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CARCINOGENIC EFFECTS OF ANTIMONY TRIOXIDE AND ANTIMONY ORE CONCENTRATE IN RATS

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This study was initiated because of a suspected increase in incidence of lung cancer in antimony smelter workers in England. Three groups of 8-mo-old Wistar-derived rats (90 males and 90 females per group) were exposed by inhalation to either Sb_2O_3 [time-weighted average (TWA) 45 mg/m^3], Sb ore concentrate (TWA 36 + 40 mg/m^3), or filtered air (controls) for 7 h/d, 5 d/wk, for up to 52 wk and sacrificed 20 wk after terminating exposures. Serial sacrifices (5 rats/sex/group) were performed at 6, 9, and 12 mo. Autopsies and histopathological examinations were performed on all animals. The dusts and animal tissues were analyzed for Sb, arsenic, and other inorganic elements by atomic absorption and proton-induced X-ray emission methods. The most significant findings were the presence of lung neoplasms in 27% of females exposed to Sb_2O_3 and 25% of females exposed to Sb ore concentrate ($p < 0.01$). None of the male rats in any group or the female controls developed lung neoplasms. There were no significant differences in incidences of cancer of other organs between exposed and control rats. These results were compared with other published results, including an animal inhalation study with Sb_2O_3 in which lung tumors were also induced.

Higher concentrations of arsenic were found in tissues from female rats than from male rats. For example, arsenic levels in blood of control males, control females, Sb_2O_3 males, Sb_2O_3 females, Sb ore males, and Sb ore females were 60, 123, 115, 230, 71, and 165 μg arsenic/g dry blood, respectively, 9 mo after initiating exposures.

This project was performed partially under contract with Midwest Research Institute (NIOSH contract 210-77-0156) and partially in-house.

Mention of brand names does not constitute an endorsement by NIOSH or the authors of this article.

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INTRODUCTION

In 1978, 45,138 tons of antimony were used in the United States. This metal is principally used as an alloying constituent with lead and other metals in storage batteries, power transmission and communication equipment, type metal, solder, and ammunition. The most commercially important compound of antimony is antimony trioxide (Sb_2O_3). In 1978, 9030 tons were used as flame retardants in plastics [e.g., polyvinyl chloride (PVC)] and other materials (Rathjen, 1980). NIOSH estimated in 1978 that 1.4 million U.S. workers were potentially exposed to antimony in their occupational environment (National Institute for Occupational Safety and Health, 1978).

Antimony is derived from antimony ore, which is principally antimony trisulfide (Sb_2S_3, stibnite). The ore is mined in South Africa, Canada, and Bolivia. It is concentrated and shipped to smelters, where it is processed further. Antimony trioxide is obtained by simply heating the concentrate and recondensing the fumes.

In the 1970s a report by the Factory Inspectorate in Great Britain suggested that the lung-cancer incidence in men who worked in an antimony smelter was higher than in the general population (National Institute for Occupational Safety and Health, 1978). Because of this finding and the lack of animal carcinogenicity studies, a project was initiated by NIOSH in 1975 to determine the chronic effects of the inhalation of Sb_2O_3 and antimony ore concentrate (Sb ore conc.) in rats.

METHODS

Chemicals

Antimony trioxide (Sb_2O_3, KR grade, sample 670-004-22, lot D5-7) and antimony ore concentrate (Sb ore, Canadian, code 10274, lot CAN-11) were supplied by Harshaw Chemical Company, Cleveland, Ohio. The antimony compounds were analyzed for their concentrations of trace elements and antimony by proton-induced X-ray emission, atomic absorption spectrophotometric, or neutron activation methods. These analyses were performed under contract by the College of William and Mary, Virginia Associated Research Campus. The results appear in Table 1.

Exposure Chambers

Six Rochester-type, 3.45-m^3, stainless steel exposure chambers with glass windows on two sides were used (Young & Bertke, Cincinnati, Ohio). Each chamber contained a diffusion plate, two small squirrel-cage fans mounted on opposite sides of the top cone to aid in the distribution of the particulate, and four stainless steel wire-mesh shelves to support animal cages.

TABLE 1. Elemental Analysis of Antimony Compounds[a]

Element	Concentration of element (μg/g)	
	Sb_2O_3	Sb ore concentrate
Be[b]	<0.04	<0.06
Al[c]	66	4830
Mg	<5	359
Ti	<30,000	<40,000
V[c]	5.3	52
Cr[b]	9	14
Mn[c]	<10	83
Fe	<30	3340
Co[b]	<0.4	1.1
Ni[b]	1.6	13
Cu	<15	600
Zn	<9	530
As	40	792
Se	<6	<10
Br	23	<10
Zr	<9	6
Cd[b]	4.4	<0.3
Sn	2100	1600
Sb	80%	46%
Te	<0.4	<0.4
I	<700	<400
Pb	2300	2500
Ce	140	<60
Au	<17	30

[a] Analyses performed by proton-induced X-ray emission on samples dissolved in acid, except where indicated.

[b] Analyses performed by atomic absorption spectrophotometry.

[c] Analyses performed by neutron activation analysis.

The air supply for the chambers was drawn from a stack on the roof of the building, passed through a coarse filter, and then over coils for heating, cooling, and dehumidifying. The air then passed through an absolute filter (99.97–99.99% retention of particles >0.3 μm in diameter) and into the plenum of the chamber. Air temperature was maintained at 75 ± 5°F, and humidity was monitored. Airflow through the chambers was kept at 12–15 air changes per hour and was monitored by a critical orifice plate connected to a Magnahelic gauge and an Autotronics 100-SSX airflow transducer. All chambers were operated at a slight negative pressure (0.1–0.2 in. of water) to prevent leakage of particulate into the room.

Generation of Dusts

A separate Wright dust feeder was used for each chamber. After leaving the dust generator, the aerosol passed through Teflon tubing into a Thermo-Systems model 3054 aerosol neutralizer (Thermo-Systems, Inc., St. Paul, Minn.) and entered the airstream through a port aproximately 6 in upstream from the plenum of the chamber. Seven months after initiating exposures, the neutralizer was removed. This permitted higher concentrations of particulate to be attained in the chambers.

Concentrations of the dusts in each chamber were measured three times daily during the exposure periods. The air samples were collected on tared 25-mm diameter, 0.04 μm pore size, Uni-Pore polycarbonate filters. The sampling flow rate and sampling time were 5 l/min and 10 min. The samples were weighed on analytical balances accurate to 0.01 mg. The time-weighted average (TWA) concentrations were calculated for each exposure day.

The target concentrations for Sb_2O_3 and Sb ore were 50 mg/m^3. This concentration was selected because it was about the middle of the range of concentrations to which workers have been exposed (National Institute for Occupational Safety and Health, 1978). The animals were scheduled to be exposed for 7 h/d, 5 d/wk for 52 wk. On a few days, exposures did not occur because of holidays or mechanical breakdowns.

During mo 6 of exposure, chamber samples for particulate size analysis were collcted on 0.1-μm pore size Nuclepore filters. The filter preparations were attached to carbon planchets with colloidal graphite and examined at a magnification of ×4000 with a scanning electron microscope (JEOL, model JXA 50A) equipped with an automated image analyzer (LeMont Scientific model B-10). Using the backscattered electron image, all of the particles in randomly selected fields of view were automatically sized by the image analyzer.

Animals

Approximately 450 male and 450 female Wistar-derived albino rats, 8–10 wk of age, were bred and supplied by Charles River Breeding Labs, Wilmington, Mass. The supplier stated that all animals were free of endemic respiratory disease. Upon receipt, the rats were housed in polycarbonate cages with stainless-steel wire tops covered with filter tops (2 males/cage and 3 females/cage). They were fed a pelleted, commercial rat diet and tap water ad libitum. When the rats were 8 mo old, they were divided into their respective exposure groups (controls, Sb_2O_3, and Sb ore) with 90 males and 90 females per group. Since the exposure chambers could accommodate only 90 rats, 2 chambers were used for each group with equal representation of males and females in each chamber. They were housed individually in wire-meshed, stain-

less-steel caging placed on shelves within the chambers. The cages were rotated daily, according to a fixed schedule that would allow each cage to be rotated to all shelf levels and cage positions. The animals remained in the chambers 24 h/d throughout the study, except while they were being weighed and when the cages and chambers were being cleaned. Cages were cleaned weekly. Food was provided ad libitum except during the exposure periods. Tap water was provided at all times. All rats were identified with numbered ear tags and numbered cages. One day prior to initiating exposures, and at wk 1, 2, 3, and 4, and monthly thereafter, the rats were weighed. Group means were calculated, and exposed groups were compared with controls using Dunnett's multiple-comparison procedure. They were examined twice daily: in the morning prior to initiating exposures, and in the afternoon at the end of the exposure periods. On weekends and holidays, they were examined once daily.

At 6, 9, and 12 mo after initiating exposures, 5 male and 5 female rats from each group were sacrificed and autopsied. All animals that died or were sacrificed because of ill health were autopsied. At 71–73 wk after initiating exposures (18–20 wk after termination of exposures), all animals were sacrificed and autopsied. At autopsy, all organs were examined grossly, and sections of tissue from the liver, kidneys, pancreas, spleen, adrenal, thyroid, pituitary, bladder, brain, eye, bone marrow, skin, mesenteric and tracheobronchial lymph nodes, stomach, ascending and descending colon, and lungs from each rat; from the testicle and prostate from males and mammary gland, ovary, uterus, and cervix from females; and from any abnormal tissue were fixed in buffered 10% formalin, embedded, sectioned, stained with hematoxylin and eosin, and examined by light microscopy. In addition, at the final sacrifice, heart tissue was sampled and examined by light microscopy.

At the serial sacrifices (6, 9, and 12 mo), portions of liver, lungs, kidneys, brains, spleens, and blood from 5 male and 5 female rats in each group were sampled for analysis for their concentrations of Sb and other trace elements by atomic absorbtion and proton-induced X-ray emission (PIXE) methods.

RESULTS

Chamber Concentrations

The mean daily TWA concentrations and ranges for each chamber appear in Table 2. The plots of the daily TWAs appear in Figs. 1–4. The mean TWAs were determined by adding all the daily TWAs and dividing by the number of exposure weeks multiplied by 5. (Since the animals were exposed during 53 wk, this number is 5 × 53 or 265. This includes some days on which there were no exposures.)

TABLE 2. Chamber Concentrations

Chamber	Compound	Mean daily TWA[a] (mg/m^3)	Range of daily TWAs (mg/m^3)
2E	Sb ore conc.	36.0	0–83.2
2W	Sb ore conc.	40.1	0–91.1
3E	Sb_2O_3	45.0	0–118.5
3W	Sb_2O_3	46.0	0–191.1

[a] Calculated by adding all the daily TWAs and dividing by number of exposure weeks multiplied by 5. This includes some days on which there were no exposures.

Considerable difficulty was experienced initially in generating the target concentration (50 mg/m^3) for the Sb ore and the Sb_2O_3. For example, during the first 9 wk the mean daily TWA for Sb ore was only 7.4 mg/m^3 in chamber 2E. Therefore, modifications were made in the equipment and procedures. The air passageway through the delivery shaft and the hole in the center of the cutting blade and blade holder in the Wright dust feeder were enlarged. The Sb ore was dried in an oven overnight before packing into the dispenser. As a consequence, the chamber concentrations were increased to about 30–50 mg/m^3. However, it was not until the aerosol neutralizer was removed (mo 7) that consistently high concentrations ($\geq$50 mg/m^3) could be achieved.

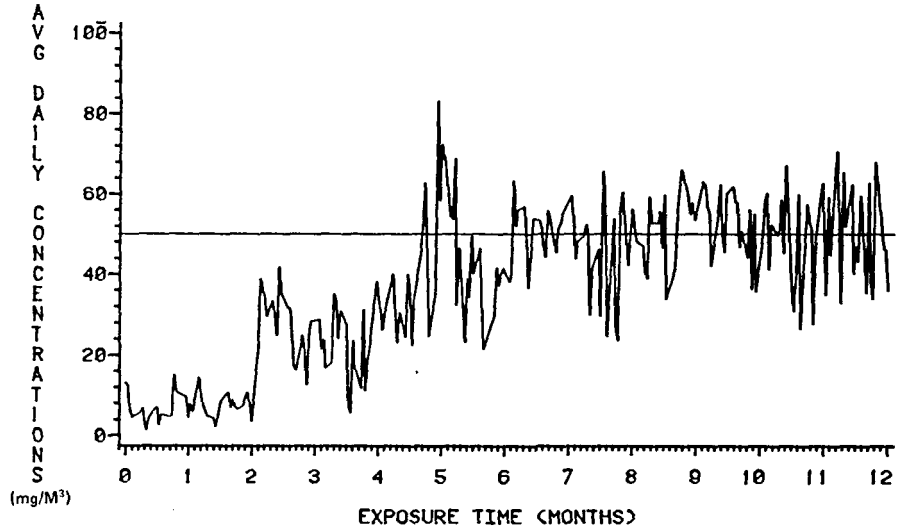

FIGURE 1. Antimony ore concentration, chamber 2E.

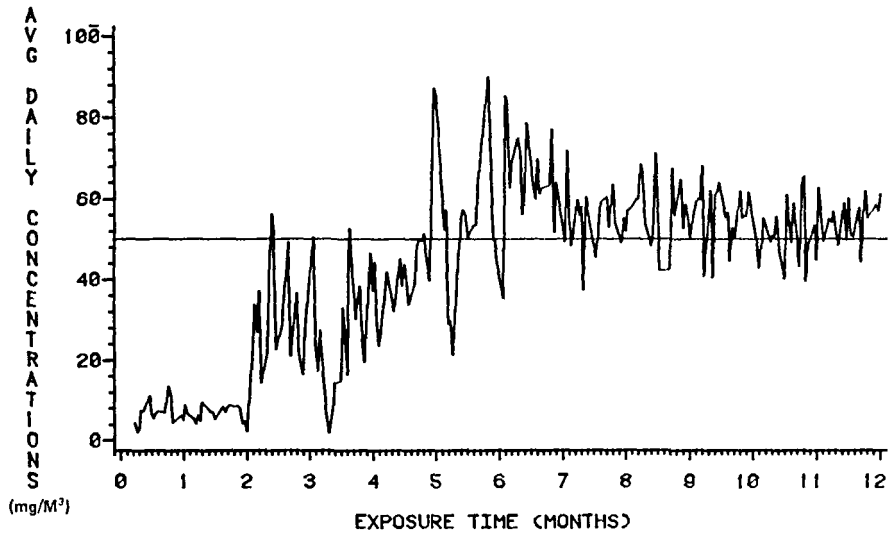

FIGURE 2. Antimony ore concentration, chamber 2W.

Particle Sizing

The particle-size data appear in Table 3. The circular area equivalent diameter is defined as the diameter of a circle that has the same area as that determined for the particle. The mass median diameters were calculated for each particle using the circular area equivalent diameters and the densities of the bulk compounds with the assumption that the

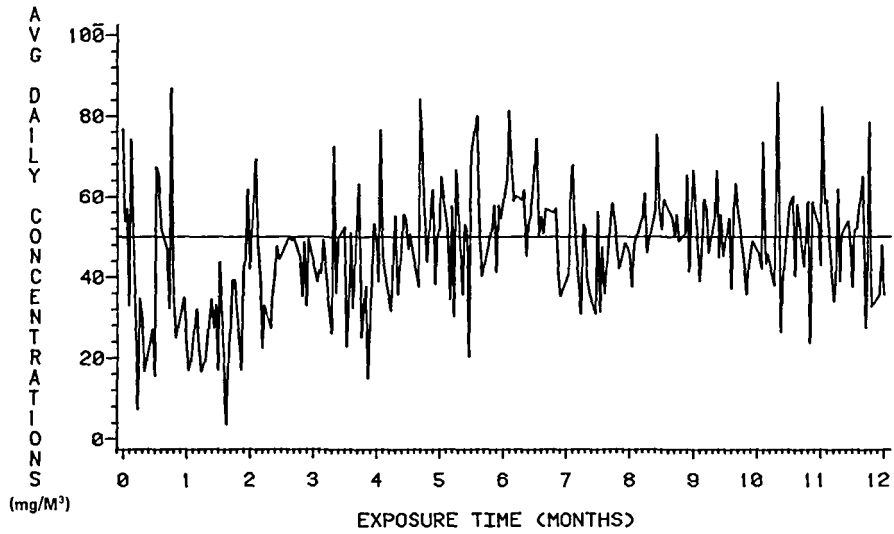

FIGURE 3. Antimony trixoide concentration, chamber 3E.

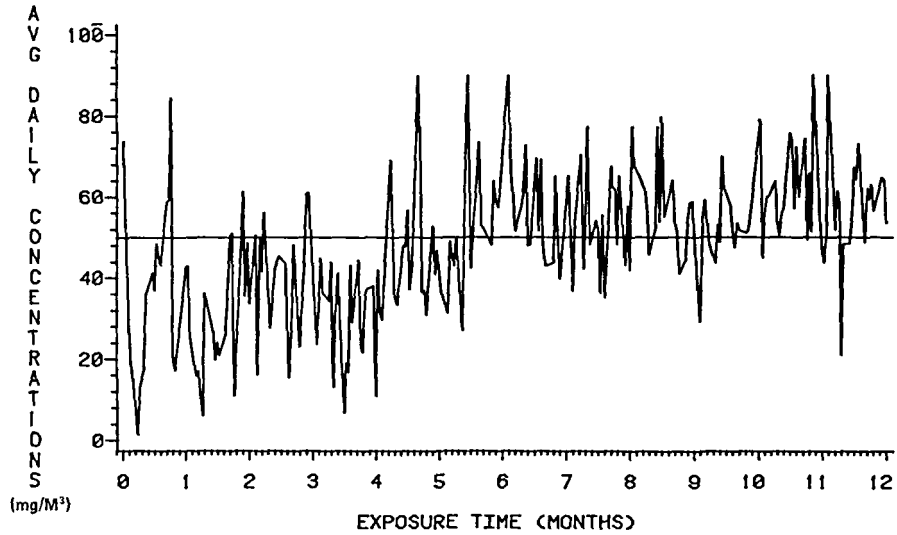

FIGURE 4. Antimony trioxide concentration, chamber 3W.

particles were spherical. Since the Sb ore was primarily the natural ore, Sb_2S_3, the density for that was used. The mass median aerodynamic diameter for each particle was calculated by multiplying the square root of the density of the bulk compound by the mass median diameter. This latter measurement is the one that is customarily used to help determine the percentage of an aerosol that is deposited in the lungs during inhalation (Heyder et al., 1980).

Clinical Observations

The most commonly observed clinical sign was hemorrhage around ears in all rats during the first 2 mo. This was caused by the metal ear tags. Sporadic bleeding from eyes and hematuria occurred in rats in all groups, but appeared to occur more frequently in the Sb_2O_3 and Sb ore groups.

The mean body weights for five intervals are shown in Table 4. From wk 26 to wk 50, the Sb_2O_3 males weighed significantly less than the control males. The maximum difference, however, was no greater than 6.2% of the control weight. From wk 26 to wk 50, the Sb ore female rats weighed less than their respective controls, and the maximum difference was no greater than 6.4% of the control weight.

Mortality

The cumulative survival distributions were estimated using the Kaplan-Meier method (Kaplan and Meier, 1958). The survival curves were compared using the Breslow test (Breslow, 1970). The survival curves

TABLE 3. Particle Sizing Data

Compound	Density of bulk compound (g/cm^3)	Fields of view examined[b]	Number of particles analyzed	Median circular area equivalent diameter (μm)	Mass median diameter (μm)	Mass median aerodynamic diameter (μm)
Sb_2O_3	5.20	17	1948	0.347	1.23	2.80
Sb ore	4.64	25	1179	0.395	2.22	4.78

[a] Fields of view were selected by use of a random-number generator for coordinates.

TABLE 4. Mean Body Weights in Grams (SD)

Week on experiment	Females			Males		
	Controls	Sb_2O_3	Sb ore	Controls	Sb_2O_3	Sb ore
0	300 ± (23)	303 (19)	307 (21)	555 (39)	564 (41)	567 (46)
26	373 (40)	353 (33)[a]	359 (33)[a]	614 (55)	596 (43)[a]	631 (49)[a]
34	386 (41)	375 (32)	372 (30)[a]	634 (52)	602 (49)[a]	623 (51)
42	406 (45)	387 (36)[a]	382 (33)[a]	623 (56)	588 (54)[a]	614 (54)
50	408 (57)	394 (41)	382 (40)[a]	607 (72)	577 (70)[a]	601 (70)

[a] Body weights are significantly different from controls at this interval ($p < 0.05$, Dunnett's multiple-comparison procedure).

did not differ significantly between control and exposure groups. However, for each of the groups, the female survival rate was significantly greater than the male counterparts. The plots of accumulative mortality appear in Figs. 5 and 6.

Elemental Concentrations in Tissues

The concentrations of Sb in the tissues of male and female rats in the 3 groups after 9 mo of exposure and in the blood after 12 mo of exposure appear in Table 5. The concentration of Sb in the lungs of the male rats (38,300 μg Sb/g) exposed to Sb_2O_3 was significantly greater than that in the female rats (25,600 μg Sb/g) exposed to Sb_2O_3 ($p \leq 0.03$). The lungs of both the male and the female rats exposed to Sb_2O_3 contained considerably more Sb than the lungs of the males and females exposed to Sb ore (males, 5.4 times as much, females 5.7 times as much, $p \leq 0.01$). There were no other statistically significant differences between males and females in each group or between exposure groups for each sex.

The concentrations of arsenic (As) in the tissues of male and female rats in the 3 groups after 9 mo of exposure and in the blood after 12 mo of exposure appear in Table 6. Lungs of male rats exposed to Sb_2O_3 contained significantly more arsenic (213 μg As/g) than lungs of female rats exposed to Sb_2O_3 (150 μg As/g). The lungs of both the male and

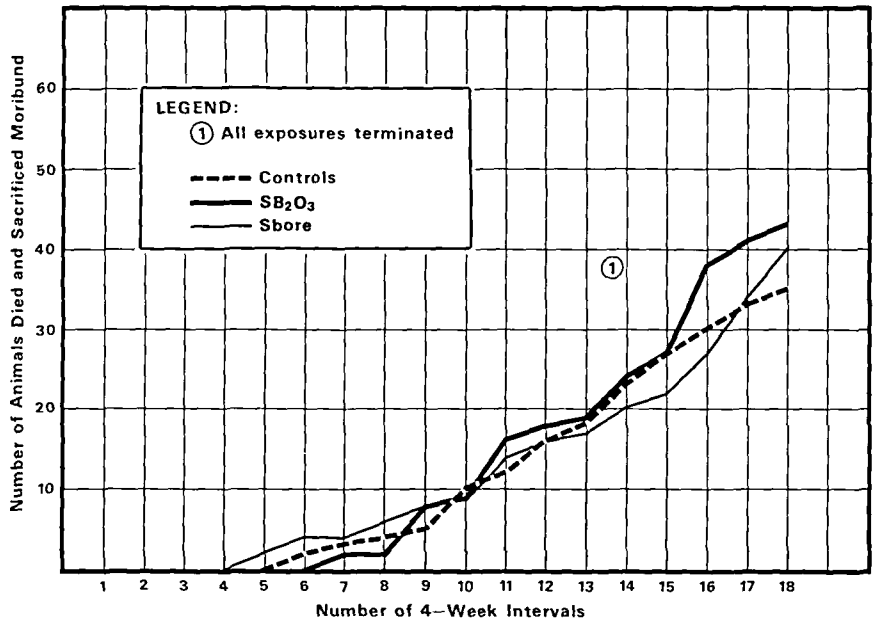

FIGURE 5. Cumulative mortality, female rats.

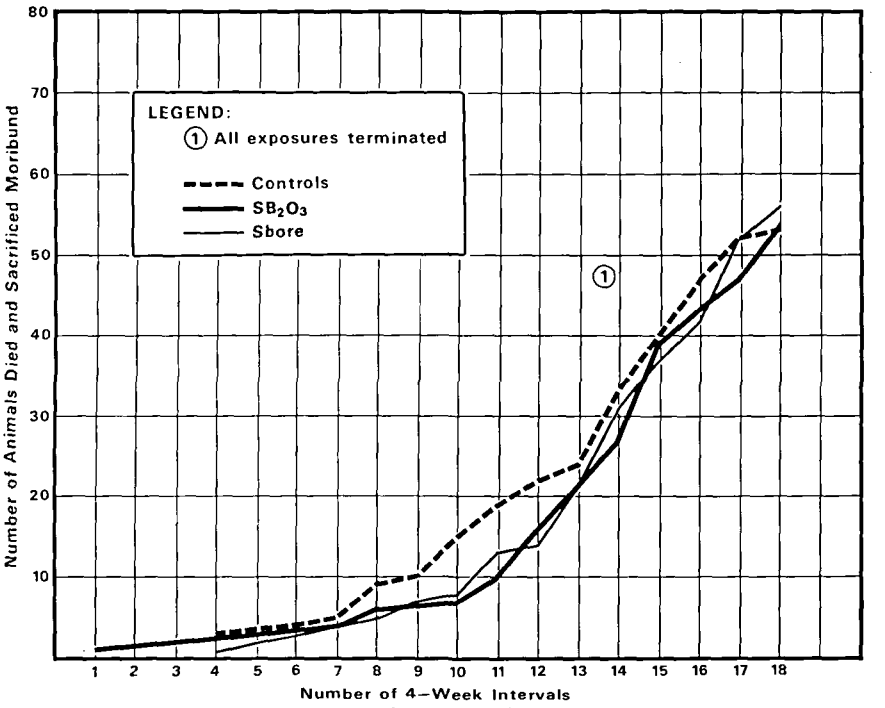

FIGURE 6. Cumulative mortality, male rats.

female rats exposed to Sb_2O_3 contained considerably more arsenic than the lungs of the males and females exposed to Sb ore (males, 21 times as much; females, 10.8 times as much, $p \leq 0.05$). In each group, all other tissues from the female rats contained higher concentrations of arsenic than the tissues from the male rats except for the femurs, in which most of the values were below the limit of detection.

Analyses of the tissues from other sacrifice intervals are not included, because the tissue was fixed in formalin instead of frozen. This resulted in the Sb dissolving into the formalin and reabsorbing in all the other tissues, resulting in concentrations of 4–17 times higher than those obtained with the frozen tissue.

Gross Pathology

At the final sacrifice, the lungs of all animals from both exposure groups contained slightly elevated, confluent, white and yellow foci on the pleural surfaces of all lobes. No lung tumors were clearly identified at autopsy. The lungs were inflated with formalin and dissected when they were processed for histopathology.

TABLE 5. Sb in Tissue at 9 Months of Exposure—Analyses by Atomic Absorption (μg Sb/g Dried Tissue, Means ± SD)[a]

Tissue	Sb_2O_3 exposure		Sb ore exposure		Control	
	Males (4 rats)	Females (5 rats)	Males (5 rats)	Females (5 rats)	Males (5 rats)	Females (5 rats)
Lung	38,300[b,c] ± 901	25,600[c] ± 429	7140[b] ± 1140	4520 ± 853	9.2 ± 0.98	10.5 ± 5.3
Liver	37 ± 15	69 ± 27	39 ± 5.6	50 ± 18	<0.3	<0.6
Kidney	37 ± 15	47 ± 15	63 ± 31	46 ± 7.3	<0.7	<1.3
Spleen	157 ± 66	226 ± 54	215 ± 40	239 ± 43	<1.7	<1.9
Brain	11 ± 7.7	17 ± 6.6	40 ± 41	13 ± 5.3	<0.4	<0.4
Femur	5.9 ± 4.1	6.0 ± 3.5	8.8 ± 4.0	7.9 ± 2.9	3.1 ± 2.0	2.2 ± .61
Blood[d]	1160 ± 189	1034 ± 126	938 ± 137	954 ± 61	12 ± 7.6	9.6 ± 1.0

[a] All values in exposed animals (except the brain and femur concentrations in the Sb_2O_3 females) are significantly different from the control values, $p \leq 0.05$.

[b] Significantly different from the corresponding value in females ($p \leq 0.03$) in the same exposure group.

[c] Significantly different from the concentrations in the same sex in the Sb ore conc. group, $p < 0.01$.

[d] Blood was taken after 12 mo of exposure.

TABLE 6. Arsenic in Tissues at 9 Months of Exposure (μg As/g Freeze-Dried Tissue)[a]

Tissue	Sb_2O_3 exposure		Sb ore exposure		Control	
	Males (4 rats)	Females (5 rats)	Males (5 rats)	Females (5 rats)	Males (5 rats)	Females (5 rats)
Lung	213 ± 41[b,c]	150 ± 14[c]	10.1 ± 1.2[b]	13.9 ± 2.6	6.5 ± 2.5	18.5 ± 2.5
Liver	4.5 ± 1.3[b,c]	10.8 ± 2.2[c]	2.6 ± 0.23[b]	6.6 ± 1.3	2.1 ± 0.59	4.9 ± 0.91
Kidney	5.2 ± 1.5[b]	10.7 ± 2.9[c]	3.6 ± 1.1[b]	6.7 ± 1.7	2.0 ± 0.70	6.6 ± 1.9
Spleen	21 ± 7.2[b]	37 ± 8.2	14.2 ± 1.5[b]	30 ± 7.1	9.4 ± 3.3	19.6 ± 5.93
Brain	1.4 ± 0.57[b]	2.6 ± 0.56[c]	0.66 ± 0.29[b]	1.6 ± 0.32	<0.5	1.0 ± 0.25
Femur	<1.1	<0.66	0.76 ± 0.25	<1.1 ± 0.54	<0.8	<0.5
Blood[d]	115 ± 17[b,c]	230 ± 42[c]	71 ± 22[b]	165 ± 6.1	60 ± 22	123 ± 9.50

[a] All analyses performed using PIXE.
[b] Values for males significantly different from females in the group ($p \leq 0.02$).
[c] Values significantly different from the concentrations in the same sex in the Sb-ore group ($p \leq 0.05$).
[d] Blood was from rats at the 12-mo sacrifice.

Histopathology—Nonneoplastic Changes

Lungs. *Sb_2O_3 Females.* At 6 mo, the lungs from the female rats exposed to Sb_2O_3 contained particles evenly scattered throughout all lobes of the lung and in more than 90% of the alveoli. Several dense particle aggregates about the size of macrophages were present in about 10% of the alveoli. Individual macrophages were obscured by the particles. In some alveoli, the particles were embedded in dense, pink, homogeneous protein. Alveolar-wall thickening, consisting of interstitial fibrosis and alveolar-wall cell hypertrophy and hyperplasia, appeared in about 50% of the alveolar duct regions, affecting about 5–10% of all alveoli. Cuboidal and columnar cell metaplasia occurred in some of these foci.

Up through the 12-mo sacrifice, the density of the particles, amount of dense, pink, homogeneous alveolar protein, areas of interstitial fibrosis, and cuboidal and columnar cell metaplasia increased. In addition, foci containing cholesterol clefts were seen. The first lung neoplasms were seen at the 12-mo sacrifice. One was a bronchioalveolar adenoma and the other a squamous-cell carcinoma.

At the sacrifice at 16–17 mo (4–5 mo postexposure), the density of particles and amount of protein in the alveoli had significantly decreased. The extent of the interstitial fibrosis, however, had increased. In some rats it affected over 80% of the alveoli. The number of foci containing cholesterol clefts had also increased to over 20 per lung section in some rats. In some rats, dense scars that appeared to be confluent areas of interstitial fibrosis were present. Occasionally, neoplasms arose from these sites.

Sb Ore Females. The histopathology of the lungs in female rats exposed to Sb ore was qualitatively similar to that seen with the Sb_2O_3-exposed females. There were fewer particles and less alveolar protein visible at all sacrifice intervals. However, the extent of the interstitial fibrosis and of the cuboidal and columnar cell metaplasia was the same. Some of the particulates were birefringent under polarized light and appeared to be silicates.

The tracheobronchial lymph nodes contained fewer particles than those in the Sb_2O_3-exposed rats. In addition, they contained mononuclear cell granulomas, similar to those that are seen in the early stages of silicosis or sarcoidosis.

Sb_2O_3 Males. At 6 mo, the lungs of male rats exposed to Sb_2O_3 had the same amount of interstitial thickening as the females; however, there was less alveolar protein. At 12 mo, the severity of the interstitial fibrosis in males was the same as in the females. In some interstitial areas, there was, in addition, dense, eosinophilic material with the appearance of amyloid. The cuboidal, alveolar-wall cell metaplasia was not as extensive as in the females. In addition, there were fewer foci with cholesterol clefts. At 4 mo postexposure, there appeared to be

some diminution in the amount of alveolar-wall metaplasia, and the metaplasia was less severe than that observed in females. Again, there were fewer foci with cholesterol clefts. The amount of alveolar protein was less than at 12 mo and much less than that present in the females. In several interstitial spaces, there appeared to be more mononuclear cells, lymphocytes, and plasma cells than seen in the female rat lungs. Some of the amyloid-appearing material was still present. There did not appear to be a significant difference in the extent and severity of the interstitial fibrosis between the males and the females.

Sb Ore Males. The alterations in the lungs were similar to those seen with Sb_2O_3. The only differences were fewer particles and the presence of some birefringent particles, as well as granulomas in tracheobronchial lymph nodes (as was observed in the female rats exposed to Sb ore).

Controls. No significant pathological alterations were seen in any of the control lungs. Occasional foci containing lymphocytes, typical of chronic pneumonia, were seen in a few rats.

Other Organs. Lesions typical of this strain and age of rats were present in all groups, but not significantly increased in incidence in any exposed group compared to the controls.

Lung Neoplasms. No lung tumors were seen in the control rats of either sex or in the male rats exposed to either compound. Both the Sb_2O_3 and Sb ore exposed female rats developed lung neoplasms. The first lung tumor was observed in a female rat that died after 41 wk of exposure to Sb ore. The first lung tumor in the Sb_2O_3 group was seen in a rat that was killed in the serial sacrifice at 53 wk. The incidence of lung tumors at each interval and the total incidence of lung tumors appear in Table 7. If only the animals at risk (those alive at the time the first lung tumor was found) and subsequently examined are used in the calculations, then the incidence of lung tumors for Sb_2O_3-exposed rats is 27% and that for Sb-ore rats is 25%.

TABLE 7. Female Rats with Lung Tumors—Rats Examined at Specified Intervals

Interval (in weeks on experiment)	Controls	Sb_2O_3	Sb ore
18–40 (died and serial sacrifice)	0/15	0/14	0/14
40 (serial sacrifice)	0/5	0/5	0/5
41–53 (died)	0/10	0/11	1/9
53 (serial sacrifice)	0/5	2/5	2/5
54–71 (died)	0/15	5/23	3/21
71–73 (serial sacrifice)	0/39	12/31 (39%)[a]	11/33 (33%)[a]
Total (18–73 wk)	0/89	19/89 (21%)[a]	17/87 (20%)[a]
41–72 wk	0/69	19/70 (27%)[a]	17/68 (25%)[a]

[a] Significantly greater incidences of lung tumors than the controls, $p \leq 0.001$.

Each lung tumor was measured in each rat. The mean tumor size for each group was calculated by using the diameter of the largest tumor in each rat. The mean diameter of the lung tumors in the Sb_2O_3-exposed rats was 0.43 cm, and in the Sb-ore rats it was 0.25 cm. This difference in size is not statistically significant.

The tumor types included squamous-cell carcinomas, bronchioloalveolar adenomas, bronchioloalveolar carcinomas, and scirrhous carcinomas. The latter tumor type consisted principally of scar tissue, probably confluent areas of interstitial fibrosis in which spaces were lined usually by cuboidal tumor cells and less frequently by squamous tumor cells. There was no significant difference in the incidence of any tumor type between the Sb_2O_3 and Sb-ore groups. Squamous-cell carcinomas were present in 9/19 of the Sb_2O_3 rats and in 9/17 of the Sb-ore rats with lung tumors. Scirrhous carcinomas were present in 5/19 of the Sb_2O_3 rats and in 4/17 of the Sb-ore rats with lung tumors. Bronchioloalveolar adenomas and carcinomas were present in 11/19 of the Sb_2O_3 and 6/17 of the Sb-ore rats with lung tumors. In six of the Sb_2O_3 rats and in three of the Sb-ore rats, the lung tumors were multiple (two to four per rat).

Other Neoplasms. The controls, as well as the exposed males and females, developed a variety of different types of tumors that are typical for this strain of rat. The incidences of the most common tumors appear in Tables 8 and 9. In the ratios that apear in the tables, the numerator is the number of rats with a tumor in the specified organ and the denominator is the number of rats in which that organ was examined. Tumors of the thyroid included follicular-cell carcinomas, follicular-cell adenomas, follicular-cell adenocarcinomas, c-cell adenomas, and malignant mixed tumors. Tumors of the mammary glands included adenomas, fibroadenomas, fibromas, and adenocarcinomas. Tumors of the pituitary included adenomas and adenocarcinomas. Tumors of the adrenals included pheochromocytomas, cortical adenomas, and ganglioneuromas. Tumors of the skin-subcutis included fibromas, fibrosarcomas, basal-cell carcinomas, a neurofibrosarcoma, a papilloma, a sebaceous adenocarcinoma, a lymphangiosarcoma, and a squamous-cell carcinoma. The incidences of these tumors in the exposed groups were not significantly elevated above those in the controls.

DISCUSSION

Watt (1983) reported the induction of lung neoplasms in female Charles River CDF rats after inhalation of Sb_2O_3 at a mean concentration of 4.2 mg Sb/m^3 of air, 6 h/d, 5 d/wk for 1 yr. The incidence of lung neoplasms was 62% (21/34), if one includes only those animals alive and examined after 12 mo of exposure. The last animal was autopsied 27 mo after initiating exposures and was about 33 mo old. A higher incidence of lung neoplasms was induced in that study than in ours. For compar-

TABLE 8. Incidence of Some Common Neoplasms: Male Rats

Weeks on study	Tissue and group[a]											
	Thyroid			Skin–subcutis			Pituitary			Adrenal		
	1	2	3	1	2	3	1	2	3	1	2	3
0–27	0/8	0/9	2/8	2/9	1/9	1/8	0/6	1/6	0/8	0/9	0/9	0/8
28–40	0/13	0/7	1/10	2/14	1/8	0/10	3/11	0/7	0/7	0/13	0/8	0/9
41–53	0/16	0/19	2/21	3/17	1/20	1/21	2/13	4/11	1/13	0/17	0/19	0/20
54–73	12/47[b]	15/48[b]	10/47[b]	4/49	2/48	3/47	9/29	10/38	11/31	2/45	1/45	1/43
0–73	12/84	15/83	15/86	11/89	5/85	5/86	14/59	15/62	12/59	2/84	1/81	1/80

[a] Group 1, controls; group 2, Sb_2O_3; group 3, Sb ore.
[b] Significantly higher incidences of thyroid tumors than females in the same group and time period, $p \leq 0.01$.

TABLE 9. Incidence of Some Common Neoplasms: Female Rats

Weeks on study	Tissue and group[a]											
	Thyroid			Mammary gland			Pituitary			Adrenal		
	1	2	3	1	2	3	1	2	3	1	2	3
0–27	0/8	0/5	0/8	0/8	0/5	1/9	2/8	0/2	4/8	0/8	0/5	0/9
28–40	1/11	0/13	0/8	2/11	3/14	1/10	6/12	5/14	5/10	0/11	0/13	0/10
41–53	0/14	1/15	1/14	3/14	6/15	6/14	7/13	10/14	4/12	1/14	0/15	1/14
54–73	4/53	5/55	2/54	22/55	17/55	19/54	31/48[b]	35/47[b]	29/47[b]	5/55	2/51	0/52
0–73	5/86	6/88	3/84	27/88	26/89	27/87	46/81	50/77	42/77	6/88	2/84	1/85

[a] Group 1, controls; group 2, Sb_2O_3; group 3, Sb ore.
[b] Significantly higher incidences of pituitary tumors than in males in the same group and time period, $p \leq 0.02$.

ison purposes, if only the animals examined after 53 weeks are included in our study, the incidence of lung neoplasms was 19/59 or 32%. The reasons for this difference might be explained on the basis that Watt allowed his rats to live a longer period of time (15 mo postexposure) than we did (5 mo postexposure), and he used a different strain of rats than we did. In both studies, the rats were exposed for 12 mo.

Another Sb_2O_3 inhalation study in rats was reported by Gross et al. (1955a,b) and deserves some comment. They found interstitial fibrosis and lipoid pneumonia in rats exposed to 100–125 mg Sb_2O_3/m^3 air for 100 h/mo for up to 14.5 mo. Serial sacrifices were performed, and animals died during the exposures. No mention was made of the sex of the rats or how many animals were examined histologicaly after 12 mo of exposure, so no evaluation of the apparent absence of lung tumors can be made.

In addition to discovering that Sb_2O_3 induces lung neoplasms, we found that Sb ore concentrate (principally Sb_2S_3) can also induce the same effect at about the same incidence. These findings support the observations of an increased incidence of lung cancer in workers exposed during the refining of Sb ore in the production of Sb_2O_3 (Davies, 1973).

The finding that female rats developed lung cancer but male rats did not in the same time period suggests that female rats are more susceptible to the induction of lung cancer by Sb_2O_3 and Sb ore. The lungs from male rats contained higher concentrations of antimony than the females, so the tumor response does not appear to be a function of lung tissue concentration of Sb. We found that the tissues and blood of female rats (controls, as well as exposed) contained higher concentrations of arsenic (with the exception of lungs) than the males. This has been reported by others in control rats (Schroeder et al., 1968). Since arsenic has been shown to effect immunological systems (Funderburk et al., 1969), it is conceivable that the immunological responsiveness in female rats is not as adequate as in male rats. It should also be noted that the lung concentration of As was higher in males than females exposed to Sb_2O_3 (but not in those exposed to Sb ore). If As is to be implicated in promoting the carcinogenic effect of Sb, then either it is not related to a local tissue effect in the lung or the As in the Sb_2O_3 is not readily biologically available. It is possible that the systemic concentration of As is more critical in affecting the carcinogenic response. The fact that the female rats had a higher incidence of pituitary tumors than the male rats might also be related.

A curious finding was the higher concentration of arsenic in the lungs of the male and female rats exposed to Sb_2O_3 than in those exposed to Sb ore. The Sb ore contained much more arsenic than the Sb_2O_3 (20 times as much), and there was only 5–6 times as much Sb in

the lungs of the animals exposed to Sb_2O_3 compared to those exposed to Sb ore. A possible explanation is that the arsenic in the Sb_2O_3 was in a more insoluble form than that in the Sb ore and remained in the lungs for a longer period of time. An alternative explanation is that the rats received much more Sb_2O_3 than is reflected in the differences in the Sb concentrations between the Sb_2O_3 and Sb ore groups, and that the Sb_2O_3 was more rapidly cleared and/or dissolved than the Sb_2S_3, whereas the arsenic compound was not.

The high concentration of Sb in the lungs of the exposed rats was expected, since the exposure concentrations were relatively high. Our finding of 38,300 μg Sb/g dry lung in male rats was comparable to the concentrations of Sb found by Gross et al. (1955a,b) in their rats. They found 3182 μg Sb_2O_3/g wet weight of lung after 9 mo of exposure. Since dry weight is about 10% of wet weight in lung tissue, the result can be written 31,820 μg Sb_2O_3/g dry weight. Since 83.53% of Sb_2O_3 is Sb by weight, then the lungs contained 26,579 μg Sb/g dry weight.

Day and Hickling (1967), utilizing a unique method for measuring Sb in lung tissue of living patients, estimated the concentration of Sb in the lungs of two industrial antimony workers. They estimated 1.4 and 2.0 g antimony in the lungs of two patients. Since human lungs weigh about 500 g, and dry weight is about 10% of wet weight, the concentrations of Sb in these two lungs were 28,000 and 40,000 μg Sb/g dry weight. These concentrations are quite close to what we found in the rat lungs. These results indicate that the workers were probably exposed to concentrations similar to those we used in our experiment.

Are these high concentrations of Sb necessary to induce lung cancer? Probably not. Watt (1983) induced lung tumors in rats with much lower concentrations of Sb_2O_3 than we used (4.2 mg Sb/m^3 versus 45 mg Sb_2O_3/m^3). Although Watt did not report analyses of the rat lung tissue in his experiment, we had the opportunity of examining the histopathology slides from his study. Based on light microscopic examinations, rats exposed to Sb_2O_3 in his experiment had less than 10% of the amount of particulate in their lungs than did rats in our study.

We have found interstitial fibrosis to be a frequent precursor to the induction of lung cancer in rats with a variety of particulates. This has been true with several different beryllium compounds (Groth et al., 1980) and quartz (Groth et al., 1986). We also found this to be true with Sb_2O_3 and Sb ore. The other interesting finding is that even though the male rats exposed to Sb_2O_3 and Sb ore developed pulmonary interstitial fibrosis, they did not develop lung cancer. Perhaps they were not allowed to live long enough for the cancer to develop or for them to become more susceptible.

Several different Sb compounds have been shown to be positive in

short-term in vitro tests as well. They have caused chromosome breakage (Paton and Allison, 1972), enhanced cell transformation (Casto et al., 1979), and mutations (Kanematsu et al., 1980).

Based on our results as well as those of others referred to above, we can conclude that antimony compounds are carcinogenic and mutagenic.

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