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Osteosarcoma Risk after Simultaneous Incorporation of the Long-Lived Radionuclide ^{227}Ac and the Short-Lived Radionuclide ^{227}Th[1,2]

W. A. MÜLLER, A. B. MURRAY, U. LINZNER, AND A. LUZ

GSF—Institut für Pathologie, D-8042 Neuherberg, Federal Republic of Germany

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The effect of injection of 1.85 kBq/kg of the long-lived radionuclide ^{227}Ac on the induction of osteosarcomas in female NMRI mice by different dose levels (18.5, 74, and 185 kBq/kg) of the short-lived radionuclide ^{227}Th was investigated. The highest absolute osteosarcoma incidence was observed with the highest doses of ^{227}Th. Addition of ^{227}Ac resulted in an additional osteosarcoma incidence only at the lowest dose of ^{227}Th and did not affect the osteosarcoma incidence resulting from higher doses of ^{227}Th. The longest times to tumor appearance were observed with ^{227}Ac alone. The latent period in two different age groups (4 weeks and 10–12 weeks) appeared to be similar following injection with combined doses of ^{227}Th and ^{227}Ac but different after injection of each radionuclide alone.

INTRODUCTION

The chance of incorporating several radionuclides simultaneously is not negligible; such an occurrence is possible in accidents in the occupational field, with medical treatment, and also at the natural level. For this reason most national and international authorities have issued risk calculations for mixtures of incorporated radionuclides and have suggested measures for the decorporation of radionuclide mixtures. In general the conservative estimate assumes that any resulting effect would be no more harmful than the addition of the known effects of the single components (*1–3*).

The precise effect of combined radiation (additive, less than additive, etc.) probably depends on a number of factors including the radiation quality and/or site of irradiation, dose, dose rate, the state of the biological target, and, in the case of organisms, their age. In the special case of bone-seeking α-emitters considered here, preliminary results from earlier experiments indicated that the incorporation of low levels of a long-lived radionuclide, as might occur from contamination, could have a marked effect on the induction of osteosarcoma by higher doses of a short-lived radionuclide (*4, 5*). The following experiments were designed to test whether addition of a low dose from the long-lived radionuclide ^{227}Ac to varying but much higher doses from the short-lived ^{227}Th had an effect in the long term on the induction of osteosarcoma by the major component. The effects were found to depend on the level of ^{227}Th.

MATERIALS AND METHODS

(a) Animals

Female NMRI mice 4 weeks of age were used in the first experiment. They were injected with ^{227}Ac (1.85 kBq/kg), ^{227}Th (185 kBq/kg), or a mixture of the two radionuclides.

In the second experiment, 10-week-old animals were used to avoid the differences in retention which may occur during the extensive skeletal growth in the juvenile. The ^{227}Ac and ^{227}Th injections were separated by a period of 2 weeks, to allow determination of ^{227}Ac and ^{227}Th retention by whole-body measurements. Three different levels of ^{227}Th were administered alone or together with ^{227}Ac: 18.5, 74, and 185 kBq/kg. The activity of ^{227}Ac was the same in all experiments (1.85 kBq/kg).

The animals were kept under standardized conditions and were checked 6 days each week. The animals were killed when moribund, i.e., when a severe neoplastic or nonneoplastic lesion of any type (including, for example, lymphomas and lung tumors) was detected clinically. All animals were autopsied. Only those osteosarcomas that could be detected by radiographs and/or by clinical examinations were used to compare the incidences in different groups. All osteosarcomas were confirmed histologically.

(b) Injection Solutions and Dosimetry

Two solutions were prepared for the combination experiments:

(1) Actinium-227, a long-lived β-emitter (half-life 21.7 years) with α-emitting daughter products (^{227}Th–^{223}Ra, etc.), was purchased from Amersham–Buchler, Inc. The solution was allowed to reach equilibrium before injection, thus ensuring that the daughter activities followed the half-life of the long-lived parent ^{227}Ac. The activity was calibrated by means of the γ-line of ^{227}Th at 238 keV with a Ge-Semiconductor device. The solution was prepared in citrate form (pH 3.5) as is usual for actinide injections (*6, 7*).

(2) Thorium-227, an α-emitter with an 18.7-day half-life, was separated from the ^{227}Ac stock solution using the method described in (*8*). The solution for injection was also in citrate form.

[1] In association with EURATOM (Contract No. BI6-0080-D).

[2] This paper is dedicated to Professor Wolfgang Gössner on the occasion of his 70th birthday.

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TABLE I
Activities, Doses, and Final Tumor Incidences in the Young (4-Week-Old) Animal Groups

Groups	Nuclides	Activity (kBq/kg)	No. of animals	Skeletal dose (Gy)	Bone sarcomas % unadjusted	Bone sarcomas % corrected for competing risks[a]
C	Controls	0	50	0	0	0
A	^{227}Ac	1.85	50	1.2	12	18.1
T	^{227}Th	185	50	10.0	54	60.8
A T	^{227}Ac + ^{227}Th	1.85 + 185	50	11.2	68	64.8

[a] Value for 10 surviving animals (see text).

All injections were carried out intraperitoneally. The skeletal doses were calculated as mean average values as described in earlier papers (*9, 10*). Whole-body measurements were performed to check the retention of the radionuclides.

The injection schedules are shown in Tables I and III. The results are shown in the tables in terms of unadjusted percentages or crude incidences, that is, the number of osteosarcoma-bearing animals per total number of animals at the start of the experiment, and also final percentages corrected for competing risks according to Kaplan and Meier (*11*). Figures 1 and 3–7 show the cumulative incidence of osteosarcoma, corrected according to Kaplan and Meier (*11*). The unadjusted percentages are of qualitative interest but can be misleading since they do not take into account the effects of earlier deaths from other causes, i.e., the loss of animals that might otherwise have developed a bone tumor. The correction for competing risks allows for the effect of earlier deaths in some groups. The causes of early death were similar in all the experimental groups. The analyses were stopped when only 10 animals survived since the last cases are always overvalued when using a correction formula, resulting in a probability of 100% risk in the final interval if the last animal dies with a tumor (*11*).

The curves were analyzed by means of a log-rank test, based on the theories of Mantel (*12*) and Peto and Peto (*13*) as modified by Kellerer and Chmelevsky[3] (*14*). The mean tumor appearance times (identical to median times in all groups) are given in Tables II and IV, together with the P values from the log-rank tests.

RESULTS

(a) Four-Week-Old Animals

Table I shows data and results from this experiment and Table II the results of the statistical assessment. The percentage of osteosarcoma-bearing animals after combined treatment was approximately additive, i.e., about the same as the sum of the values in the groups with ^{227}Th or ^{227}Ac alone. The P values indicated significant or highly significant differences in times of tumor appearance between all four experimental groups.

Figure 1 shows the cumulative incidence of osteosarcomas in the three groups. Addition of ^{227}Ac resulted in an earlier occurrence of tumors marked by a surprisingly steep increase in osteosarcoma incidence in the period 300–400 days. This was much earlier than the occurrence of osteosarcomas in the ^{227}Ac alone group which was more than 600 days after injection (Controls = 0). Osteosarcoma incidence did not start to increase in the ^{227}Th group until after 400 days after injection, so that for the first 600 days addition of ^{227}Ac resulted in a marked increase in osteosarcoma incidence, although the final cumulative incidence in the combined and ^{227}Th alone groups was about the same.

(b) Ten- to Twelve-Week-Old Animals

A second series of combination experiments was performed using different levels of the radionuclides and with some improvements and modifications as described under Materials and Methods.

The results of the whole-body measurements are given in Fig. 2. The pattern of retention in the injected material is consistent among the experimental groups.

Tables III and IV and Figs. 3–5 show details and results of these experiments.

In the group administered 1.85 kBq/kg ^{227}Ac and 18.5 kBq/kg ^{227}Th, the time to appearance of osteosarcomas was significantly ($P < 0.0260$ using the log-rank test) shorter than in the thorium-alone group (Fig. 3). There was a higher

TABLE II
Statistical Assessments of Experiments with 4-Week-Old Mice

P_i	Experimental groups	ta_i (Days)	P_j	Experimental groups	ta_j (Days)	P_{ij}
	C	No case		A	869 (819–919)	0.010
<0.0001			<0.0001			
	T	633 (585–681)		A T	560 (494–627)	0.0155

Note. ta_i, mean tumor appearance time without ^{227}Ac contamination; ta_j, mean tumor appearance time with ^{227}Ac contamination. Confidence limits in parentheses. P_i, P_j, P values from log-rank test between the vertical-listed experimental groups; P_{ij}, P values from log-rank test of the combination with ^{227}Ac. Experimental groups as in Table I.

[3] The authors thank A. Kellerer and D. Chmelevsky for valuable advice and for making their log-rank computer program available.

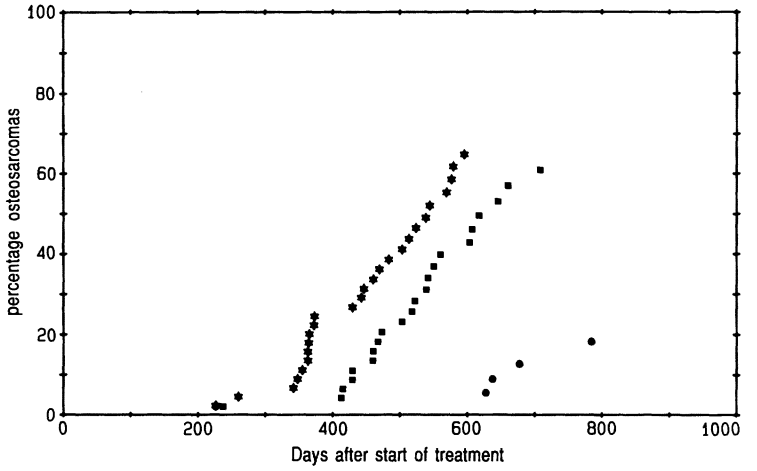

FIG. 1. Cumulative incidence of osteosarcomas in 4-week-old NMRI mice, corrected for competing risks. (●) 1.85 kBq/kg ^{227}Ac; (■), 185 kBq/kg ^{227}Th; (*) ^{227}Ac + ^{227}Th.

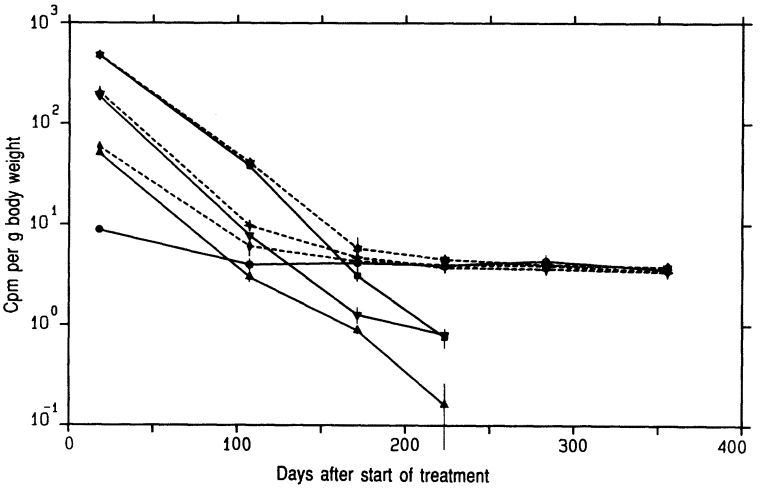

FIG. 2. Retention of ^{227}Ac and ^{227}Th in 10- to 12-week-old mice after single and combined injection of different amounts. (●) 1.85 kBq/kg ^{227}Ac; (▲) 18.5 kBq/kg ^{227}Th; (▼) 74 kBq/kg ^{227}Th; (■) 185 kBq/kg ^{227}Th; (♦) 1.85 kBq/kg ^{227}Ac + 18.5 kBq/kg ^{227}Th; (+) 1.85 kBq/kg ^{227}Ac + 74 kBq/kg ^{227}Th; (*) 1.85 kBq/kg ^{227}Ac + 185 kBq/kg ^{227}Th.

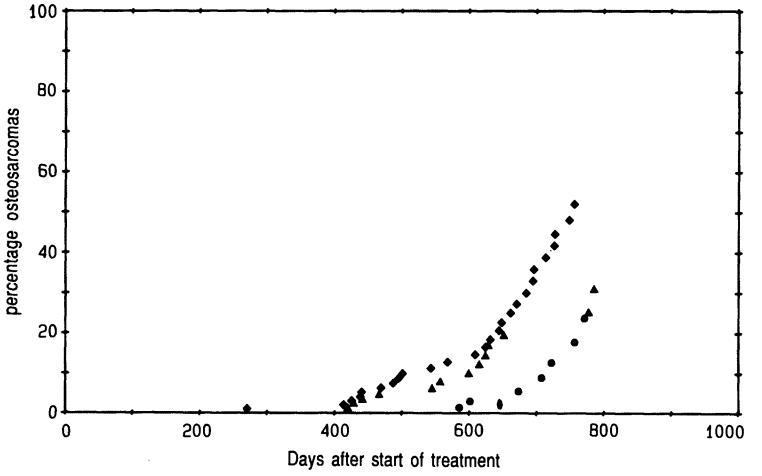

FIG. 3. Cumulative incidence of osteosarcomas in 10- to 12-week-old NMRI mice with ^{227}Ac and/or 18.5 kBq/kg ^{227}Th, corrected for competing risks. (●) Controls; (●) ^{227}Ac; (▲) ^{227}Th; (♦) ^{227}Ac + ^{227}Th.

TABLE III
Activities, Doses, and Final Tumor Incidences in the Young Adult Animal Groups

Groups	Nuclides	Activity (kBq/kg)	No. of animals	Skeletal dose (Gy)	Bone sarcomas % unadjusted	Bone sarcomas % corrected for competing risks[a]
C	Controls	0	100	0	1	2.2
A	^{227}Ac	1.85	101	1.2	8	23.6
T1	^{227}Th	18.5	100	1.0	16	30.7
A T1	^{227}Ac + ^{227}Th	1.85 + 18.5	103	2.2	30	52.0
T2	^{227}Th	74	101	4.0	47	70.6
A T2	^{227}Ac + ^{227}Th	1.85 + 74	101	5.2	45	64.8
T3	^{227}Th	185	94	10.0	41	59.6
A T3	^{227}Ac + ^{227}Th	1.85 + 185	104	11.2	42	63.1

Note. Age of mice: 10 weeks at ^{227}Ac injection; 12 weeks at ^{227}Th injection.
[a] Value for 10 surviving animals (see text).

rate of tumor appearance between 700 and 800 days in the combined group than in the other two groups added together (Fig. 3). Interestingly, the time of appearances in the thorium-alone group showed a bimodal distribution: before 650 days and after 750 days.

The addition of 1.85 kBq/kg ^{227}Ac, which adds a dose of about 1.2 Gy, to the dose of 4 Gy from 74 kBq/kg ^{227}Th, had no effect on either the corrected cumulative tumor incidence (Fig. 4), the crude incidence (Table III), or the tumor appearance time (Table IV).

In the group administered 18.5 kBq/kg ^{227}Th, the addition of 1.85 kBq/kg ^{227}Ac resulted in a slightly steeper but not significant increase in osteosarcoma incidence in the second half of the exposure period (Fig. 5, Table IV), but there was no effect on the final corrected cumulative osteosarcoma incidence or the crude incidence (Table III).

TABLE IV
Statistical Assessments of Experiments with 10- to 12-week-old Mice

P_i	Experimental groups	ta_i (Days)	P_j	Experimental groups	ta_j (Days)	P_{ij}
	C	985 (1 animal)		A	906 (847–965)	0.0159
<0.0001			<0.0001			
	T1	805 (765–844)		A T1	749 (704–794)	0.0260
<0.0001			<0.0001			
	T2	629 (581–677)		A T2	612 (583–640)	n.s.
n.s.			0.0150			
	T3	579 (540–617)		A T3	554 (522–585)	n.s.

Note. ta_i, mean tumor appearance time for control and thorium-treated groups; ta_j, mean tumor appearance time for actinium-treated and combination groups. Confidence limits in parentheses. P_i, P_j, P values from log-rank test between the vertical-listed groups; P_{ij}, P values from log-rank test between single and combined groups; n.s., not significant, i.e., $P > 0.1$. Experimental groups as in Table III.

DISCUSSION

With thorium alone, tumor incidence was markedly higher in the 74 kBq/kg group than after 18.5 kBq/kg, but no further increase occurred in either the corrected cumulative incidence or percentage of tumor-bearing animals in the 185 kBq/kg group (Fig. 6, Table III). The mean time to tumor appearance in the low-dose group was also longer than in the medium- and high-dose groups. Thus it seems that for ^{227}Th (with half-life of 18.7 days), applications of radioactivities above the level of 74 kBq/kg do not induce higher osteosarcoma incidences. A similar relationship was observed earlier in a large-scale experiment using single injected doses of ^{227}Th between 18.5 and 1850 kBq/kg (*15, 16*).

Addition of a low level of ^{227}Ac affected the corrected cumulative incidence of osteosarcoma induction by ^{227}Th only at the lowest level of ^{227}Th tested. The combination effect at this level was the same as or lower than the sum of the effects of the two radionuclides given separately. It seems likely that addition of a low level of radioactivity of a long-lived component can affect the osteosarcoma incidence only in the range in which induction by the short-lived component is still increasing, i.e., at low levels of the short-lived component. As Fig. 5 shows, the only effect of adding a low level of ^{227}Ac to the highest level of ^{227}Th was a shortening of the latent period in the late survival period. The lack of effect of combining low levels of ^{227}Ac with the two highest thorium levels, as shown in Figs. 4 and 5, is also reflected in the p_{ij} log-rank values in Table IV, which are not significant.

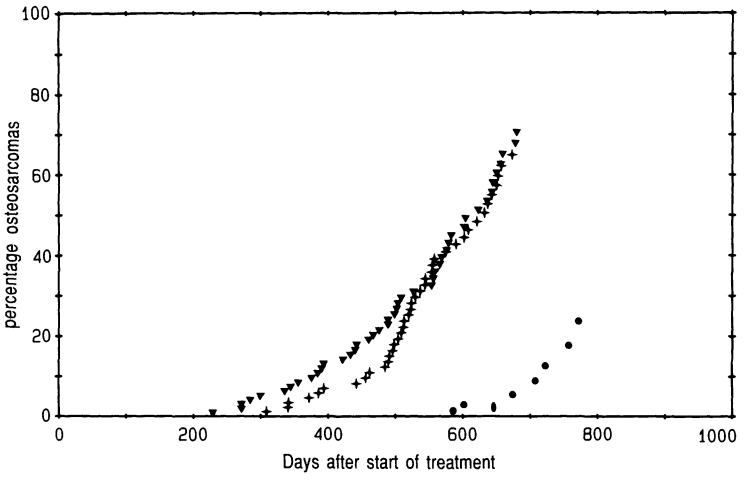

FIG. 4. Cumulative incidence of osteosarcomas in 10- to 12-week-old NMRI mice with ^{227}Ac and/or 74 kBq/kg ^{227}Th, corrected for competing risks. (●) Controls; (●) ^{227}Ac; (▼) ^{227}Th; (+) ^{227}Ac + ^{227}Th.

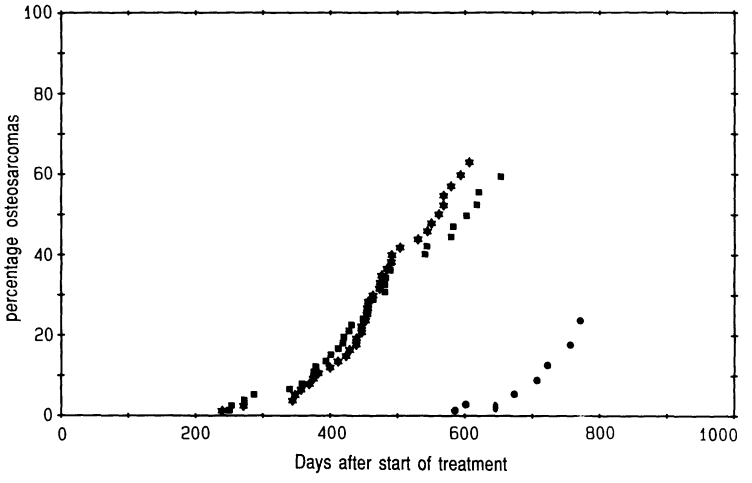

FIG. 5. Cumulative incidence of osteosarcomas in 10- to 12-week-old NMRI mice with ^{227}Ac and/or 185 kBq/kg ^{227}Th, corrected for competing risks. (●) Controls; (●) ^{227}Ac; (■) ^{227}Th; (*) ^{227}Ac + ^{227}Th.

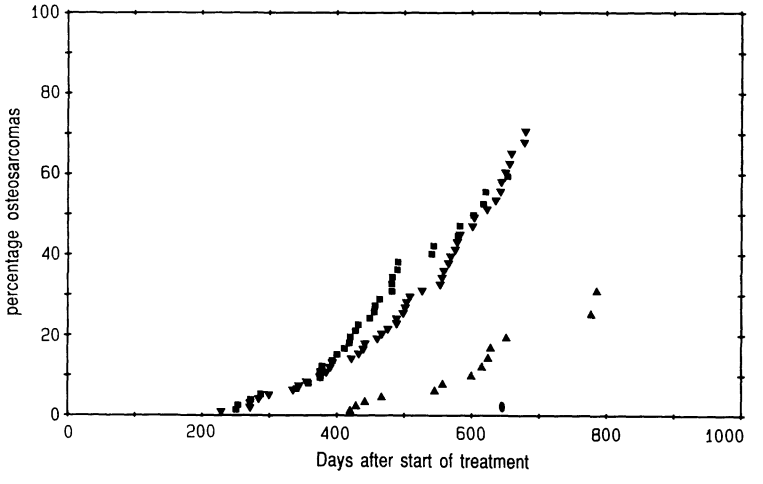

FIG. 6. Cumulative incidence of osteosarcomas in mice after injection of different doses of thorium alone, corrected for competing risks. (●) Controls; (▲) 18.5 kBq/kg ^{227}Th; (▼) 74 kBq/kg ^{227}Th; (■) 185 kBq/kg ^{227}Th.

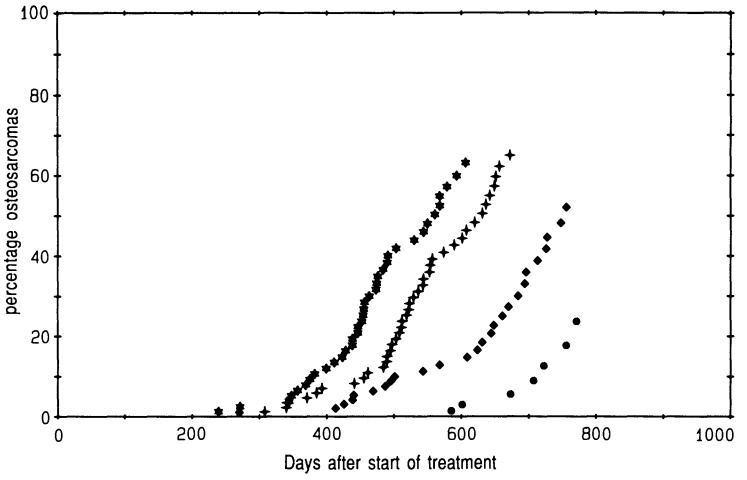

FIG. 7. Cumulative incidence of osteosarcomas in mice after injection of the different ^{227}Ac/^{227}Th dose combinations used in this experiment, corrected for competing risks. (●) ^{227}Ac alone; (♦) ^{227}Ac + 18.5 kBq/kg ^{227}Th; (+) ^{227}Ac + 74 kBq/kg ^{227}Th; (*) ^{227}Ac + 185 kBq/kg ^{227}Th.

Figure 6 shows the corrected cumulative incidences in all the experimental groups which received different levels of ^{227}Th alone. There was no clear dose–response relationship with tumor appearance time; similarly, in the log-rank test (p_i values in Table IV), the p value between groups T_2 and T_3 was not significant. In contrast, there was a clearly dose-dependent action for all levels of thorium when combined with a low level of ^{227}Ac, i.e., a shorter latent period was observed with increasing ^{227}Th dose, as shown in Fig. 7. In other words, the effect of a particular dose from actinium on osteosarcoma induction by thorium depends on the dose from thorium. These relationships were confirmed by the significant log-rank values (p_j in Table IV).

The corrected cumulative osteosarcoma incidence was the same in the groups treated with 185 kBq/kg thorium alone at 4 or 12 weeks, although the unadjusted percentage of tumor-bearing animals was higher in the younger group, but the time to tumor appearance in the older group was somewhat shorter, resulting in a similar age at appearance of the tumors (Tables II and IV). This effect has been described previously (*17*). Actinium alone also resulted in the same tumor incidence in both age groups, but in a longer time to tumor appearance in the older animals. Combination of the two radionuclides resulted in a "canceling out" of the opposing effects; in other words, the tumor appearance time after administration of a combination of the two radionuclides was the same in both age groups. The effects of age on tumor induction have been discussed in more detail in a recent paper (*18*).

The results of these experiments show that the combined incorporation of two α-emitting radionuclides at the levels of radioactivity studied has a lower biological effect than the sum of the effects of the components administered singly. However, the effect of adding a low level of a long-lived component to a higher level of a short-lived component is variable, and depends on the level of the short-lived component. The less-than-additive effect observed is in good agreement with experiments dealing with incorporation of a mixture of β-emitters, in which the effects are also less than additive (*19, 20*).

Thus our results show that the recommendation of the ICRP (*1*), which is known to be conservative for β-emitters, is also conservative in the case of α-emitters, at least at the levels of radioactivity studied.

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REFERENCES

1. ICRP, *Limits for Intakes of Radionuclides by Workers.* Pergamon, Oxford, 1978.
2. N. D. PRIEST and A. BIRCHALL, The calculation of bone doses from alpha-emitting bone surface seeking radionuclides for radiological protection purposes. Background: Problems of bone dosimetry. *Radiat. Environ. Biophys.* **23,** 149–153 (1984).
3. V. VOLF, *Treatment of Transuranium Elements,* Technical Report Series 184. IAEA, Vienna, 1978.
4. W. A. MÜLLER, E. H. SCHÄFFER, U. LINZNER, and A. LUZ, Incorporation experiments with combined application of different bone seekers. *Radiat. Environ. Biophys.* **23,** 113–115 (1984).
5. A. LUZ, U. LINZNER, W. A. MÜLLER, E. DE FRIES, and W. GÖSSNER, Osteosarcoma induction by simultaneous incorporation of ^{227}Th and ^{227}Ac. In *Biological Implications of Radionuclides Released from Nuclear Industries,* pp. 141–151. IAEA, Vienna, 1979.
6. G. N. TAYLOR, C. W. MAYS, R. D. LLOYD, P. A. GARDNER, L. R. TALBOT, and S. MCFARLAND, Comparative toxicity of ^{226}Ra, ^{239}Pu, ^{241}Am, ^{249}Cf, and ^{252}Cf in C57Bl/Do black and albino mice. *Radiat. Res.* **95,** 584–601 (1984).

7. N. D. PRIEST, G. R. HOWELLS, D. GREEN, and J. W. HAINES, Initial deposition pattern of actinides 91 to 96 in the skeleton of the rat. In *Metals in Bone* (N. D. Priest, Ed.), pp. 175–197. MTP Press, Lancaster, 1985.
8. W. A. MÜLLER, Präparative Arbeiten über ^{227}Ac und seine Folgeprodukte. *Radiochim. Acta* **9,** 181–186 (1968).
9. W. A. MÜLLER, Studies on short-lived internal alpha-emitters in mice and rats. Part II. ^{227}Th. *Int. J. Radiat. Biol.* **3,** 233–243 (1971).
10. A. LUZ, W. A. MÜLLER, W. GÖSSNER, and O. HUG, The dependence of late effects in mice on the temporal distribution of skeletal alpha-doses from ^{224}Ra and ^{227}Th. In *Biological Effects of ^{224}Ra* (W. A. Müller and H. G. Ebert, Eds.), pp. 135–148. Nijhoff, The Hague/Boston, 1978.
11. E. L. KAPLAN and P. MEIER, Nonparametric estimation from incomplete observations. *J. Am. Statistical Assoc.* **53,** 457–481 (1958).
12. N. MANTEL, Evaluation of survival data and two new rank order statistics arising in its consideration. *Cancer Chemother. Rep.* **50,** 163–179 (1966).
13. R. PETO and J. PETO, Asymptotically efficient rank invariant test procedures. *J. Roy. Statistical Soc. Ser. A* **135,** 185–206 (1972).
14. A. M. KELLERER and D. CHMELEVSKY, Small-samples properties of censored-data rank tests. *Biometrics* **39,** 675–682 (1983).
15. W. A. MÜLLER, W. GÖSSNER, O. HUG, and A. LUZ, Late effects after incorporation of the short-lived alpha-emitters ^{224}Ra and ^{227}Th in mice. *Health Phys.* **35,** 33–55 (1978).
16. W. GÖSSNER, Biological effects of thorium. In *Radionuclide Metabolism and Toxicity* (P. Galle and R. Masse, Eds.), pp. 273–280. Masson, Paris, 1982.
17. A. LUZ, W. A. MÜLLER, E. SCHÄFFER, A. B. MURRAY, U. LINZNER, and W. GÖSSNER, The sensitivity of female NMRI mice of different ages for osteosarcoma induction with ^{227}Thorium. *Strahlentherapie,* Suppl. **80,** 178–182 (1986).
18. A. LUZ, W. A. MÜLLER, U. LINZNER, A. B. MURRAY, R. R. WICK, and W. GÖSSNER, Die Zeit als Kofaktor des Strahleninduzierten Knochentumors. In *Fortschritte der Osteologie in Diagnostik und Therapie* (F. H. W. Heuck and E. Keck, Eds.), pp. 119–125. Springer-Verlag, Berlin/Heidelberg, 1988.
19. A. S. MONAKHOV, A cytogenetic study of rat lymphocytes affected by ^{45}Ca and ^{131}I delivered separately and in combination. *Radiobiologyia* **28,** 381–384 (1988).
20. V. L. SHVEDOV, Radiation affection caused by a combination of ^{89}Sr and ^{131}I repeatedly delivered to body. *Radiobiologyia* **28,** 415–419 (1988).