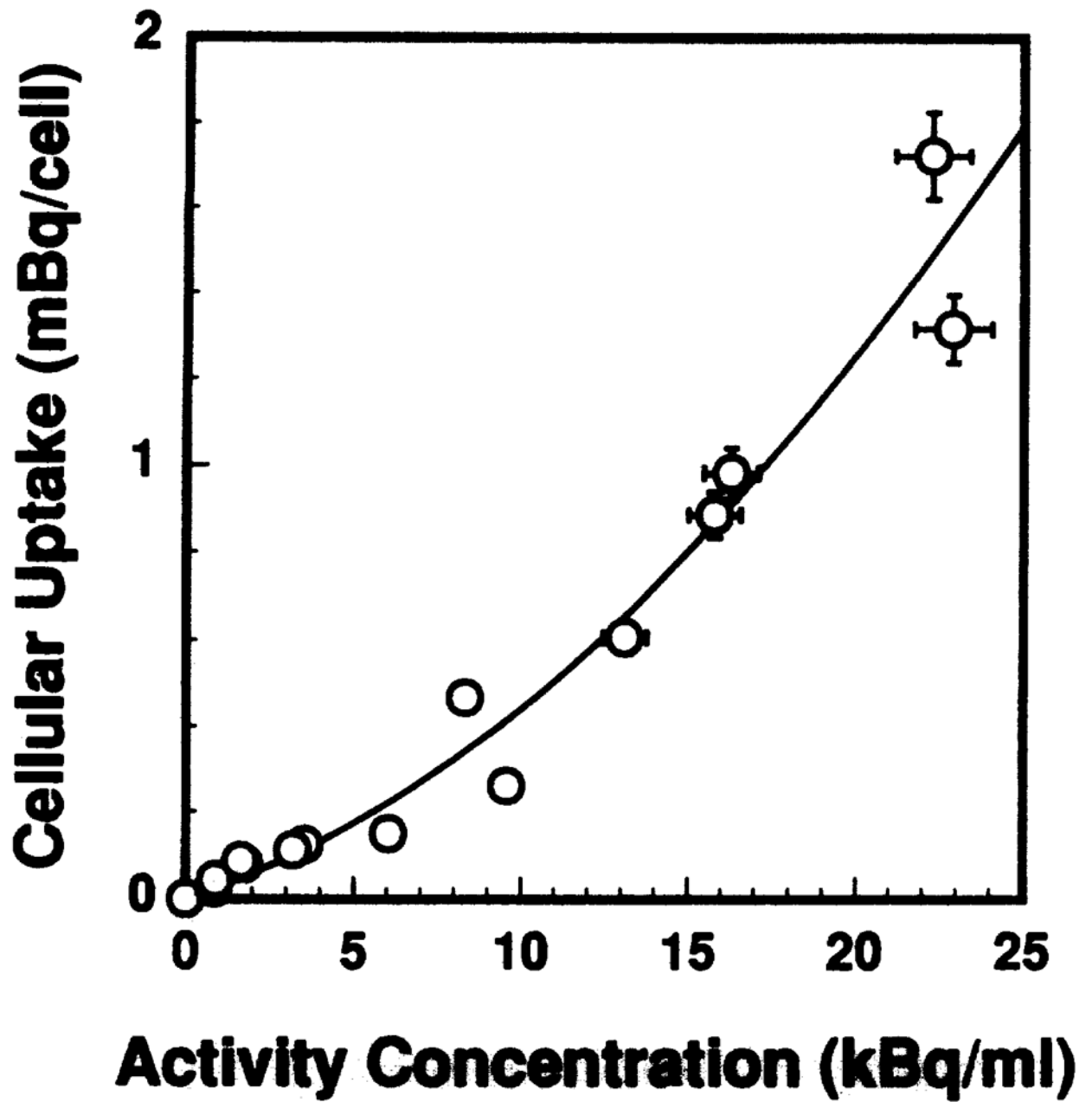
A: Molecular structure of pyrrolidinedithiocarbamate anion used as the ligand in the synthesis of  $\text{Pb}(\text{PDC})_2$ . B: Proposed molecular structure of  $\text{Pb}(\text{PDC})_2$  in solution. The structure is a pseudo-5-coordinate trigonal bipyramidal structure with a non-bonding electron pair located in the trigonal plane.



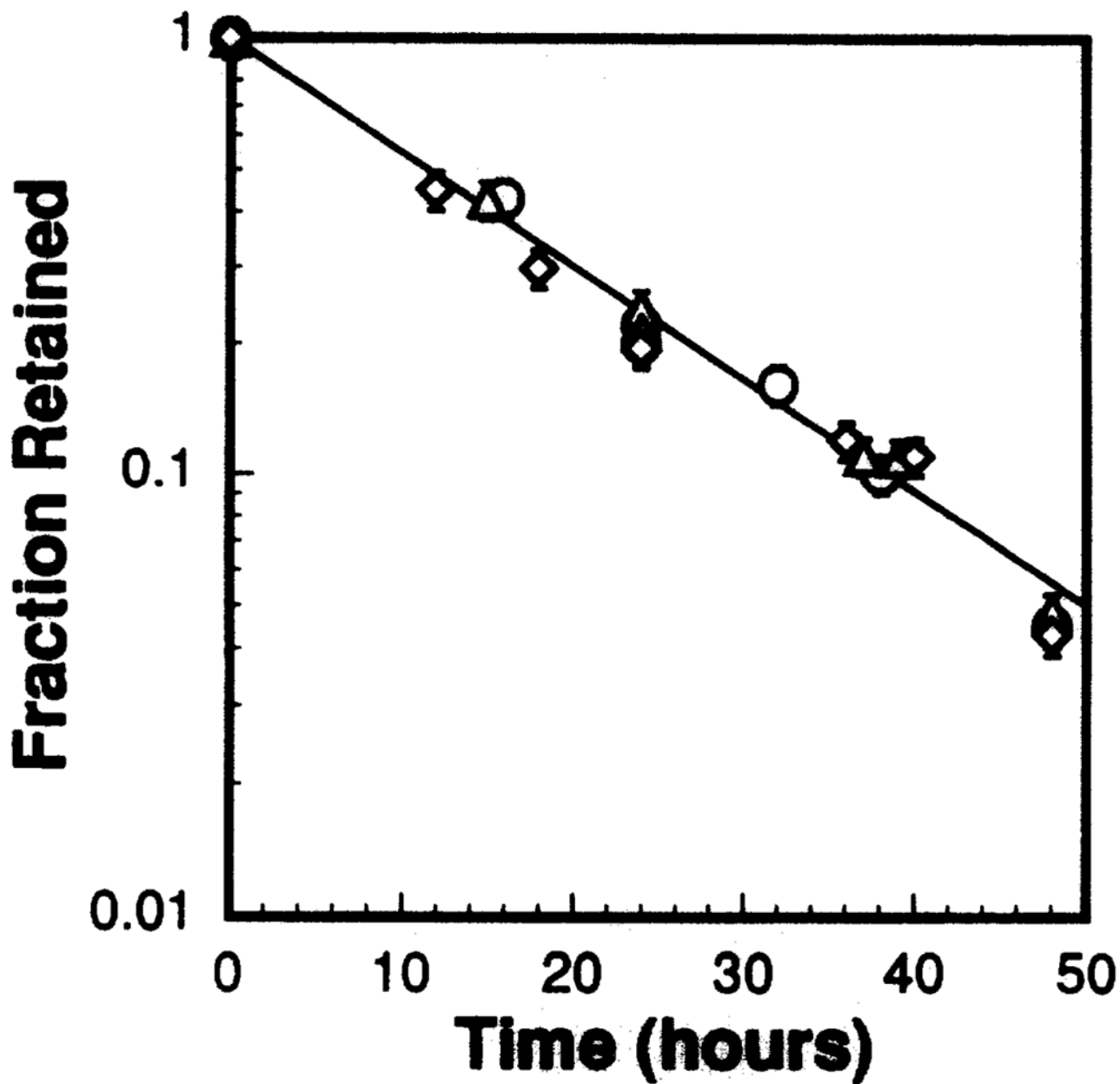
**FIG. 3.** Ultraviolet absorption spectra for (A) ammonium pyrrolidine dithiocarbamate in absolute ethanol, (B) nonradiolabeled  $\text{Pb}(\text{PDC})_2$  complex and (C) radiolabeled  $^{212}\text{Pb}(\text{PDC})_2$ .



**FIG. 4.** Effective uptake kinetics of  $^{212}\text{Pb}$  in V79 cells. The experiment was performed at three different activity concentrations of  $^{212}\text{Pb}(\text{PDC})_2$  in the culture medium: 35.3 ( $\circ$ ), 16.8 ( $\Delta$ ), and 9.67 ( $\diamond$ ) kBq/ml. Over this range, the kinetics of uptake was independent of concentration. Error bars represent the standard deviation of the mean.

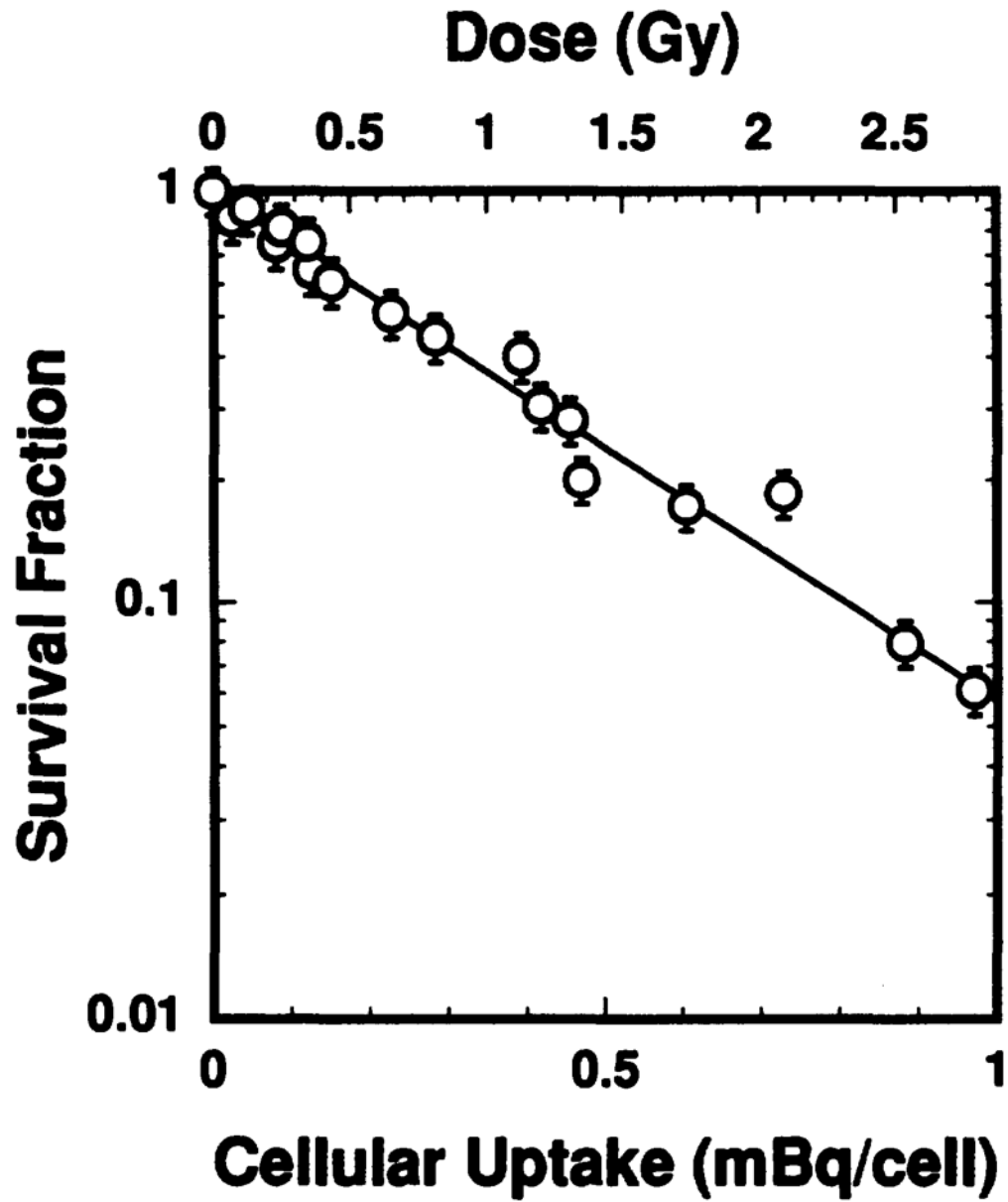


**FIG. 5.** Mean cellular uptake of  $^{212}\text{Pb}$  as a function of extracellular activity concentration of  $^{212}\text{Pb}(\text{PDC})_2$  in the culture medium. The error bars are the standard deviation of the mean.



**FIG. 6.** Kinetics of biological retention of  $^{212}\text{Pb}$  after exposure of V79 cells to three different activity concentrations of  $^{212}\text{Pb}(\text{PDC})_2$  in the culture medium. The kinetics of biological retention was independent of extracellular activity concentration over the range of activities administered: 35.3 ( $\circ$ ), 16.8 ( $\Delta$ ), 9.67 ( $\diamond$ ) kBq/ml. The error bars represent the standard deviation of the mean.





**FIG. 7.** Surviving fraction of V79 cells as a function of mean cellular uptake of radioactivity (lower abscissa) and mean absorbed dose to the cell nucleus (upper abscissa) after exposure to  $^{212}\text{Pb}(\text{PDC})_2$ . The dose response is exponential with a mean lethal uptake of 0.35 mBq/cell and mean lethal dose of 1.0 Gy when the recoil energy of the  $^{212}\text{Pb}$  daughters is ignored.

**TABLE I**Elemental Analysis of Pb(PDC)<sub>2</sub>

<b>Element</b>	<b>Theoretical percentage</b>	<b>Experimental percentage</b>
Lead	41.6	42.0
Carbon	24.0	23.6
Nitrogen	5.60	5.50
Sulfur	25.6	24.8
Hydrogen	3.20	4.10

TABLE II

Cellular Dosimetry for <sup>212</sup>Pb and Daughters

Radionuclide	Branching ratio (16)	$S_{N-N} \text{ (Gy/Bq s)}^{a,c}$	$S_{N-Cy} \text{ (Gy/Bq s)}^{b,c}$	$f_N S_{N-N} + f_C S_{N-Cy} \text{ (Gy/Bq s)}$	Fraction of total cellular dose ( $q_i$ )
<sup>212</sup> Pb	—	$3.29 \times 10^{-3}$	$9.38 \times 10^{-4}$	$2.63 \times 10^{-3}$	0.028
<sup>212</sup> Bi	1	$4.40 \times 10^{-2}$	$1.98 \times 10^{-2}$	$3.72 \times 10^{-2}$	0.40
<sup>212</sup> Po	0.64	$9.83 \times 10^{-2}$	$4.45 \times 10^{-2}$	$5.33 \times 10^{-2}$	0.57
<sup>208</sup> Tl	0.36	$4.96 \times 10^{-4}$	$2.18 \times 10^{-4}$	$1.51 \times 10^{-4}$	0.0016
Total	—	—	—	$9.33 \times 10^{-2}$	1

<sup>a</sup> Dose to the V79 cell nucleus per unit cumulated activity in the cell nucleus. Data taken from ref. (21).

<sup>b</sup> Dose to the V79 cell nucleus per unit cumulated activity in the cytoplasm. Data taken from ref. (21).

<sup>c</sup>  $S_i$  values are for V79 cells (diameter of cell = 10 μm, diameter of cell nucleus = 8 μm, ref. 11).