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The Fate of Inhaled Azodicarbonamide in Rats

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The Fate of Inhaled Azodicarbonamide in Rats. MEWHINNEY, J. A., AYRES, P. H., BECHTOLD, W. E., DUTCHER, J. S., CHENG, Y. S., BOND, J. A., MEDINSKY, M. A., HENDERSON, R. F., AND BIRNBAUM, L. S. (1987). *Fundam. Appl. Toxicol.* **8**, 372-381. Azodicarbonamide (ADA) is widely used as a blowing agent in the manufacture of expanded foam plastics, as an aging and bleaching agent in flour, and as a bread dough conditioner. Human exposures have been reported during manufacture as well as during use. Groups of male F344/N rats were administered ADA by gavage, by intratracheal instillation, and by inhalation exposure to determine the disposition and modes of excretion of ADA and its metabolites. At 72 hr following gavage, 30% of the administered ADA was absorbed whereas following intratracheal instillation, absorption was 90%. Comparison between groups of rats exposed by inhalation to ADA to achieve body burdens of 24 or 1230 μg showed no significant differences in modes or rates of excretion of [^{14}C]ADA equivalents. ADA was readily converted to biurea under physiological conditions and biurea was the only ^{14}C-labeled compound present in excreta. [^{14}C]ADA equivalents were present in all examined tissues immediately after inhalation exposure, and clearance half-times on the order of 1 day were evident for all tissues investigated. Storage depots for [^{14}C]ADA equivalents were not observed. The rate of buildup of [^{14}C]ADA equivalents in blood was linearly related to the lung content as measured from rats withdrawn at selected times during a 6-hr inhalation exposure at an aerosol concentration of 25 μg ADA/liter. In a study extending 102 days after exposure, retention of [^{14}C]ADA equivalents in tissues was described by a two-component negative exponential function. The results from this study indicate that upon inhalation, ADA is rapidly converted to biurea and that biurea is then eliminated rapidly from all tissues with the majority of the elimination via the urine.

Azodicarbonamide (ADA, $NH_2OC-N=N-CONH_2$, CAS No 123-77-3) is produced as a condensation product of urea and hydrazine. ADA is manufactured predominantely as a fine yellowish powder milled to particle sizes in the 2 to 10 μm range (Slovak, 1981). ADA is widely used as a blowing agent in the manufacture of expanded foam plastics, as an aging and bleaching agent in cereal flour, and as a bread dough conditioner. Production of ADA in the United States was between 1 and 10 million pounds in 1977 and it has been estimated that 232,000 workers are occupationally exposed to ADA in the form of respirable dust (Perry, 1980). Azodicarbonamide has been implicated in the appearance of an asthmatic reaction of the respiratory tract in workers involved in grinding ADA during manufacture (Ferris *et al.*, 1977; Slovak, 1981).

The safety of use of ADA in baked goods was established in a series of feeding studies in rats and dogs (Oser *et al.*, 1965). Because ADA is readily reduced to biurea during the baking process and because the incorporation of ADA in bread dough could not be accomplished above 75 ppm (10 times the normal level of ADA), these studies were largely focused on feeding studies in which biurea was added to the bread diet at levels of 100,

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316, and 1000 times the normal use levels (7.5 ppm ADA in bread dough). Since these first feeding studies elicited no adverse response for the 2-year study period, a second series of studies were accomplished in which dogs and rats were fed commercial ration with addition of 5 or 10% biurea for 1 year. No adverse effects were observed in rats whereas in dogs evidence of renal pathology developed after about 4 months. All dogs died or were moribund between the 28th and 45th week on test. Histopathologic examinations showed deposition of calculi in the kidney, ureter, and bladder of most of the dogs. The calculi were shown analytically to consist principally of biurea.

Additional studies (Oser *et al.*, 1965) of the fate of an oral dose of biurea in rats disclosed that 83 to 99% of the dose was excreted in the feces or remained in the gastrointestinal tract during the first 24 hr, while 7 to 10% of the dose was excreted in urine over the same period. Only trace amounts of [^{14}C]-biurea equivalents were detected in respiratory CO_2 or in organs other than the GI tract. The LD50 for a single oral feeding in rats was estimated to be in excess of 6400 mg/kg body wt.[1]

The disposition of orally administered [*carbonyl*-^{14}C]azodicarbonamide was investigated in a 48-hr study.[2] Groups of three male F344/N rats were gavaged with suspensions of ADA:water at 11.7, 117, and 1170 mg/kg body wt and one group was gavaged with a suspension of ADA:corn oil at the highest concentration. The report states that less than 10% of the ADA:water or ADA:corn oil dose was excreted in urine, with virtually all of the dose being excreted in feces by 24 hr after administration.

The acute inhalation toxicity of ADA has been studied with inconclusive results.[3] In that study, 5 male and 5 female rats were exposed (whole body) in a glass chamber for a 4 hr period to an average concentration of 6.1 mg ADA/liter of air. The particle size was 5.8 μm mass median aerodynamic diameter (MMAD) and the geometric standard deviation was 2.25 as determined by cascade impactor. Rats were sacrificed 14 days after exposure. The only clinical or pathological sign observed was dyspnea in 8 of the 10 rats immediately after removal from the exposure chamber. Histopathological examination of tissues after sacrifice showed no abnormalities.

The research reported in this manuscript was designed to define the disposition of inhaled ADA dusts in rats. Two types of studies were carried out in sequential fashion. First, a study was conducted to determine the fate of ADA administered by gavage or by intratracheal instillation. Second, four inhalation exposure studies were carried out to determine the influence of the level of initial body burden upon the uptake and retention of ADA in tissues and the rates and modes of excretion, to determine the relationship of ^{14}C buildup in blood and lung during inhalation exposure, and to determine the temporal relationships of uptake and retention of ADA in tissues at selected times through 102 days after exposure.

MATERIALS AND METHODS

Chemical. The ^{14}C-labeled ADA was obtained from Pathfinder Laboratories (St. Louis, MO) and the unlabeled ADA from Midwest Research Institute (Kansas

[1] National Polychemicals, Inc., Technical Bulletin No. OKE-02-0766.

[2] International Research and Development Corporation, Biological Disposition of Orally Administered Azodicarbonamide in Male Rats, Unpublished Report to the National Toxicology Program, Report No. 5703-125, 1982.

[3] International Research and Development Corporation, Acute Inhalation Toxicity Test with Azodicabonamide in Rats, Unpublished Report to the National Toxicology Program, Report No. 5703-115, 1982.

City, MO). The purity of both the unlabeled and ^{14}C-labeled ADA determined by the high-performance liquid chromotography (HPLC) method was greater than 97%. The ^{14}C-labeled ADA was diluted with unlabeled ADA to obtain the specific activity required. The dilution was accomplished by dissolving ADA in dimethyl sulfoxide (DMSO), to which 5% water (by volume) was added, mixing appropriate volumes of the labeled and unlabeled solutions, and precipitating the combined ADA using methylene chloride (radiochemical recovery > 80%). The addition of water was necessary to reduce the static charge on the dried ADA and to provide the proper consistency of the precipitate for packing in the aerosol generation system. The specific activity used in each of the studies ranged from 0.29 to 26 mCi/g and was adjusted so that approximately equal amounts of ^{14}C were deposited in animals at all exposure concentrations. A sample of the ADA particles collected during the inhalation exposure of rats was also measured as having greater than 97% purity.

Animals. All rats used in these studies were male F344/N rats, born and reared in this Institute's barrier maintained colony. Rats were 11 to 15 weeks of age (200 to 250 g) at the initiation of each study. Prior to exposure, rats were housed in polycarbonate cages (size 20 × 25 × 48 cm, 2 rats per cage) with hardwood chip bedding (Murphy Forest Products, Rochelle Park, NJ) and filter caps. Bedding was changed weekly. Animal rooms were maintained at 21 ± 1°C with a relative humidity between 20 and 50% and a 12-hr light/12-hr dark cycle starting at 0600 hr. Food (Lab Blox, Allied Mills, Chicago, IL) and water were provided *ad libitum.*

All rats in these studies were sacrificed by intraperitoneal injection of 1 ml of T61 euthanasia solution (Taylor Pharmacal Co., Decatur, IL) and tissues were dissected and weighed immediately. Blood samples were obtained by heart puncture using heparinized syringes and needles.

Experimental design. The experimental design for the several studies is summarized in Table 1. Rats in the gavage and intratracheal instillation studies were dosed and placed in metabolism cages for a period of 72 hr, then sacrificed. Rats in the 3-hr inhalation studies were either sacrificed immediately upon cessation of the exposure (3 rats in normal exposure tubes and 5 rats in plethysmograph tubes) or placed into metabolism cages for a 72-hr period, then sacrificed (4 rats). For the 5.5-hr inhalation exposure, groups of 3 rats were withdrawn from the exposure apparatus at times of 20, 40, 60, 90, 120, 180, 240, 300, or 330 min after the beginning of the exposure to determine the rate of buildup of ^{14}C in blood and lung. Additionally, 8 rats were sacrificed immediately upon cessation of the exposure (3 from normal exposure tubes and 5 from plethysmograph tubes) and 4 rats were placed into metabolism cages for 96 hr, then sacrificed. After the 6-hr exposure, 3 rats were sacrificed immediately after cessation of the exposure, 4 rats were placed into metabolism cages for a 96-hr period, then sacrificed, and 36 rats were scheduled for sacrifice in groups of 3 at 0.02, 0.17, 0.33, 0.66, 1, 2, 4, 8, 12, 16, 24, and 102 days after cessation of the exposure to determine the time course of tissue distribution of the [^{14}C]-ADA or its metabolites.

Compound administration and aerosol generation. A saline suspension of the precipitated [^{14}C]ADA (0.1 mg ADA/0.5 ml saline) containing 5 μCi [^{14}C]ADA was administered to each rat by either gavage or by intratracheal instillation. Gavage of ADA in rats was accomplished using a curved, bulb-ended cannula mounted on a syringe. Intratracheal instillation was done by anesthetizing the rat with halothane (5% in O_2), placing the animal in a vertical position, and inserting into the trachea a blunt-ended needle attached to a syringe.

For inhalation exposures a Wright dust feeder (BGI Incorporated, Waltham, MA) operated with an air flow of 20 liters/min was used to generate aerosols of ADA. The aerosol was conveyed into a small-animal, nose-only, inhalation exposure chamber (Raabe *et al.,* 1973). The desired aerosol concentrations within the exposure chamber were obtained by regulation of the speed at which the material reservoir cup rotated in relation to the scraper blade of the dust feeder.

For each exposure except the one of 6 hr duration, five rats were exposed in specially designed exposure tubes which served as whole-body plethysmographs (Medinsky *et al.,* 1985). This allowed measurement of the breathing rate and minute volumes of these rats throughout the exposure period and subsequent calculation of the percentage of the aerosol deposited in the rats.

Aerosol characterization. During each inhalation exposure of rats, the aerosol concentration and particle size were determined. The relative concentration was monitored continuously by a real-time aerosol monitor (GCA Corp., Bedford, MA, Model RAM-S). The aerosol concentration was also measured twice per hour throughout the exposure period by collection on glass fiber filters (Gelman Instrument Co., Ann Arbor, MI, Type A/E) for periods of 10 min. Aerosol size was determined by periodic sampling using cascade impactors (Mercer *et al.,* 1970). Quantitation of ^{14}C-labeled ADA on filter samples or cascade impactor stages was by liquid scintillation spectroscopy (Packard Instrument Co., Downers Grove, IL, Model 460).

Metabolism collections. Immediately after administration of ^{14}C-labeled ADA, randomly selected rats were placed in glass metabolism cages (Stanford Glass, Palo Alto, CA) for separate collection of urine, feces, and exhaled metabolites. A vacuum pump was used to pull 0.5 liter/min of filtered room air through the cages and two bubblers containing 0.5 M KOH, in series. Urine and feces were collected in glass vials placed on dry ice to prevent microbial activity. The collection vials and the trap-

TABLE 1

EXPERIMENTAL DESIGN FOR STUDIES OF THE FATE OF INTERNALLY ADMINISTERED AZODICARBONAMIDE

Mode of administration[a]	Number of animals				
	Metabolism cages	Plethys-mographs	Immediate sacrifice	Postexposure sacrifice	Scheduled blood samples
Gavage	3				
Intratracheal	3				
Inhalation (3 hr)					
Low level	4	5	3		
High level	4	5	3		
Inhalation (5.5 hr)	4	5	3		27[b]
Inhalation (6.0 hr)	4		3	36[c]	

[a] The dose administered by gavage or intratracheal instillation was 0.1 mg/animal. Projected inhalation exposure concentrations were 1.5 mg/m^3 (low), 150 mg/m^3 (high), and 20 mg/m^3 (5.5 and 6-hr studies).

[b] Groups of three rats withdrawn from the exposure apparatus at selected times after the initiation of the exposure.

[c] Groups of three rats sacrificed at selected times after cessation of the exposure.

ping solutions were changed at 3, 6, 9, 18, 24, 48, 72, and 96 hr after administration of the compound.

Quantification of ^{14}C in Excreta and Tissue samples. Quantification of ^{14}C in urine or exhaled air was accomplished by addition of duplicate aliquots of 0.5 ml of urine or trapping solution directly to scintillation cocktail and counting in the liquid scintillation spectrometer (Packard Instrument Co.). Feces and large tissue samples were prepared for counting by addition of water and formation of a slurry (feces) or homogenization in a blender. Duplicate aliquots of a slurry or homogenate were placed in cellulose cones and the ^{14}C content was oxidized to $^{14}CO_2$ using a tissue oxidizer (Packard Instrument Co., Model 306B). The $^{14}CO_2$ formed upon oxidation was trapped in liquid scintillation cocktail (Packard, Permaflour V) and the ^{14}C content was determined by liquid scintillation counting. Small tissue samples (< 0.2 g) were oxidized *in toto* and counted in the same fashion.

Metabolite identification. A high-performance liquid chromatography method for separating ADA from its major metabolite, biurea, in urine and feces was developed. The conditions were: silica gel column (Alltech Chemical Co., Deerfield, IL, 600 SIB, 25 cm by 4.6 mm, 10 μm); 80:20 ethyl acetate:methanol (Burdick and Johnson, Muskegon MI, distilled in glass); 1 ml/min flow rate; and a refractive index or radioactive flow monitor/detector. Retention times of compounds under these conditions were 7.1 min (ADA), 11.9 min (urea), and 19.0 min (biurea). For spiked urine, an aliquot was dried under nitrogen and the residue taken up in DMSO and injected onto the column. Eluant from the HPLC was collected and assayed for ^{14}C. Recovery for this procedure was 90%. For feces, approximately 0.5 g of feces was homogenized with 2.5 g silica gel and DMSO. This slurry was added to a small glass column and 10 ml DMSO was used to elute ^{14}C from the sample. The eluant was collected and counted for ^{14}C and the remainder lyophilized to dryness. The residue was then redissolved in a small volume of DMSO and analyzed by HPLC, as described for urine. Recovery of radioactivity as eluted from the silica gel was 89%. Only one peak eluted from the HPLC with a retention time equivalent to biurea.

To establish whether ADA or biurea was present in rat blood after inhalation exposure, an ancillary experiment was conducted to determine the stability of ADA in rat whole blood. Duplicate 1 ml fresh blood samples were spiked with a known amount of [^{14}C]ADA/DMSO solution (about 5 μg ADA), mixed, and immediately frozen in liquid nitrogen. The samples were lyophilized to dryness. One aliquot was reconstituted in 1 ml of DMSO; the second was reconstituted with 1 ml of a solution containing 5 mg/ml unlabeled-ADA dissolved in DMSO.

Data analysis. The initial body burden (IBB) for rats in each inhalation exposure was determined by sacrifice at the end of the exposure of three rats exposed in standard exposure tubes and sacrifice of five rats exposed in plethysmograph tubes. The ^{14}C content of all tissues, excluding the pelt, was summed to estimate the IBB. For rats maintained in metabolism cages, the tissue content at sacrifice (excluding the pelt) was summed with the ^{14}C content measured in all excreta for that animal to estimate the IBB. The ADA content in any animal or tissue was calculated from the ^{14}C content of the sample and the specific activity was measured for the bulk material in the Wright dust feeder reservoir (mean of triplicate determinations).

TABLE 2

^{14}C IN EXCRETA AND WHOLE BODY OF RATS AT 72 hr FOLLOWING INTRATRACHEAL INSTILLATION OR GAVAGE OF ^{14}C-LABELED AZODICARBONAMIDE

Mode of administration	Percentage of the initial body burden ($\bar{x} \pm$ SE)[a]				
	Urine	Feces	Exhaled air	Total excretion	Body burden at sacrifice
Gavage	30 ± 2.8	67 ± 2.5	2.1 ± 0.88	99 ± 0.36	0.90 ± 0.36
Intratracheal	88 ± 2.0	9.8 ± 2.4	0.81 ± 0.20	99 ± 0.53	1.0 ± 0.50

[a] Initial body burden (dose) was 5 μCi [^{14}C]ADA (0.1 mg).

Fitting of retention curves to data sets was accomplished using a nonlinear least-squares method. The function fitted to excreta and tissue data was either a one-, two-, or three-component negative exponential. Data for tissue content were expressed as percentages of the IBB. Excretion data were expressed as percentages of the IBB excreted per hour. The likelihood ratio statistic (Gallant, 1975) was used to determine if the addition of a second or third term of the typical negative exponential function was appropriate. An F statistic was used to determine if the data subsets were from a single population. When data sets were combined, the mean values for each collection period were obtained and subsequent fitting of such data was accomplished using a weighted nonlinear routine where the weights were the reciprocal of the variance of each mean value. Student's t distribution was used in estimating 95% confidence intervals. Data values for excretory products in the urine, feces, or exhaled air were analyzed and plotted using the midpoint of the collection interval as the time axis.

RESULTS

Metabolite Identification

In the ancillary studies designed to determine the form of the ^{14}C in urine, feces, and blood samples following gavage or intratracheal instillation of ^{14}C-labeled ADA, the ^{14}C label was always associated with the metabolite biurea. When ^{14}C-labeled ADA was added to fresh blood samples taken from control rats, the conversion occurred within a period of 5 min, the time required for sample preparation for analysis by HPLC. No evidence was found for other potential metabolites. Only when fresh blood samples were pretreated with relatively massive quantities of ADA (5 mg/ml) prior to additon of ^{14}C-labeled ADA was the ^{14}C associated with the parent compound. These results indicate that the conversion from ADA to biurea in blood can be saturated, but only at unrealistic levels of ADA content in blood.

Gavage and Intratracheal Administration

The cumulative percentages of the IBB of [^{14}C]ADA equivalents measured in urine and feces and present in tissues of rats at 72 hr after either gavage or intratracheal instillation are presented in Table 2. The actual percentages of the administered ADA absorbed (sum of total urinary excretion, amount exhaled in respired air, and content in tissues at sacrifice) may be slightly underestimated by this method because it does not account for the potential role of biliary excretion. However, in rats biliary excretion would be expected to result in fecal excretion of at most 5% of the administered dose for compounds with molecular weights of less than 120 (Smith, 1973). Within this limitation, absorption of ADA was 33% of the administered dose following gavage and 90% following intratracheal installation. Urinary excretion rate of [^{14}C]ADA equivalents expressed as a percentage of IBB per hour was much greater

TABLE 3

INITIAL BODY BURDENS (IBB) FOR FOUR INHALATION EXPOSURES OF RATS TO GRADED LEVELS OF ^{14}C-LABELED ADA AND EXCRETION PATTERNS OVER A 72 hr PERIOD AFTER EXPOSURE

Exposure concentration[a] (μg/liter)	AMAD[b] (μm)	σ_g[b]	Exposure duration (hr)	Initial body burden[c]		Percentage of the IBB at 72 hr[d]		
				nCi	μg	Feces	Urine	Whole body
1.5 ± 1.5	1.8 ± 0.12	1.7 ± 0.1	3	624 ± 167	24	75 ± 4	24 ± 4	1.2 ± 0.2
150.0 ± 9.1	2.1 ± 0.19	1.7 ± 0.2	3	353 ± 87	1230	84 ± 3	11 ± 2	4.1 ± 1.4
20.2 ± 2.9	3.1 ± 0.14	1.6 ± 0.1	5.5	533 ± 246	468	58 ± 14	34 ± 14	5.7 ± 4.0
25.1 ± 4.5	3.4 ± 0.10	1.6 ± 0.1	6	945 ± 255	602	68 ± 3	27 ± 1	1.6 ± 0.5

[a] $\bar{x} \pm$ SD, $n > 6$.
[b] $\bar{x} \pm$ SD, $n = 6$.
[c] $\bar{x} \pm$ SE, $n = 3$.
[d] $\bar{x} \pm$ SE, $n = 4$.

for intratracheal instillation compared to gavage. Fecal excretion rates were not different for these two modes of administration. Only a small percentage (less than 2%) of the IBB was converted to $^{14}CO_2$ and exhaled, regardless of the mode of administration. At 72 hr after gavage, the gastrointestinal tract and carcass contained 0.06 and 0.6% of the IBB, respectively. After intratracheal instillation these values were 0.2 and 1.0%, respectively. The lung contained 0.5% of the IBB at 72 hr after intratracheal instillation.

Inhalation Exposures

The aerosol characteristics measured during each of the four inhalation exposures of rats to ADA are summarized in Table 3. The particle size ranged from 1.8 to 3.4 μm AMAD. The particle sizes measured during the two exposures conducted to determine the potential role of the level of IBB upon subsequent retention and clearance of [^{14}C]-ADA equivalents were not different (1.8 and 2.1 μm AMAD). The particle size for the exposures conducted to determine the rate of buildup of ^{14}C in blood or the tissue retention of [^{14}C]ADA equivalents were also not different from each other (3.1 and 3.4 μm). The measured geometric standard deviation (σ_g) for all four exposures was either 1.6 or 1.7. Deposition, expressed as the mean (±SD, $n = 5$) percentage of the inhaled material deposited in the respiratory tract, was 24 ± 8, 38 ± 12, and 33 ± 13% after exposure to 1.8, 2.1, and 3.1 μm AMAD aerosols, respectively. The mean aerosol concentrations measured during each exposure are also presented in Table 3. The IBBs attained for each of the four inhalation exposures and the cumulative percentages of the IBB excreted in urine and feces or retained in the body at 72 hr after the exposure are also shown.

The rate of urinary excretion of [^{14}C]ADA equivalents by rats for each exposure was not different for the 72-hr period after exposure when the three data sets were compared using the F statistic. Similarly, the rate of fecal excretion was not different among the four exposures.

The rate of buildup of [^{14}C]ADA equivalents in blood of rats withdrawn from the exposure apparatus at selected times during the 5.5-hr exposure was a linear function. The rate of buildup of [^{14}C]ADA equivalents in lung of these same rats was also linear. Consequently, the ratio of [^{14}C]ADA in total lung to the [^{14}C]ADA ml of blood was a linear

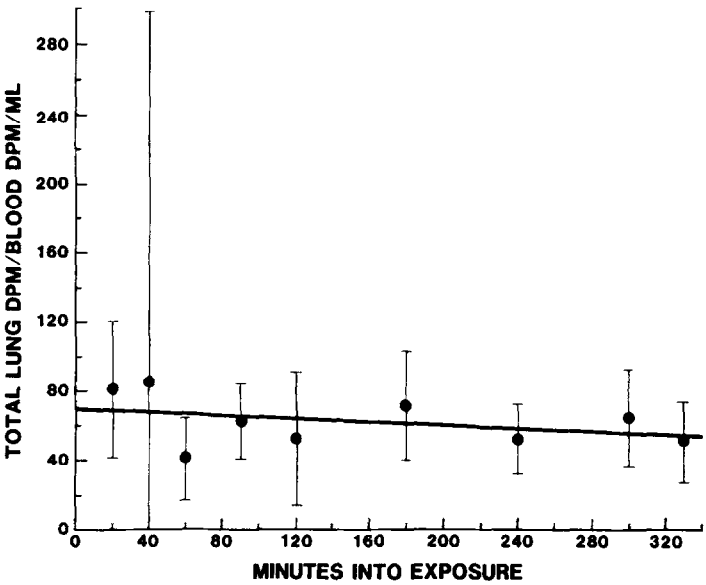

FIG. 1. The ratio of lung content of [^{14}C]ADA equivalents to the blood concentration of [^{14}C]ADA equivalents in the same animals. Values are the mean with 95% confidence interval. The curve represents a linear function fitted to the data.

function with a slope indistinguishable from zero (Fig. 1).

The [^{14}C]ADA equivalents (as % IBB) present in tissues of rats at 72 hr after exposure to aerosols of ADA were comparable for all four exposures (data not shown). At sacrifice, the gastrointestinal tract contained <1.1%, the carcass <2.7%, all other tissues <0.06%, and the lung <0.2% of the IBB.

The time course of uptake and retention of [^{14}C]ADA equivalents in tissues over the 102-day period after exposure for the group of rats exposed for 6 hr are presented in Table 4. The concentrations of [^{14}C]ADA equivalents in these same tissues are presented in Table 5.

DISCUSSION

These studies indicate that ADA is readily absorbed following deposition in the respiratory tract. Clearance of inhaled ADA from the body occurs rapidly, such that only small fractions of the initial burden are present by 72 hr after administration. ADA is rapidly converted to its metabolite biurea under physiological conditions such that the transport and clearance kinetics quantified in these studies should be viewed as typical of biurea, rather than ADA.

In ancillary studies the ^{14}C measured in selected urine and fecal samples of rats administered ADA by gavage or intratracheal instillation was determined by HPLC to be in the form of biurea; no evidence for the presence of ADA was found in these samples. The form of the ^{14}C in blood of rats after inhalation exposure could not be determined directly due to the extremely low concentration of ^{14}C in these samples. In additional ancillary studies designed to determine the form of the ADA in blood, it was determined that only when the blood was pretreated by addition of substantially larger quantities of ADA than could possibly reach blood via normal absorption routes, could spikes of [^{14}C]ADA be detected as associated with ADA rather than biurea.

TABLE 4

TISSUE CONTENT OF [^{14}C]ADA EQUIVALENTS IN RATS AT SELECTED TIMES AFTER INHALATION EXPOSURE TO 25 μg/liter ADA FOR A 6-hr PERIOD (% IBB, $\bar{x} \pm$ SE, $n = 3$)

Sacrifice time (days)	Kidney	Liver	GI tract	Trachea	Lung	Turbinates	Skull	Carcass
0.02	0.27 ± 0.017	1.6 ± 0.98	78 ± 6.4	0.26 ± 0.07	1.2 ± 0.6	0.33 ± 0.11	1.7 ± 0.33	9.9 ± 3.3
0.17	0.14 ± 0.044	0.32 ± 0.12	64 ± 18	0.019 ± 0.006	0.27 ± 0.04	0.20 ± 0.09	0.74 ± 0.14	4.6 ± 0.29
0.33	0.074 ± 0.013	0.14 ± 0.03	57 ± 5.4	0.0082 ± 0.0021	0.38 ± 0.14	0.26 ± 0.14	0.72 ± 0.11	4.9 ± 1.3
0.67	0.049 ± 0.003	0.13 ± 0.01	38 ± 11	0.012 ± 0.003	0.21 ± 0.04	0.062 ± 0.012	0.32 ± 0.03	5.9 ± 3.1
1	0.041 ± 0.004	0.11 ± 0.03	21 ± 9	0.0081 ± 0.0024	0.34 ± 0.10	0.11 ± 0.051	0.56 ± 0.15	2.1 ± 0.26
2	0.017 ± 0.003	0.058 ± 0.012	5.9 ± 2.7	0.0015 ± 0.0002	0.12 ± 0.014	0.031 ± 0.012	0.19 ± 0.03	1.4 ± 0.34
4	0.0069 ± 0.0009	0.037 ± 0.005	0.92 ± 0.10	0.0007 ± 0.0002	0.045 ± 0.013	0.0074 ± 0.0016	0.080 ± 0.015	0.52 ± 0.10
8	0.0031 ± 0.0008	0.016 ± 0.006	0.12 ± 0.04	ND[a]	0.0094 ± 0.0010	0.0014 ± 0.0001	0.079 ± 0.029	0.36 ± 0.07
12	0.0017 ± 0.0005	0.014 ± 0.010	0.065 ± 0.012	0.0005[b]	0.0048 ± 0.0025	0.0005 ± 0.0001	0.068 ± 0.021	0.44 ± 0.03
16	0.0011 ± 0.0004	0.020 ± 0.006	0.088 ± 0.021	0.0004 ± 0.0001	0.0052 ± 0.0020	0.0008 ± 0.0001	0.27 ± 0.09	0.33 ± 0.12
24	0.0024[b]	0.024 ± 0.005	0.040 ± 0.014	ND	0.0029 ± 0.0007	ND	0.12 ± 0.06	0.13 ± 0.03
102	0.0017 ± 0.0005	0.0038 ± 0.0012	0.014 ± 0.003	ND	0.0028 ± 0.0009	ND	0.026 ± 0.011	0.13 ± 0.04

[a] Not detectable.
[b] $n = 1$.

TABLE 5

TISSUE CONCENTRATION OF [^{14}C]ADA EQUIVALENTS IN TISSUES OF RATS AT SELECTED TIMES AFTER EXPOSURE TO 25 μg/liter ADA FOR A 6-hr PERIOD (% IBB PER g TISSUE, $\bar{x} \pm$ SE, $n = 3$)

Sacrifice time (days)	Kidney	Liver	GI tract	Trachea	Lung	Turbinate	Skull	Carcass
0.02	0.76 ± 0.08	0.83 ± 0.54	96 ± 34	13 ± 2.9	7.0 ± 3.4	9.6 ± 2.0	0.76 ± 0.13	0.25 ± 0.03
0.17	0.38 ± 0.11	0.17 ± 0.06	76 ± 29	0.89 ± 0.33	1.5 ± 0.2	5.6 ± 2.4	0.34 ± 0.07	0.18 ± 0.01
0.33	0.21 ± 0.04	0.076 ± 0.019	36 ± 5.5	0.45 ± 0.16	1.8 ± 0.6	7.0 ± 3.4	0.33 ± 0.04	0.19 ± 0.05
0.67	0.13 ± 0.01	0.058 ± 0.004	28 ± 8.3	0.44 ± 0.14	1.0 ± 0.3	1.8 ± 0.4	0.14 ± 0.02	0.23 ± 0.12
1	0.13 ± 0.01	0.064 ± 0.019	26 ± 1.8	0.41 ± 0.12	1.5 ± 0.4	2.8 ± 1.1	0.24 ± 0.08	0.089 ± 0.012
2	0.059 ± 0.021	0.038 ± 0.011	11 ± 6.1	0.070 ± 0.006	0.60 ± 0.02	0.96 ± 0.29	0.11 ± 0.02	0.064 ± 0.027
4	0.022 ± 0.003	0.024 ± 0.004	1.5 ± 0.3	0.040 ± 0.011	0.24 ± 0.07	0.24 ± 0.05	0.041 ± 0.008	0.024 ± 0.006
8	0.008 ± 0.002	0.008 ± 0.003	2.9 ± 0.8	ND[a]	0.047 ± 0.005	0.047 ± 0.010	0.037 ± 0.013	0.013 ± 0.003
12	0.004 ± 0.001	0.006 ± 0.004	0.10 ± 0.02	0.025[b]	0.024 ± 0.013	0.011 ± 0.004	0.031 ± 0.009	0.016 ± 0.001
16	0.003 ± 0.001	0.009 ± 0.003	0.089 ± 0.011	0.015 ± 0.004	0.024 ± 0.010	0.027 ± 0.009	0.13 ± 0.04	0.011 ± 0.004
24	0.006[b]	0.011 ± 0.003	0.12 ± 0.05	ND	0.014 ± 0.004	ND	0.047 ± 0.024	0.004 ± 0.001
102	0.004 ± 0.001	0.002 ± 0.001	0.022 ± 0.003	ND	0.013 ± 0.004	ND	0.007 ± 0.003	0.004 ± 0.001

[a] Not detectable.
[b] $n = 1$.

The observation of biurea as the only metabolite was not unexpected. A potential mechanism is the reduction of ADA by glutathione. Support for such oxidation/reduction reactions has been observed in that ADA exerts an improving effect in bread dough by oxidizing bread protein sulfhydryl, presumably to disulfide linkages (Tsen, 1963). This reaction reached completion in 2.5 min. When ADA reacts with either glutathione or flour SH, mole ratios (GSH/ADA) for the reaction were 1.79 or 1.75, respectively. These values are comparable to the expected mole ratio of 2.0 for a pure oxidation reaction.

The absorption of ADA expressed as the percentage of the initially administered quantity was greater following intratracheal instillation (about 90%) than for gavage (about 33%). The tissue content of [^{14}C]ADA equivalents was virtually identical for both these modes of administration, indicating that distribution in the body was similar. The rates of urinary excretion following administration by these two modes were very similar while the percentage of the IBB eliminated in urine was greater following intratracheal instillation. The conversion of [^{14}C]ADA to $^{14}CO_2$ was very low for both modes of administration. The results are in agreement with previous results following oral administration of ADA or biurea to rats[2] (Oser *et al.*, 1965).

The particle size measured during the inhalation studies was comparable to that measured in the work place where humans may be exposed to ADA dusts (Slovak, 1981). However, the size (about 2.0 μm AMAD) for the first two exposures was less than the size (3.1 μm AMAD) measured during the latter two exposures. The difference in size did not result in measurable differences in the initial desposition of ADA in the respiratory tract. Deposition, expressed as the mean (±SD, n = 5) percentage of the inhaled material deposited in the respiratory tract, was 24 ± 8, 38 ± 12, and 33 ± 13% after exposure to 1.8, 2.1, and 3.1 μm AMAD aerosols, respectively. Also, the apparent difference in particle size among these exposures was not great enough to result in measurable differences in the rate of uptake or in the rate of elimination. The rapid transport of [^{14}C]ADA equivalents to all tissues examined in rats sacrificed immediately after cessation of the exposures indicated that the [^{14}C]ADA equivalents were probably transported in blood. The percentages of the IBB excreted in urine, representing uptake, are not significantly different for the four inhalation exposures (Table 3). The similarity in excretion patterns following inhalation compared to oral dosing was expected since a large fraction of material deposited in the respiratory tract during inhalation exposure is cleared by the mucociliary process to the gastrointestinal tract. The percentage of the IBB present in the tissues examined at 72 hr after the exposure was not different among the four exposures.

The content of [^{14}C]ADA equivalents in blood increased in a linear fashion during the exposure, as did the lung content. The ratio of [^{14}C]ADA equivalents in blood and in lung of the same rats indicated that no saturation of a absorption pathway was reached (Fig. 1). The rapid uptake of [^{14}C]ADA equivalents in all tissues examined can be observed from the data in Table 4, noting that, for all tissues, the highest percentage of the IBB was measured at 0.02 day (30 min) after exposure. The rapid rate of elimination of [^{14}C]ADA equivalents was discerned from the fitting of exponential function to the tissue content data. In all cases the first or intermediate component of retention had a half-time of 1.3 days or less. The long-term component of retention in all tissues examined involved only a small percentage of the IBB (<0.35% for carcass, <0.1% for all other tissues). The retention half-times for the long-term components were not reliable, largely due to the extremely low levels of ^{14}C measured in these tissues at times greater than a few days after exposure. However, a long-term component of retention of approximately 50 days would appear reasonable. Importantly, none of the tissues

examined indicated attainment of a high concentration of [^{14}C]ADA equivalents (Table 5).

The studies described in the report indicated that inhaled ADA was rapidily absorbed, and probably converted to the metabolite biurea, either prior to absorption or immediately thereafter. ADA was transported rapidly to all tissues and elimination was also rapid. After inhalation exposure, approximately 71% of the IBB was eliminated in feces and 25% was eliminated in urine. The level of IBB, between 24 and 1230 μg of ADA, did not result in significant changes in the rate of elimination in urine, nor in the content of tissues (as % IBB) at any time after exposure. The rate of conversion of ^{14}C-labeled ADA to $^{14}CO_2$ was less than 2% of the IBB for any mode of administration. No saturation of absorption pathways was discernible from the blood uptake rate, the tissue content or the tissue concentration of [^{14}C]ADA equivalents.

The results of these studies indicate that inhalation of ADA dusts at levels up to 75 times the concentrations reported in ADA manufacturing (Ferris *et al.*, 1977) do not result in accumulation or storage of the metabolite biurea in tissue. Rapid elimination of the majority of the initial body burden via the kidney to urine might suggest a potential for kidney toxicity due to the relative insolubility of biurea if chronic exposure occurred over long periods.

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