Inhalation of 2,3,5-Triiodobenzoic Acid by Normal and Sulfur Dioxide-Exposed Rats

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Abstract □ Exposure to ^{125}I-labeled 2,3,5-triiodobenzoic acid resulted in deposition of the material in the lungs of rats. The initial uptake was 20.85% followed by removal at an exponential rate. The residency half-life in the lungs was 17.7 min. Exposure of animals to sulfur dioxide for 4 consecutive days, 4 hr/day, prior to inhalation of the labeled compound did not provide statistically significant differences in the parameters studied.

Keyphrases □ 2,3,5-Triiodobenzoic acid, radiolabeled—inhalation and half-life in lungs, normal and sulfur dioxide-exposed rats □ Sulfur dioxide—effect on residency time of 2,3,5-triiodobenzoic acid in lungs of rats □ Inhalation—radiolabeled 2,3,5-triiodobenzoic acid, normal and sulfur dioxide-exposed rats, residency time in lungs

The substance 2,3,5-triiodobenzoic acid has been shown to affect the growth and development of certain plants. Greer and Anderson (1) reported that treatment of soybean plants with this chemical at the beginning of flowering resulted in an increased seed yield.

While the uptake and fate of 2,3,5-triiodobenzoic acid following oral administration have been studied in various species (2–5), no information is available relevant to lung uptake and retention following inhalation of the compound. In the present investigation, the degree of uptake and loss of inhaled ^{125}I-labeled 2,3,5-triiodobenzoic acid in the lungs of rats was studied. In addition, the consequence of exposure to sulfur dioxide was determined. Sulfur dioxide-induced alterations in the uptake and rate of removal of inhaled particles have been studied with varying results (6–8). Sulfur dioxide-induced alterations in the physiological response of the lung could affect the uptake, loss, and subsequent hazard of 2,3,5-triiodobenzoic acid from inhalation.

EXPERIMENTAL

For this investigation, 2,3,5-triiodobenzoic acid labeled in the 2-position with ^{125}I was employed in the production of the dry labeled aerosol. The labeled material was synthesized in a manner similar to that described previously (4, 9). Approximately 100 mCi of ^{125}I in the form of sodium iodide[1] was used in the synthesis. Purification of the final material was achieved by TLC (3, 4), employing a fluorescent silica gel[2] and a developing solution consisting of petroleum ether (bp 30–60°)–propionic acid (10:1). The labeled material was removed from the gel with hot ethanol and was found to be 97.2% pure. Approximately 0.25 g of labeled 2,3,5-triiodobenzoic acid containing about 10 mCi of ^{125}I was dissolved in 10.0 ml of ethanol, and the resulting solution was used in the aerosol generator.

The radioactive aerosol was produced with a Lucite jet and generator in a manner similar to that of Johnson and Ziemer (10). Measurement of aerosol diameter was performed with a seven-stage cascade impactor[3]. Calibration and operation of the impactor were performed according to methods described previously (10, 11).

The sampling flow rate through the impactor was 105–120 ml/min, which approximates the breathing rate of rats (12). The aerosol was sampled for 3 min during each exposure period. Each cover slip from each stage in the cascade impactor was removed and analyzed by crystal scintillation spectrometry for the amount of impinged radioactive aerosol. The percent less than stated size for each impactor stage was calculated from the results obtained for the cover slips, and the activity median aerodynamic diameter and corresponding geometric standard deviation of the aerosol were determined from these data.

For the entire investigation, the activity median aerodynamic diameter values ranged between 0.31 and 0.51 μm, with the standard deviations ranging from 2.45 to 3.09 μm. The average value was 0.41 μm and the average of the standard deviations was 2.73 μm. The standard deviation of the average activity median aerodynamic diameter value was 0.074 μm at the 68% confidence level. The diameter of the aerosol for each aerosol run was within 2 *SD* of the average. Therefore, variation in biological data obtained in the various portions of the investigation probably was not caused by different particle sizes.

A Lucite chamber (38 × 38 × 15 cm) was used for exposing the animals to sulfur dioxide. The environmental chamber was divided into five segments, thus allowing five animals to be kept separated during exposure. Compressed air mixed with 10% sulfur dioxide[4] was passed through the closed chamber at a flow rate of about 4.5 liters/min. The exhaust air–gas mixture was analyzed 3 hr after initiation of sulfur dioxide exposure by passing the exhaust through a trapping solution. The trapping solution was analyzed colorimetrically by the West-Gaecke method in a similar manner as described by Stern (13).

The major portion of the investigation was conducted in two phases. Phase I dealt with the determination of the percent uptake and residency half-life of inhaled 2,3,5-triiodobenzoic acid in the lungs of 200–300-g male laboratory rats[5] exposed only to an aerosol of the labeled material. For each run, a group of five animals was anesthetized with a freshly prepared solution of sodium pentobarbital and chloral hydrate (10). The anesthetized animals were placed in a special small animal aerosol exposure chamber[6]. Operational procedures for the exposure chamber were conducted in a similar manner as described previously (10, 14).

After a 6-min aerosol inhalation period, an animal was decapitated at 15, 30, 45, 60, and 75 min postexposure. The amount of inhaled ^{125}I, expressed as corrected counts per minute, in the skinned head, excreta, left and right lungs, and residual carcass was determined by crystal scintillation spectrometry. Although the chamber design prevented contamination of the body of the animal, the skin was removed from the head because it became contaminated with the radioactive aerosol during the exposure period. The total inhaled radioactivity for each animal was determined by the summation of counts per minute for all samples composing that animal.

The percent of total inhaled radioactivity remaining in the left and right lungs at the various postexposure time periods was calculated. Percents at the various time periods were plotted on semilog paper, and a line of best fit was statistically determined along with the equation describing the line. From the equation of the

[1] New England Nuclear Corp., Boston, Mass.
[2] Adsorbosil-1P, Applied Science Laboratories, Inc., State College, Pa.
[3] Supplied by the Lovelace Foundation, Albuquerque, N.M., who also supplied the effective cut-off diameters for the various stages.
[4] Matheson Co., Inc., Joliet, Ill.
[5] Sprague–Dawley strain supplied by Laboratory Supply, Indianapolis, Ind.
[6] Designed by the Lovelace Foundation.

Table I—Percent Lung Retention[a] of Labeled 2,3,5-Triiodobenzoic Acid following Inhalation

Post-exposure Time, min[b]	Study A			Study B			Study C		
	Left Lung	Right Lung	Total Lung	Left Lung	Right Lung	Total Lung	Left Lung	Right Lung	Total Lung
Labeled Compound Only									
15	4.23	7.05	11.28	6.07	9.33	15.40	7.50	4.06	6.56
30	1.79	3.29	5.08	1.68	3.26	4.94	1.78	3.21	4.99
45	0.67	1.27	1.94	1.63	2.56	4.19	1.34	2.71	4.05
60	0.53	0.80	1.33	0.43	1.00	1.41	0.69	1.18	1.81
75	0.20	0.31	0.51	0.19	0.89	1.08	0.25	0.38	0.63
Sulfur Dioxide and Labeled Compound[c]									
15	5.14	7.38	12.52	6.97	7.84	14.81	2.48	4.45	6.93
30	2.60	4.37	6.97	2.20	3.40	5.60	1.51	2.54	4.05
45	1.36	1.75	3.11	0.76	1.30	2.06	1.07	1.83	2.90
60	0.87	1.79	2.66	0.70	1.21	1.91	0.31	0.55	0.86
75	0.52	0.80	1.32	0.47	0.61	1.08	0.30	0.49	0.79

[a] Percent in each lung was based on the total amount of inhaled labeled 2,3,5-triiodobenzoic acid found in the entire animal, including excreta and excluding skin on the head. [b] One animal at each time period for each study. [c] The average concentration of four sulfur dioxide exposures for Studies A, B, and C was 429, 370, and 325 ppm, respectively.

line, the initial percent uptake at time zero was determined and the residency half-life was calculated from the slope of the line. Slope of the line was also used to express the rate of removal of radioactive compound from the lungs. Three values for each time period were obtained by repeating Phase I three times.

Phase II was the determination of the uptake and residency half-life of inhaled labeled aerosol in the lungs of rats after the animals were exposed to sulfur dioxide. The animals were subjected to the sulfur dioxide gas in the chamber described previously. The concentration of sulfur dioxide in the exhaust air was approximately 400 ppm for each sulfur dioxide exposure. Each group of five animals was exposed to the gas for 4 hr daily for 4 consecutive days.

One hour following the fourth exposure to sulfur dioxide, each group of five animals was subjected to the aerosol of labeled 2,3,5-triiodobenzoic acid. Following the same procedures as in Phase I, the initial percent uptake and residency half-life of inhaled labeled aerosol in the lungs were determined for animals in Phase II. Three values for each time period were obtained by conducting Phase II three times.

Gross autoradiography techniques were employed to determine the approximate location and loss of inhaled labeled 2,3,5-triiodobenzoic acid within the lungs at the various postexposure time periods. Two separate groups of five animals were treated identically as the animals in Phases I and II. The lungs from each animal were exposed to X-ray film[7] while frozen. All photographic processing procedures were kept constant to allow correlation in intensity of the darkened X-ray with the amount of inhaled labeled aerosol.

A pathology study was performed to determine if either 2,3,5-triiodobenzoic acid or sulfur dioxide produced any pathoanatomic alterations of the lung tissue. One group of five animals was exposed to the labeled aerosol while another group received sulfur dioxide and the labeled aerosol as described previously. Lungs were removed and prepared for sectioning. The tissue slices were stained with eosin–hematoxylin and a glycoprotein stain. The staining solutions and techniques employed were described by Luna (15). Lungs from control animals were treated similarly for comparative purposes.

RESULTS AND DISCUSSION

Table I lists the percent lung retention of 2,3,5-triiodobenzoic acid at the various postexposure time periods for animals exposed only to the labeled compound and animals exposed to sulfur dioxide previous to 2,3,5-triiodobenzoic acid administration. Studies A, B, and C represent the three individual runs performed for each phase. It can be seen from the data that inhaled compound reached both the left and right lungs. Also, the amount of labeled compound in the lungs decreased as time elapsed following exposure. The data indicate that the amount of inhaled compound was always greater in the right lung than in the left lung. This occurrence may be explained by the greater mass of the right lung.

The data in Table I were expressed as individual exponential equations for the left, right, and total lungs for Studies A, B, and C. The general equation $Y = Ae^{-BX}$ represents each line, where Y is the percent retention, A is the ordinate intercept, B is the slope of the line, and X is time in minutes. The intercept of the equation is equal to the initial percent uptake at postexposure time zero.

For rats exposed only to labeled 2,3,5-triiodobenzoic acid, the percent uptake for the total lung for Studies A, B, and C was 22.75, 24.72, and 15.09%, respectively. The average of these values was 20.85 ± 5.09% at the 68% confidence level. The residency half-life of labeled 2,3,5-triiodobenzoic acid in the lung was calculated from individual exponential equations. The slope of each line from each equation was found to be equal; thus, the residency half-life for each line was equal. The average of the nine residency half-life values was 17.7 min.

From the nine equations for animals exposed to sulfur dioxide previous to labeled 2,3,5-triiodobenzoic acid, the average residency half-life value was 15.8 min. The total percent uptake at time zero, as obtained from the intercept of the equation, was 20.30, 21.47, and 13.07% for Studies A, B, and C, respectively, with an average value of 18.28 ± 4.55% at the 68% confidence level. The uptake of 2,3,5-triiodobenzoic acid for animals exposed only to labeled compound was statistically ($p = 0.10$) equal to the value obtained for animals receiving sulfur dioxide previous to inhalation of the labeled material. Sulfur dioxide produced no observable effect upon the percent uptake of inhaled compound at time zero in the lungs of rats.

Figure 1 represents a compilation of the data for animals exposed to labeled 2,3,5-triiodobenzoic acid only as well as those receiving sulfur dioxide before exposure to the labeled compound. Each line in Fig. 1 was obtained by statistically determining a line of best fit for the corresponding 15 data points from Studies A, B, and C (Table I). Because of the magnitude of values, the 90 data points were omitted from the figure.

Statistical comparisons between the total, right, and left lungs within and between each phase were conducted by a reported method (16). These comparisons showed that the slopes of the lines within each phase and between each phase were equal. Thus, the slopes of the lines representing the rate of removal of 2,3,5-triiodobenzoic acid from the lungs were equal not only among the total, right, and left lungs within each phase but also between each phase. Because the slopes were equal, the residency half-life value of 17.7 min for animals exposed to labeled 2,3,5-triiodobenzoic acid only was equal to the 15.8-min half-life value for animals receiving sulfur dioxide previous to inhalation of the labeled compound.

The fact that inhaled 2,3,5-triiodobenzoic acid was removed from the lungs in all animals exposed to the labeled compound was substantiated by the gross autoradiography study. The intensity of the darkened area on the X-ray film was the greatest at the 15-min postexposure time period, and there was a continuous decrease in

[7] Kodak No-Screen, Eastman Kodak Co., Rochester, N.Y.

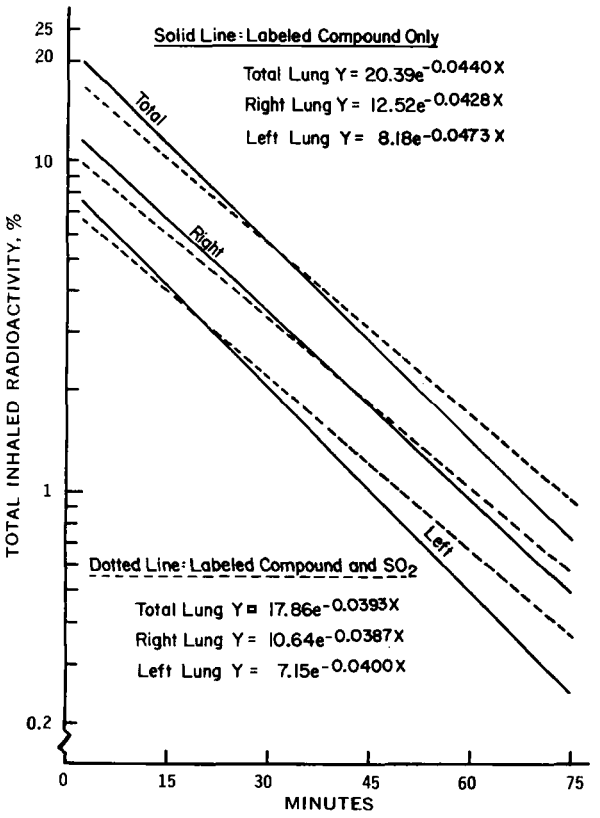

Figure 1—*Percent of the total labeled 2,3,5-triiodobenzoic acid remaining in the lung at various postexposure time periods for pooled values of all three studies in each phase. Each line represents 15 data points.*

intensity for each subsequent time period. Since the intensity for the 60- and 75-min postexposure time periods was negligible, satisfactory photographs for publication were not feasible.

Histopathological examination of lung tissues from animals exposed to either labeled 2,3,5-triiodobenzoic acid or sulfur dioxide was performed. No pathoanatomic alterations were observed when the lung tissues were compared to the controls. It was concluded that neither the labeled compound nor sulfur dioxide at these experimental conditions produced pathological changes in the lungs of rats exposed to either substance.

Since other studies (17, 18) showed that sulfur dioxide at the concentrations used in this investigation will immobilize ciliary movement, it would seem that ciliary movement is not responsible for the removal of the dry labeled aerosol from the lungs in the present study. However, the rate of removal of labeled 2,3,5-triiodobenzoic acid was rapid and may be due to the biological solubility of the substance. Although 2,3,5-triiodobenzoic acid may be a possible environmental pollutant, damage to the lung stemming from inhalation of the compound is not likely since the substance is removed rapidly from the lungs.

The results of the investigation show that inhalation of 2,3,5-triiodobenzoic acid is a very important route by which the compound may be assimilated during application to crops. Furthermore, sulfur dioxide inhalation did not influence the uptake and subsequent removal of the compound from the lung.

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ACKNOWLEDGMENTS AND ADDRESSES

Received October 15, 1973, from the *Department of Bionucleonics, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907*

Accepted for publication June 17, 1974.

Supported in part by the U.S. Public Health Service, Food and Drug Administration, Bureau of Radiological Health, and conducted under the auspices of the Institute for Environmental Health, Purdue University.

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