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Carcinogenic Responses of Transgenic Heterozygous *p53* Knockout Mice to Inhaled $^{239}PuO_2$ or Metallic Beryllium*

GREGORY L. FINCH, THOMAS H. MARCH, FLETCHER F. HAHN, EDWARD B. BARR, STEVEN A. BELINSKY, MARK D. HOOVER, JOHN F. LECHNER, KRISTEN J. NIKULA, AND CHARLES H. HOBBS

Inhalation Toxicology Laboratory, Lovelace Biomedical and Environmental Research Institute, Lovelace Respiratory Research Institute, Albuquerque, New Mexico 87185

ABSTRACT

The transgenic heterozygous $p53^{+/-}$ knockout mouse has been a model for assessing the tumorigenicity of selected carcinogens administered by noninhalation routes of exposure. The sensitivity of the model for predicting cancer by inhaled chemicals has not been examined. This study addresses this issue by acutely exposing $p53^{+/-}$ mice of both sexes by nose-only inhalation to either air (controls), or to 1 of 2 levels of $^{239}PuO_2$ (500 or 100 Bq ^{239}Pu) or beryllium (Be) metal (60 or 15 μg). Additional wild-type $p53^{+/+}$ mice were exposed by inhalation to either 500 Bq of $^{239}PuO_2$ or 60 μg of Be metal. These carcinogens were selected because they operate by differing mechanisms and because of their use in other pulmonary carcinogenesis studies in our laboratory. Four or 5 of the 15 mice per sex from each group were sacrificed 6 mo after exposure, and only 2 pulmonary neoplasms were observed. The remainder of the mice were held for life-span observation and euthanasia as they became moribund. Survival of the $p53^{+/-}$ knockout mice was reduced compared to the $p53^{+/+}$ wild-type mice. No lung neoplasms were observed in $p53^{+/-}$ mice exposed to air alone. Eleven of the $p53^{+/-}$ mice inhaling $^{239}PuO_2$ developed pulmonary neoplasms. Seven $p53^{+/+}$ mice exposed to $^{239}PuO_2$ also developed pulmonary neoplasms, but the latency period for pulmonary neoplasia was significantly shorter in the $p53^{+/-}$ mice. Four pulmonary neoplasms were observed in $p53^{+/-}$ mice exposed to the higher dose of Be, whereas none were observed in the wild-type mice or in the heterozygous mice exposed to the lower dose of Be. Thus, both $p53^{+/-}$ and $p53^{+/+}$ mice were susceptible to ^{239}Pu-induced carcinogenesis, whereas the $p53^{+/-}$ but not the $p53^{+/+}$ mice were susceptible to Be-induced carcinogenesis. However, only 2 pulmonary neoplasms (1 in each of the $^{239}PuO_2$ exposure groups) were observed in the 59 $p53^{+/-}$ mice that were sacrificed or euthanatized within 9 mo after exposure, indicating that the $p53^{+/-}$ knockout mouse might not be appropriate for a 6-mo model of carcinogenesis for these inhaled carcinogens.

Keywords. Inhalation exposure; acute exposure; lung; pulmonary neoplasm; cancer incidence; latency, carcinogenesis; survival; mice, transgenic; *p53* tumor suppressor gene

INTRODUCTION

Rodent bioassays conducted by the National Toxicology Program (NTP) have been the standard toxicologic tool for identifying potential carcinogenic hazards to humans. However, these bioassays are expensive and time consuming. Therefore, attention has been focused on developing useful, shorter-term alternatives to the chronic rodent bioassay used to identify carcinogenesis hazard. One such alternative being studied is the heterozygous, transgenic *p53* knockout mouse model ($p53^{+/-}$), in which 1 *p53* allele has been inactivated. The extent to which this model system can enhance or partially replace the standard rodent carcinogenesis bioassays is the subject of debate (1, 5, 30, 32).

The $p53^{+/-}$ knockout mouse model has been used to examine the carcinogenicity of several chemicals (12, 33, 34), and another $p53^{+/-}$ mouse construct has been used to study the carcinogenicity of exposures to radiation (18). Two potentially significant benefits of transgenic mouse models are that fewer numbers of animals can be used and that tumorigenicity can be detected in a shorter period than in the standard NTP bioassay. Test chemicals have been administered by dermal and oral routes, and tumors have been observed within approximately 6 mo in a variety of tissues including skin, bladder, kidney, liver, ovaries, and mammary tissues (33, 34). This $p53^{+/-}$ knockout model has been shown to be sensitive in detecting genotoxic carcinogens (33, 34).

Inhalation is a major route of exposure in environmentally and occupationally induced cancer. The current study was designed to extend studies of the $p53^{+/-}$ knockout mouse model to the inhaled carcinogens ^{239}Pu and beryllium (Be) metal. Plutonium-239 is an α-emitting radionuclide that, when administered by inhalation in a relatively insoluble form ($^{239}PuO_2$), delivers a protracted, high linear energy transfer (LET) ionizing radiation dose to lung (22). The effects of median body burdens of approximately 500 Bq of ^{239}Pu have not been well characterized in Manhattan Project workers (22, 36), although recent data indicate that mean ^{239}Pu lung burdens of several kBq in Russian workers confer a significant risk for lung cancer (35). The carcinogenicity of inhaled $^{239}PuO_2$ has been demonstrated in rodents (20, 22) and most likely stems from the induction of gene mutations and chromosomal aberrations by the ionizing radiation (22). Beryllium and its compounds are classified as suspected human carcinogens in the United States (6) and as demonstrated human carcinogens in Europe (17). Inhaled Be metal is a strong carcinogen in rats (7, 23) and a weak carcinogen in mice (2, 24, 25). The potential mutagenic

* Address correspondence to: Dr. Gregory L. Finch, Lovelace Respiratory Research Institute, P.O. Box 5890, Albuquerque, New Mexico 87185; e-mail: gfinch@lrri.org.

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TABLE I.—Experimental design for study of carcinogenicity of inhaled Pu or Be in $p53^{+/-}$ and $p53^{+/+}$ mice.

Exposure and target initial lung burden	Mouse used[a]	Carcinogenicity evaluation[b]		Initial lung burden measurement[b]	
		Males	Females	Males	Females
Sham exposure	$p53^{+/-}$	15	15		
$^{239}PuO_2$ 500 Bq	$p53^{+/-}$	15	15		
	$p53^{+/+}$	15	15	3	3
$^{239}PuO_2$ 100 Bq	$p53^{+/-}$	15	15		
	$p53^{+/+}$			3	3
Be metal 60 μg	$p53^{+/-}$	15	15		
	$p53^{+/+}$	15	15	3	3
Be metal 15 μg	$p53^{+/-}$	15	15		
	$p53^{+/+}$			3	3

[a] Heterogygous knockout ($p53^{+/-}$) or wild-type ($p53^{+/+}$) mice.
[b] Number of mice assigned per group.

and genotoxic effects of Be have been examined in a variety of systems (2, 17), and Be is generally not considered to be directly genotoxic (17). For both exposures, accidental human exposures are appropriately modeled by single, acute inhalation exposures of animals. Moreover, because of the limited solubility of both $^{239}PuO_2$ and metallic Be particles, a single acute inhalation exposure results in a protracted exposure as a result of significant lung retention.

We used the $p53^{+/-}$ mouse construct of Donehower et al (4), in which knockout mice derived from the mouse strain 129 were back-crossed into the C57Bl/6 lineage. Heterozygous $p53^{+/-}$ knockout mice were exposed by inhalation to 1 of 2 levels of $^{239}PuO_2$ or Be metal, or to air as controls, and $p53^{+/+}$ wild-type mice were exposed to the highest levels of $^{239}PuO_2$ or Be metal. The principal end point examined was lung cancer.

Materials and Methods

Animals and Experimental Design. This study used 150 heterozygous TSG-*p53*® knockout mice ($p53^{+/-}$; GenPharm International, Mountain View, CA) and 84 wild-type littermates ($p53^{+/+}$). Equal numbers of male and female mice were used. Mice were housed (2-wk quarantine, temperature of 20–24°C, humidity of 40–70%, 12-hr light cycle) in polycarbonate cages with hardwood bedding; males were housed singly and females were housed 2 or 3/cage. Food (Wayne Lab Blox, Allied Mills, Chicago, IL) and water were available *ad libitum* except during the exposure. Approximately 7 days before exposure, mice were weighed (PathTox, Xybion, Ceder Knolls, NJ), randomly assigned by weight to treatment groups, and identified by tail tattoo. All mice were weighed again before exposure and monthly thereafter. Animals were visually observed twice daily for health status, and moribund animals were euthanatized as described below.

The experimental design is given in Table I. Mice designated for initial lung burden (ILB) analysis were sacrificed 7 days postexposure (dpe) for measurement of Pu or Be in the lung as described below. Mice designated for the carcinogenicity evaluation were initially scheduled for sacrifice 6 mo postexposure, and either 4 or 5 of the mice in each group and sex were sacrificed at this time. Because a significant lung carcinogenic effect was not evident, the study was extended to life-span observation of the remaining mice.

Exposures. The selection of 500 Bq as the ILB for ^{239}Pu was based on previous work at our institute in which female C57BL/6J mice inhaled $^{239}PuO_2$ by the nose-only route (20). Exposure to 20, 90, 460, or 2,300 Bq ^{239}Pu decreased survival only at the highest ILB, and tumor incidence was maximum (approximately 5% incidence) in the groups exposed to 90 and 460 Bq. The maximum Be metal ILB of 60 μg was based on studies in our laboratory in which mice were exposed by single acute Be metal inhalation exposure to achieve mean lung burdens ranging from 47 μg (A/J mice) to 64 μg (C3H mice) Be (24, 25). For both carcinogens, 2 dose levels were used to provide dose–response information on lung carcinogenicity. The lower exposure levels were chosen to provide a lung burden several times less than the high-level group while at the same time providing a level that might yield carcinogenic responses.

Mice were exposed in groups to either $^{239}PuO_2$ or Be metal or were sham exposed to filtered air at 8 to 10 wk of age. All exposures were single, nose-only inhalation exposures of durations from 1 to 3 hr, with the exception of the 60-μg target ILB of the Be group, which required daily exposures averaging 2.3 hr/day on 3 consecutive days. All exposures were conducted in 96-port rodent nose-only inhalation exposure chambers (15).

Aerosols of $^{239}PuO_2$ were prepared using the method of Raabe et al (28). Briefly, the ^{239}Pu was precipitated as a colloidal hydroxide, suspended in 0.6 M HCl, then nebulized. Droplets were passed successively through a 300°C tube furnace for drying and a 1,150°C tube furnace for oxidation to $^{239}PuO_2$. The aerosol was then diluted with filtered air and directed into the exposure chamber. Aerodynamic size of the exposure aerosols was measured using cascade impactors, and particle morphology was examined by transmission electron micrography of samples collected with a point-to-plane electrostatic precipitator. Exposure aerosol radioactivity concentrations were measured by ZnS scintillation counting of filter samples collected during each exposure. The $^{239}PuO_2$ aerosol activity median aerodynamic diameter was 1.0 μm, with σ_g = 1.6. For the 500- and 100-Bq ^{239}Pu target ILBs, exposure durations were 75 and 25 min, and mean exposure radioactivity concentrations were 1.1 and 2.0 kBq ^{239}Pu/L, respectively.

The Be metal aerosol was produced using a dry-powder generation method (11, 16). The Be metal powder (Type I-400, Brush Wellman, Elmore, OH) was continuously fed into a dry powder mill (Jet-O-Mizer, Fluid Energy Processing and Equipment Co., Hatfield, PA) to deagglomerate the particles, passed through stage 3 of an aerosol cyclone (effective aerodynamic cut-off of 1.7 μm; InTox Products, Albuquerque, NM), then delivered to the exposure chamber. Particle aerodynamic size was measured by cascade impaction, and concentration was measured gravimetrically from filter samples taken during the exposure. The nominal Be metal mass median aerodynamic diameter was 1.4 μm with σ_g = 1.8 (16). A single inhalation exposure of mice was conducted for the 15-μg Be target ILB, with an exposure duration of 112 min

and a mean exposure concentration of 34 μg Be/L. Three consecutive daily exposures were required to achieve the 60-μg Be target ILB. The mean daily exposure duration was 139 min with a mean daily concentration of 36 μg Be/L.

Initial Lung Burden Measurement. At 7 days postexposure, selected *p53*$^{+/+}$ wild-type mice (Table I) were sacrificed by an intraperitoneal injection of an overdose of sodium pentobarbital followed by exsanguination. The lungs were removed and frozen for either ^{239}Pu or Be analysis. For ^{239}Pu, tissues were alternately wet and dry ashed in 2 M HNO_3; then aliquots of the digest were analyzed for ^{239}Pu content using an extractive liquid scintillation procedure (19). For Be, samples were analyzed following evaporation to dryness, dry ashing, wet ashing, and dissolution in 2 M HNO_3. Aliquots of the digest were measured by flame atomic absorption spectroscopy (AAS; Model 306, Perkin-Elmer, Norwalk, CT) as previously described (8).

Carcinogenicity Evaluation. Either 4 or 5 of the mice in each group and gender were sacrificed 6 mo postexposure. Thereafter, animals were euthanatized when moribund. When the surviving number of *p53*$^{+/-}$ knockout or *p53*$^{+/+}$ wild-type mice reached 10% or less of the original population, the remaining animals were sacrificed and necropsied. This occurred at 19 mo postexposure for the *p53*$^{+/-}$ knockout mice and 22.5 mo postexposure for the *p53*$^{+/+}$ wild-type mice. Sacrifice or euthanasia was performed by an intraperitoneal injection of an overdose of sodium pentobarbital followed by exsanguination. All mice in this portion of the study (those sacrificed, euthanatized, or found dead) received a detailed necropsy in which body and lung weights were recorded. Lungs were inflated with 4% paraformaldehyde by airway perfusion until the pleura was smooth and fixed for at least 24 hr. All nonpulmonary gross lesions and major tissues were preserved in 10% neutral-buffered formalin. After fixation, sections of all tumors and/or standard sagittal sections along the major conducting airways of each lung lobe were cut and embedded in paraffin. A 5-μm-thick section was cut and stained with hematoxylin and eosin for histopathologic evaluation.

Statistical Evaluations. Potential treatment-related effects on body weight, lung weight, and lung-to-body weights were examined by multiple pairwise comparisons (analysis of variance, BMDP P7D, BMDP Statistical Software, Inc., Los Angeles, CA) using a Bonferroni correction for multiple comparisons. Comparisons were made among *p53*$^{+/-}$ knockout, sham-exposed mice and each of the 4 *p53*$^{+/-}$ exposure groups, between the 2 *p53*$^{+/+}$ wild-type exposure groups, and between the *p53*$^{+/-}$ and *p53*$^{+/+}$ mice exposed to either Pu or Be. Estimates of median survival times were generated using a Kaplan-Meier test (BMDP P1L) by group and by sex for mice that died spontaneously or were euthanatized as moribund. For the survival analysis, pairwise comparisons either among groups (to evaluate treatment-related effects) or between males and females within a group (to evaluate gender-related effects) were made using generalized Savage (Mantel-Cox) statistics corrected for multiple comparisons. A similar Mantel-Cox time-to-tumor model (BMDP P1L) was used to examine the difference in latency for ^{239}Pu-induced tumors between the *p53*$^{+/-}$ knockout versus the *p53*$^{+/+}$ wild-type mice. Tumor incidence data were analyzed using a Fisher's exact test (SAS Proc FREQ; SAS Institute, Cary, NC). Statistical tests used a level of significance set at $p \leq 0.05$.

RESULTS

Initial Lung Burdens

Lung burdens of ^{239}Pu and Be measured in 3 male and 3 female wild-type mice sacrificed at 7 days postexposure were approximately 75–90% of targeted levels. Mean burdens of 390 ± 50 Bq (standard deviation) and 76 ± 11 Bq of ^{239}Pu were achieved for the 500- and 100-Bq target ILBs, respectively. Mean burdens of 54 ± 6 μg and 12 ± 4 μg of Be were achieved for the 60- and 15-μg target ILBs, respectively.

Toxicity and Survival

No treatment-related effects on body weight were observed. Lung weights and lung-to-body weight ratios appeared to be elevated for *p53*$^{+/-}$ knockout mice exposed to Be metal, but this effect was only marginally significant ($0.05 < p < 0.10$) for the sham versus the 60-μg Be groups. Median survival times (MSTs) were variable and ranged from 374 to 557 days of age for *p53*$^{+/-}$ knockout mice and from 464 to 672 days of age for *p53*$^{+/+}$ wild-type mice (Fig. 1). In general, *p53*$^{+/+}$ wild-type mice tended to survive longer than *p53*$^{+/-}$ knockout mice, exposure to Be tended to decrease survival time, and males survived longer than females within the treatment group. The only statistically significant ($p < 0.05$) differences were increased MSTs in *p53*$^{+/+}$ versus *p53*$^{+/-}$ female mice receiving 500 Bq $^{239}PuO_2$ and increased MSTs of male versus female *p53*$^{+/-}$ mice receiving 100 Bq of ^{239}Pu.

Histopathology and Carcinogenicity

The incidence of pulmonary neoplasms observed are shown in Table II. Data for male and female mice in each group were pooled for statistical analysis because there were no apparent gender-related differences in neoplastic responses. The incidence of lung neoplasms resulting from exposure to Be was marginally greater in *p53*$^{+/-}$ knockout mice than *p53*$^{+/+}$ wild-type animals ($p = 0.056$). Five primary lung neoplasms were observed in the 4 neoplasm-bearing *p53*$^{+/-}$ mice exposed to the 60-μg level of Be, whereas the *p53*$^{+/+}$ mice exposed to the same ILB did not develop pulmonary neoplasms throughout the 22.5-mo length of the study. Further, none of the *p53*$^{+/-}$ knockout mice exposed to the lower level of Be developed pulmonary neoplasia. In contrast, the incidence of lung neoplasms was similar in *p53*$^{+/-}$ and *p53*$^{+/+}$ mice exposed to $^{239}PuO_2$. Contingency analysis did not detect any difference in ^{239}Pu-induced tumor incidence as a function of either dose or mouse *p53* status. Furthermore, the number of *p53*$^{+/-}$ knockout mice with pulmonary neoplasia induced by the high level of Be was not statistically different from the number of either *p53*$^{+/-}$ or *p53*$^{+/+}$ mice with tumors induced by $^{239}PuO_2$.

The latency period for the detection of pulmonary neo-

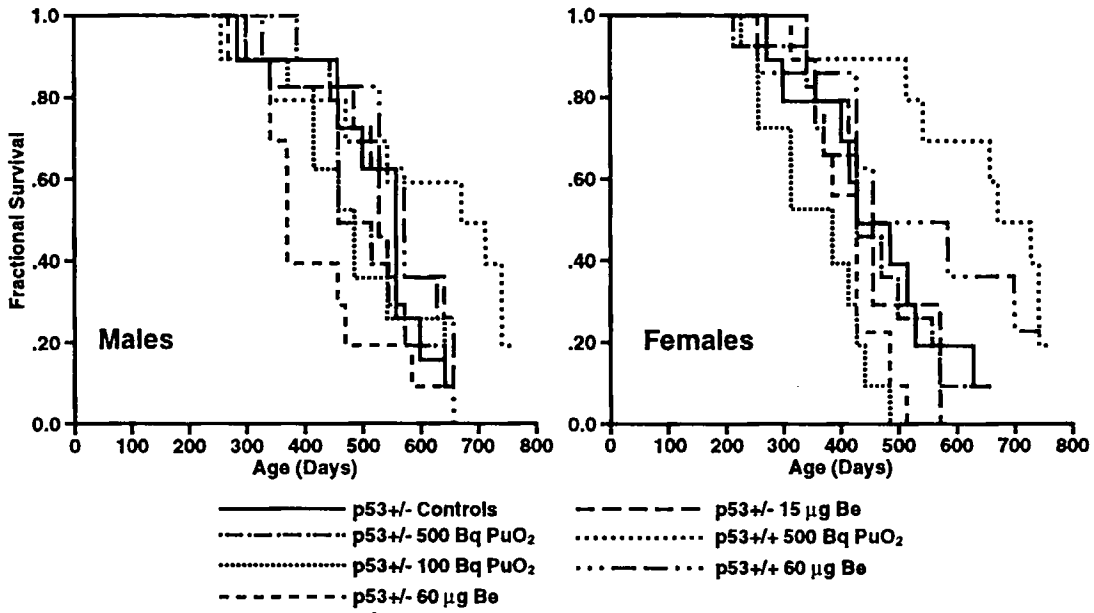

FIG. 1.—Survival curves for male and female mice expressed as fraction of mice surviving as a function of days of age. Exposed groups received $^{239}PuO_2$ or Be metal at approximately 72 days of age.

plasia in the $p53^{+/-}$ knockout mice was shorter than that in the $p53^{+/+}$ wild-type mice (Fig. 2). For ^{239}Pu-induced neoplasms, this effect was highly significant (p = 0.0001). However, only 2 of 59 $p53^{+/-}$ mice examined developed adenocarcinomas by 9 mo postexposure. The remaining tumors in the $p53^{+/-}$ mice were observed at scattered time points more than 9 mo after exposure, with most detected between 12 mo after exposure and the final sacrifice at 19 mo. Two of the 11 tumors were observed in mice at the final sacrifice. In contrast, all 7 of the tumors in the $p53^{+/+}$ wild-type mice were seen between 19 mo and the final sacrifice at 22.5 mo postexposure.

Most mice with pulmonary neoplasia had only 1 neoplasm or coalesced nodules of neoplastic tissue that involved nearly all of 1 lung lobe. Tumors in mice exposed to $^{239}PuO_2$ were usually papillary adenocarcinomas that were circumscribed and compressed adjacent structures (Fig. 3). Some encompassed airways and vessels, and a few had papillary growths of neoplastic tissue within bronchiole lumens. Whether this was a result of a bronchiologenic neoplasm or retrograde extension of a peripheral neoplasm was obscured by the extensive involvement of the lung parenchyma. In contrast to the $^{239}PuO_2$-exposed mice, most pulmonary neoplasms in Be-exposed mice were squamous cell carcinomas (SCCs). Three of 4 Be-exposed mice with pulmonary neoplasia had poorly circumscribed SCCs (Fig. 4), and all were associated with at least some degree of granulomatous pneumonia. A moderate amount of alveolar squamous metaplasia was mixed with inflammation surrounding 1 tumor. Another SCC had moderate to marked alveolar epithelial hyperplasia and septal fibrosis along with inflammation in the parenchyma surrounding and interposed with the neoplastic tissue. The SCCs sometimes abutted the epithelium of terminal bronchioles, but the degree of disruption of the parenchyma did not allow for an assessment of whether these tumors arose from bronchiolar epithelium.

Very few significant pulmonary changes other than the tumors were observed in the mice exposed to $^{239}PuO_2$. In contrast, Be exposure, particularly at the high level, caused granulomatous pneumonia in both the $p53^{+/-}$ knockout and $p53^{+/+}$ wild-type mice (Fig. 5). Overall, the

TABLE II.—Number of mice with 1 or more pulmonary neoplasms over the number of mice examined.

	Exposure groups						
	Heterozygous knockout mice ($p53^{+/-}$)					Wild-type mice ($p53^{+/+}$)	
Groups of mice	Air sham	500 Bq $^{239}PuO_2$	100 Bq $^{239}PuO_2$	60 µg Be	15 µg Be	500 Bq $^{239}PuO_2$	60 µg Be
Males	0/15	1/15	6/15	2/15	0/15	5/15	0/15
Females	0/15	3/14	1/15	2/13	0/14	2/14	0/13
Sexes combined	0/30[a,b,c]	4/29[a,d]	7/30[b]	4/28[c,e]	0/29	7/29[d]	0/28[e]

[a] Marginally significant difference between air sham versus 500 Bq $^{239}PuO_2$ $p53^{+/-}$ mice (p = 0.052).
[b] Significant difference between air sham versus 100 Bq $^{239}PuO_2$ $p53^{+/-}$ mice (p = 0.005).
[c] Significant difference between air sham versus 60 µg Be $p53^{+/-}$ mice (p = 0.048).
[d] No difference between 500 Bq $^{239}PuO_2$-exposed $p53^{+/-}$ versus $p53^{+/+}$ mice (p = 0.506).
[e] Marginally significant difference between 60 µg Be-exposed $p53^{+/-}$ versus $p53^{+/+}$ mice (p = 0.056).

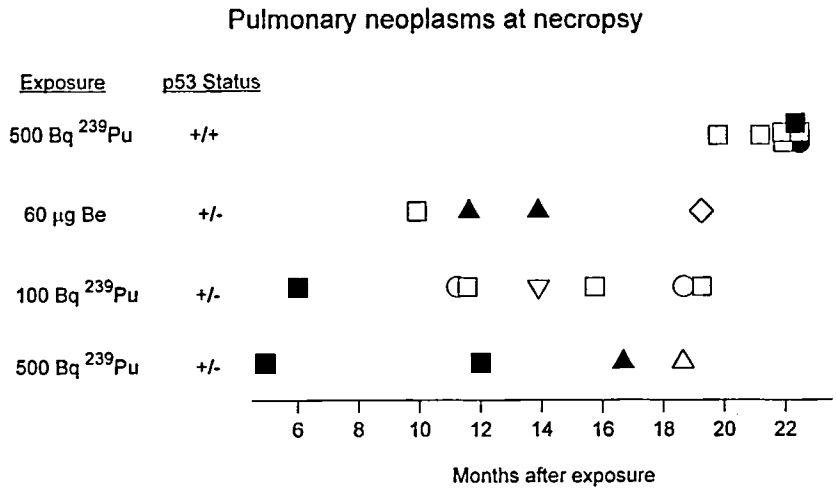

FIG. 2.—Plot showing the types of pulmonary neoplasms observed, the distribution among male and female mice, and the period of time after exposure that neoplasms were observed for the 4 exposure groups shown in Table II with mice that had pulmonary neoplasms. Key to symbols: ○, adenoma; □, adenocarcinoma; △, squamous cell carcinoma (SCC); ▽, poorly differentiated carcinoma; ◇, adenocarcinoma and SCC. Males are shown by open symbols (○), and females are shown by filled symbols (●).

severity of the pneumonia appeared to be slightly greater in the $p53^{+/+}$ mice. The severity was not correlated with length of survival. The pneumonia was distributed primarily in centriacinar regions and was characterized by alveolar septal and perivascular interstitial infiltrates of varying numbers of macrophages, lymphocytes, and plasma cells. Alveolar air spaces often contained macrophages and occasional multinucleated giant cells with foamy or vacuolated cytoplasm and intracytoplasmic Be

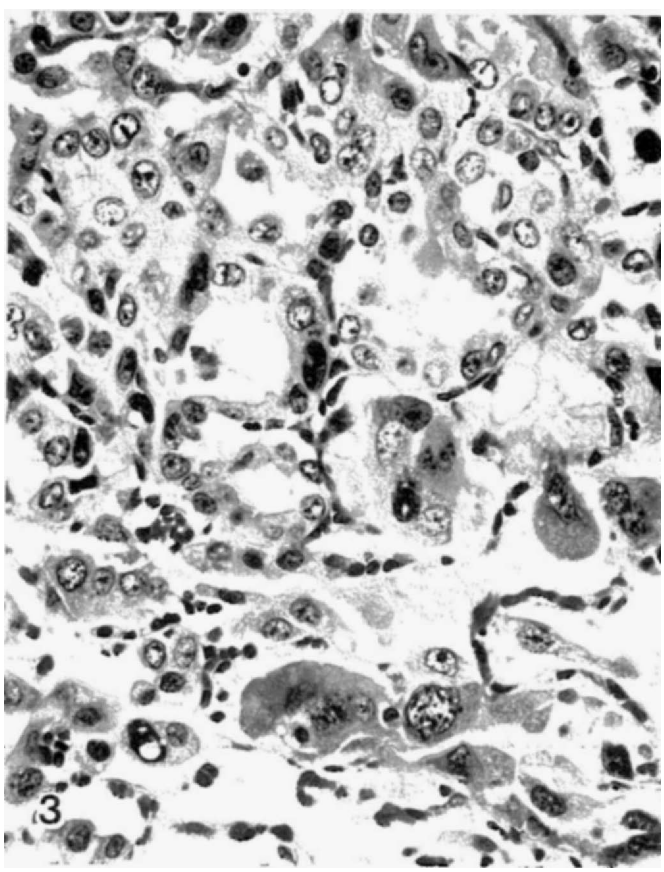

FIG. 3.—Photomicrograph of an adenocarcinoma in the lung of a $p53^{+/-}$ knockout mouse exposed to 500 Bq $^{239}PuO_2$. The tumor is composed of papillae, nests, and cords of pleomorphic cells. Many are multinucleated. H&E. ×400.

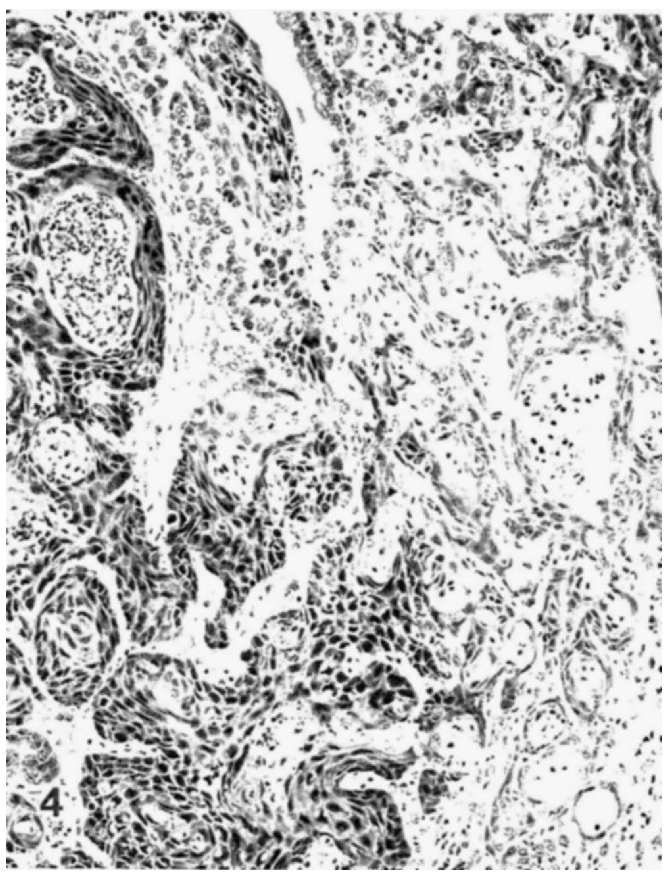

FIG. 4.—Photomicrograph of a squamous cell carcinoma in the lung of a $p53^{+/-}$ knockout mouse exposed to 60 µg Be. The neoplastic tissue extends into the lumen of an adjacent bronchiole (top). H&E. ×150.

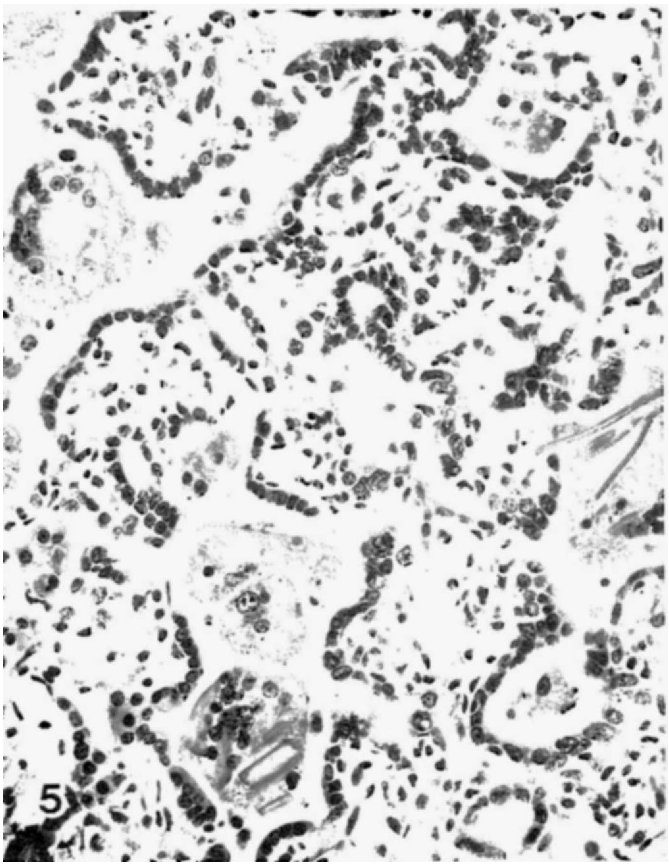

FIG. 5.—Photomicrograph of granulomatous and acidophilic macrophage pneumonia in a $p53^{+/-}$ knockout mouse exposed to 60 μg Be. Note the architectural disruption by alveolar epithelial hyperplasia and septal fibrosis. Alveoli, macrophages, and multinucleated giant cell often contain eosinophilic crystalline material. H&E. ×300.

particles. Some lungs had fairly diffuse alveolar deposits of protein. In several lungs, multiple foci of alveolar epithelial hyperplasia, some containing ciliated cuboidal cells, were associated with granulomatous and neutrophilic inflammatory cell infiltrates, septal disruption, and cell debris. The severity of the hyperplasia paralleled that of the inflammation grade. Alveolar septal fibrosis was infrequent, minimal to moderate, widely scattered, and associated with alveolar epithelial hyperplasia sometimes resembling what is termed "adenomatous" hyperplasia. Alveolar squamous metaplasia was also infrequent and was not observed in bronchioles.

Several mice exposed to Be (5 $p53^{+/-}$ knockout mice, 2 with SCC; and 1 $p53^{+/+}$ wild-type mouse) and $^{239}PuO_2$ (1 $p53^{+/-}$ mouse and 2 $p53^{+/+}$ mice, 1 with adenocarcinoma) had sporadic, fairly marked acidophilic macrophage pneumonia (AMP). This was characterized by intra-alveolar macrophages and giant cells with bright eosinophilic intracytoplasmic crystalline material. Similar oblong and needlelike crystals were also free within alveolar air spaces and the lumens of small bronchioles. The alveoli and bronchioles within the lesions were often lined by cells distended with globules of bright eosinophilic, sometimes refractile material. Neutrophils, foamy macrophages, protein, and cell debris were unevenly mixed within AMP lesions. Infiltrates of eosinophils were not a feature of the AMP.

TABLE III.—Number of mice with various neoplasms observed in each exposure group (n = 28–30 per group).

	Number of mice with neoplasms by exposure group						
	Heterozygous knockout mice ($p53^{+/-}$)					Wild-type mice ($p53^{+/+}$)	
Types of neoplasm	Air sham	500 Bq $^{239}PuO_2$	100 Bq $^{239}PuO_2$	60 μg Be	15 μg Be	500 Bq $^{239}PuO_2$	60 μg Be
Osteosarcoma	8	7	4	4	6	0	0
Lymphoma	3	2	2	1	2	1	0
Histiocytic sarcoma	1	2	3	1	1	1	0
Other neoplasia[a]	1	1	2	1	1	2	1

[a] Other neoplasms included cholangiocarcinoma, hepatocellular carcinoma, pancreatic carcinoma, mammary carcinoma, hepatic hemangiosarcoma, laryngeal spindle cell sarcoma, prostatic leiomyosarcoma, uterine stromal sarcoma, and ovarian hemangioma.

A number of nonpulmonary neoplastic lesions were observed in both the $p53^{+/-}$ and $p53^{+/+}$ mice (Table III). The most frequently occurring was osteosarcoma in the $p53^{+/-}$ knockout mice. Sometimes the osteosarcomas were multicentric and had metastasized to the lung. None appeared to be an effect of the exposures, and none were observed in the $p53^{+/+}$ wild-type animals. Similar observations were made for lymphoid neoplasia.

DISCUSSION

This is the first study to describe carcinogenesis in the heterozygous $p53^{+/-}$ knockout mouse model following inhalation exposures. This study was conducted to examine pulmonary carcinogenesis in $p53^{+/-}$ mice resulting from the inhalation of the carcinogens ^{239}Pu and Be metal. These carcinogens were chosen for study because of our interest in pulmonary neoplasia resulting from the inhalation of these carcinogens in other studies (9, 20, 24, 25) and because these carcinogens operate by different mechanisms (17, 22). Our findings indicated that both carcinogens induced pulmonary neoplasia in $p53^{+/-}$ knockout mice. Although a pronounced neoplastic response was not observed by 6 mo after carcinogen exposure, these mice did show a decreased latency period for ^{239}Pu-induced lung carcinogenicity compared to $p53^{+/+}$ wild-type mice.

Measured lung burdens of either ^{239}Pu or Be in selected $p53^{+/+}$ wild-type mice sacrificed 7 days after exposure were approximately 75–90% of targeted levels. However, approximately 20% of deposited, relatively insoluble particles can be expected to clear from the rodent alveolar region of the lung within the first 7 days after an acute inhalation exposure (31). Therefore, we conclude that actual ILBs achieved were approximately as targeted.

The survival of $p53^{+/-}$ knockout mice observed in this study was comparable to published survival curves, which suggest MSTs of approximately 50 wk of age (12) to 80 wk of age (13). The survival of the $p53^{+/+}$ wild-type mice appeared to be somewhat less than that reported previously for this mouse (12) and for another $p53^{+/+}$ wild-type mouse crossed to an NIH/Ola background (18). Indications of decreased MST for $p53^{+/-}$ mice versus $p53^{+/+}$ mice and for Be-exposed $p53^{+/-}$ mice

versus unexposed $p53^{+/-}$ mice, although not consistently statistically significant, are consistent with reports for dimethylnitrosamine treatment (12) or x-ray exposure (18).

The incidence of pulmonary neoplasms from ^{239}Pu in both the $p53^{+/-}$ knockout and $p53^{+/+}$ wild-type mice was greater than that reported by Lundgren et al (20), although the relatively low number of animals used in the present study do not permit a definitive conclusion. Over a relatively wide range of lung burdens, Lundgren et al (20) reported maximum tumor incidences of approximately 5% at 90 and 460 Bq ^{239}Pu ILBs. Similarly, Nikula et al (24, 25) reported a tumor incidence of approximately 5% in a life-span study of C3H mice receiving an ILB of 64 μg of Be metal; this incidence is less than that reported here for the $p53^{+/-}$ mice.

Our results suggest that having an inactivated *p53* allele was associated with the progression of neoplasia in the $p53^{+/-}$ knockout mice, because of the decreased latency period for ^{239}Pu-induced lung neoplasms and the significant difference in the incidence of Be-induced lung neoplasms for the $p53^{+/-}$ versus the $p53^{+/+}$ mice. However, the $p53^{+/-}$ mice were not more sensitive than the $p53^{+/+}$ mice to the induction of pulmonary neoplasms from inhaled $^{239}PuO_2$. Decreased tumor latency has been observed in $p53^{+/-}$ knockout mice exposed to the carcinogens dimethylnitrosamine (12), *p*-cresidine, 4-vinyl-1-cyclohexene diepoxide (33, 34), and x-irradiation (18). Moreover, the incidence of tumors in the treated $p53^{+/-}$ mice in these studies was greater than in the treated $p53^{+/+}$ wild-type mice. One potential explanation for the difference between our results for $^{239}PuO_2$-exposed mice and these reports could be effects on biological activity of the *p53* protein in the target tissues for these carcinogens. The *p53* protein affects gene transcription, DNA synthesis and repair, genomic plasticity, and programmed cell death [as reviewed in Greenblatt et al (10)]. Tissue differences in the activity of the remaining *p53* allele, which must perform these functions, could have a profound effect on both tumor initiation and progression. Alternatively, the fact that the *p53* gene is not generally a target in murine lung tumors (3, 14) could account for these differences. In contrast, *p53* is a target in tumors in other tissues in both the $p53^{+/-}$ mouse model and in nontransgenic mice. The *p53* wild-type allele is commonly inactivated in osteosarcomas and soft-tissue tumors in $p53^{+/-}$ mice (13, 18), and in bone, skin, and bladder cancers in nontransgenic mice (27, 29). Finally, Tennant et al (33) showed that *N*-methylolacrylamide, a liver carcinogen in a 2-yr bioassay, was not detected as a carcinogen in a 6-mo $p53^{+/-}$ mouse gavage study. Thus, more potent murine lung carcinogens (e.g., methylene chloride) might also be readily detected as carcinogens within the framework of a 6-mo $p53^{+/-}$ knockout mouse inhalation study.

The spectrum of nonpulmonary lesions observed, principally osteosarcomas with fewer numbers of lymphomas, histiocytic sarcomas, and other tumors, were similar to those observed in unexposed $p53^{+/-}$ knockout mice (12). In addition, our observation that 13 of 30 (43%) of unexposed $p53^{+/-}$ mice had a neoplasm over their life span is consistent with the data of Harvey et al (12), who reported that 53% of untreated $p53^{+/-}$ mice had a tumor by 18 mo of age.

Several nonneoplastic pulmonary lesions were of interest. Some of the mice exposed to Be or to $^{239}PuO_2$ had AMP (21, 37). These lesions are reported to be more frequent in a strain of C57BL mice known as "moth-eaten" (37) and have been described as infrequent lesions in C57BL and other strains of mice (21). Similar to observations in the moth-eaten variety of mice, infiltrates of eosinophils were not a feature of AMP in the animals of the present study. Also, neither the $p53^{+/-}$ nor the $p53^{+/+}$ mice with Be-induced granulomatous pneumonia in this study had well developed nodular lymphocytic infiltrates within the peripheral parenchyma. This contrasts with mouse strains with *k* major histocompatibility complex (MHC) class II haplotypes (e.g., A/J and C3H mice) that were exposed to Be (26). This lack of reaction is similar to findings we have observed at our institute in mice with *b* MHC class II haplotypes that had been exposed to Be (K. J. Nikula, unpublished data). The mice used in the present study were derived primarily from the C57Bl/6 strain and thus would also be expected to contain *b* MHC class II haplotypes.

In conclusion, our results demonstrated that unlike wild-type $p53^{+/+}$ mice, heterozygous $p53^{+/-}$ knockout mice were susceptible to Be-induced lung neoplasms. Both $p53^{+/-}$ and $p53^{+/+}$ mice were susceptible to pulmonary neoplasms from inhaled $^{239}PuO_2$, and the latency period to carcinogenesis was reduced in the $p53^{+/-}$ mice, thus indicating a role for *p53* in this model, which will be examined in further studies. In addition, we observed a relatively late onset of tumors in the $p53^{+/-}$ mice from inhaled $^{239}PuO_2$ or Be metal. Although our group sizes of 4 or 5 mice per group per sex at 6 mo is insufficient for definitive confirmation, our results suggest a 6-mo bioassay in $p53^{+/-}$ mice might not be an appropriate model for pulmonary carcinogenesis from these inhaled materials.

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