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DCN

Attn: 8(e) Coordinator

Dear Sir or Madam:

On November 8, 1990, the International Isocyanate Institute Inc. (III) informed you of preliminary results of a metabolic fate study of toluene-2,4-diisocyanate being conducted at the Dow Chemical Company, Mammalian and Environmental Toxicology Research Laboratory.

A copy of the final report for this study, which is initiated to define the metabolic profile following exposure of rats to toluene-2,4-diisocyanate (TDI) by the oral and inhalation routes, is enclosed.

A copy of this final report will be supplied to our member companies. Please note that a copy of this report is being submitted to Section 8(d) Health and Safety Study Reporting Rule docket.

Very truly yours,

R. K. Rigger
Managing Director

RKR/sha
Enclosure

0 0 0 2

CONTAINS NO CBI

Study Title

Metabolic Fate of ^{14}C-Toluene-2,4-Diisocyanate in Fischer 344 Rats

Data Requirement

None

Author(s)

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Study Completion Date

January 9, 1992

Performing Laboratory

The Toxicology Research Laboratory
Health and Environmental Sciences
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Sponsor

International Isocyanate Institute Inc.
Project ID- AM-AB-82

Laboratory Project Study ID

K-022862-003

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

Compound: ^{14}C-Toluene-2,4-Diisocyanate

Title: Metabolic Fate of ^{14}C-Toluene-2,4-Diisocyanate in Fischer 344 Rats

To the best of my knowledge and belief the study described in this report was conducted in compliance with the following Good Laboratory Practice Standards:

United States Environmental Protection Agency,
Title 40 Code of Federal Regulations Part 792,
1 July 1990 Edition

Japan Ministry of Agriculture, Forestry and Fisheries,
59 NohSan, Notification No. 3850,
Agricultural Production Bureau,
10 August 1984

Organization for Economic Co-Operation and Development
ISBN 92-64-12367-9, Paris 1982

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QUALITY ASSURANCE STATEMENT

Compound: ^{14}C-Toluene-2,4-Diisocyanate

Title: Metabolic Fate of ^{14}C-Toluene-2,4-Diisocyanate in Fischer 344 Rats

This study was examined for conformance with Good Laboratory Practices as published by the U.S. Environmental Protection Agency, and the Food and Drug Administration. The final report was determined to be an accurate reflection of the data obtained. The dates of Quality Assurance activities on this study are listed below.

Study Initiation Date: 5/5/89 Study Completion Date: 1/9/92

TYPE OF AUDIT:	DATE OF AUDIT:	DATE FINDINGS REPORTED TO STUDY DIRECTOR/MANAGEMENT:
Preliminary protocol	5/16/89	5/16/89
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Study Conduct (Adden. 1)	4/27/90	4/27/90
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Addendum 2	11/12/90	11/19/90
Protocol Data and Draft	7/09/91	7/10/91

ARCHIVING: Raw data and a copy of the final report are filed in the testing facility archives.

T. S. Gushow 8 Jan 92

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Midland, Michigan 48674

SIGNATURE PAGE

Compound: ^{14}C-Toluene-2,4-Diisocyanate

Title: Metabolic Fate of ^{14}C-Toluene-2,4-Diisocyanate in Fischer 344 Rats

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SUMMARY

This study was initiated to evaluate the pharmacokinetics/metabolism of ^{14}C-labeled 2,4-toluene-diisocyanate (^{14}C-2,4-TDI) following oral and inhalation exposure in the rat. For comparison, the pharmacokinetics/metabolism of 2,4-toluene-diamine (^{14}C-2,4-TDA) was also evaluated. This approach was based on the rationale that a comparative evaluation of the pharmacokinetics of ^{14}C-2,4-TDI following oral and inhalation exposure could provide needed perspective on observed route dependent differences in TDI oncogenicity. In addition, comparison with 2,4-TDA was reasonable based on the potential chemical or metabolic conversion of 2,4-TDI to 2,4-TDA, a known rodent carcinogen. Groups of 3 - 4 male rats were given either a single 60 mg/kg oral dose of ^{14}C-2,4-TDI or were exposed to ^{14}C-2,4-TDI vapors at a target concentration of 2 ppm for a 4 hr period. Additional groups of male rats were given single 3 or 60 mg/kg oral doses or a single 3 mg/kg intravenous (iv) dose of ^{14}C-2,4-TDA. All rats were sacrificed by 48 hr post-exposure.

Following oral administration of ^{14}C-2,4-TDI, >93% of the administered radioactivity was recovered in the urine, feces, cage wash and tissues. Approximately 8% of the oral dose was excreted in the urine while 81% was eliminated in the feces. It is estimated that during inhalation exposure, essentially all of the inhaled ^{14}C-2,4-TDI was retained by the animal. At 48 hr post-inhalation exposure approximately 15% and 47% of the recovered radioactivity was in the urine and feces, respectively. Following oral or inhalation exposure to ^{14}C-2,4-TDI, no radioactivity was eliminated as either expired ^{14}C-organics or $^{14}CO_2$. Comparison of the ^{14}C-2,4-TDI inhalation group with the oral ^{14}C-2,4-TDI and ^{14}C-2,4-TDA treatment groups indicated that a larger percentage of the inhaled radioactivity was in the tissues and carcass (34% vs 2 - 4%) and the clearance of radioactivity into the urine was slower ($t_{1/2}$= 20 hr vs 5 - 8 hr) following TDI inhalation. The total amount of free + acetylated TDA metabolites detected in the urine specimens following oral and inhalation exposure to ^{14}C-2,4-TDI were 1.92 and 0.05 ug eq 2,4-

TDA/g urine, respectively. No free 2,4-TDA was detected in the urine specimen from the inhalation group. In comparison, following oral administration of 60 and 3 mg/kg ^{14}C-2,4-TDA, 63 and 3.7 µg eq 2,4-TDA/g urine was detected in the urine specimen, respectively. Following ^{14}C-2,4-TDI inhalation exposure approximately 90% of the quantitated urinary metabolites existed as acid-labile conjugates. Whereas, after oral ^{14}C-2,4-TDI administration approximately 65% of the quantitated urinary metabolites existed as acid-labile conjugates. In contrast, following oral administration of ^{14}C-2,4-TDA only 16 - 39% of the quantitated urinary metabolites existed as acid-labile conjugates. These data indicate that inhalation exposure to 2,4-TDI will primarily result in the formation of acid-labile conjugates with little or no 2,4-TDA being formed. This suggests that the metabolic disposition of inhaled 2,4-TDI is quite different than the disposition observed following orally administered 2,4-TDI or iv and orally administered 2,4-TDA. The route dependent differences in 2,4-TDI disposition and metabolism may explain why TDI was not carcinogenic in rodents following inhalation exposure.

INTRODUCTION

Toluene diisocyanate (TDI) is a monomer that is used in the production of polyurethane products. It is most commonly utilized as a technical grade material consisting of 80% 2,4-TDI and 20% 2,6-TDI (80:20).

Initial concern over the possible oncogenicity of TDI was based on the potential for TDI to undergo chemical hydrolysis forming toluene diamine (TDA) which was shown to be a carcinogen in chronic feeding studies in Fischer 344 rats and $B_6C_3F_1$ mice (NCI, 1979). To evaluate the carcinogenic potential of TDI, groups of male and female Sprague-Dawley CD rats and CD-1 mice were exposed to 0.05 and 0.15 ppm of a technical grade mixture (80:20) of TDI by inhalation, 6 hr/day, 5 days/week for approximately 2 years (Loeser, 1983). The inhalation route was chosen since this represents the primary route of occupational exposure (Rampy et al., 1983). This study resulted in reduced weight gains in both rats and mice and signs of respiratory irritation in female mice. However, no tumorigenic response was observed indicating that TDI was not carcinogenic following inhalation exposure in rats and mice. A technical grade mixture (80:20) of TDI was also evaluated for carcinogenic activity in Fischer 344 rats and $B_6C_3F_1$ mice, however the test material was administered by the oral route in a corn oil vehicle. Animals were administered doses ranging from 30 to 240 mg/kg body weight, 5 days/week, for approximately 2 years (Dieter et al., 1990). This treatment resulted in over a 10% depression in body weight gains of rats in all dose groups and survival was also significantly lower than controls. However, this study did produce subcutaneous fibromas, fibrosarcomas, pancreatic islet cell adenomas, neoplastic nodules of the liver and mammary gland tumors and Dieter et al. (1990) concluded that TDI was carcinogenic in rats (male and female) and female mice, although TDI was not carcinogenic in male mice. Confounding the interpretation of this study was the fact that the TDI dose solutions had reacted with the corn oil vehicle resulting in a 10 - 23% decomposition of the TDI in these solutions (Dieter et al., 1990). Based on the observed differences

in tumorigenic response it appears that the tumorigenic potential of TDI may be associated with the route of exposure in the rat.

Two previous studies on the metabolism of the technical (80:20) mixture of ^{14}C-labeled TDI in the rat after inhalation and oral administration were performed under the sponsorship of the International Isocyanate Institute Inc. (Sacley, 1977; Stoltz et al., 1987). The results of these studies indicated that most of the radioactivity was eliminated in the urine and feces within 24 - 48 hr after either route of administration. Data from an NIEHS sponsored study (RTI, 1985) on the metabolism of 2,6-TDI in the Fischer 344 rat after oral administration (60 and 900 mg/kg) indicated that TDI was hydrolyzed and/or polymerized in the stomach. In addition, RTI reported on the urinary excretion of 2,6-bis-(acetylamino)-toluene resulting from the acetylation of 2,6-toluene diamine (2,6-TDA). The formation of TDA metabolites following TDI exposure was also supported by Rosenberg and Savolainen (1985) who reported the presence of acid-labile conjugates of TDA in the urine of rats following percutaneous (via the tail) exposure to 2,4-TDI. Although these studies suggest the formation of 2,4-TDA following 2,4-TDI exposure they do not provide sufficient data on the identity and quantity of TDI metabolites to help interpret the chronic bioassays (Loeser, 1983; Dieter et al., 1990).

The primary objective of the study described in the present report was to make a qualitative and quantitative assessment of the absorption, distribution, metabolism and excretion of ^{14}C-2,4-TDI following inhalation and oral exposure to the major isomer, 2,4-TDI. This approach was based on the rationale that a comparative evaluation of the pharmacokinetics following oral and inhalation exposure could provide needed perspective on the observed differences in TDI oncogenicity (Loeser, 1983, Dieter et al., 1990). In addition, comparison with 2,4-TDA pharmacokinetics and metabolism was reasonable based on the potential chemical or metabolic conversion of 2,4-TDI to 2,4-TDA, a known rodent carcinogen (NCI, 1979).

In response to the final Rules amending the U.S. Animal Welfare Act that were promulgated by the U.S. Department of Agriculture effective October 30, 1989, the Animal Care and Use Activity that was required for the conduct of this study has been reviewed and given full approval by the Institutional Animal Care and Use Committee.

EXPERIMENTAL

GENERAL DESIGN AND RATIONALE:

Phase I. Groups of male rats were administered ^{14}C-2,4-TDI orally at a target dose of 60 mg/kg of body weight and exposed to ^{14}C-2,4-TDI vapors at a target concentration of 2 ppm for a 4 hr period in a head-only inhalation chamber. Following oral administration of ^{14}C-2,4-TDI, three rats were housed in glass Roth-type metabolism cages and urine, feces, expired ^{14}C-volatile organics and $^{14}CO_2$ were trapped for up to 48 hr and analyzed for radioactivity. At 48 hr post-dosing the rats were sacrificed, and selected tissues were collected and analyzed for radioactivity. Pooled urine specimens were analyzed by high performance liquid chromatography (HPLC) and gas chromatography/ mass spectrometry (GC/MS). An additional group of four rats were administered ^{14}C-2,4-TDI orally and were sacrificed at approximately 2 hr post-dosing (peak ^{14}C blood concentration) and selected tissues were collected and analyzed.

Two groups of four male rats each were exposed to a target concentration of 2 ppm ^{14}C-2,4-TDI for a 4 hr period in a head-only inhalation chamber under dynamic air-flow conditions. Following exposure, one group of rats was placed in glass Roth-type metabolism cages, while the second group was sacrificed at the termination of the exposure. The collection of excreta, blood and tissues was as previously described for the groups administered the oral dose of ^{14}C-2,4-TDI.

Male rats were utilized throughout this study since there was no indication of a sex-related difference in the toxicity of 2,4-TDI or 2,4-TDA. An oral dose of 60 mg/kg was selected since this represented the highest oral dose possible which would not result in an excessive amount of polymerization of TDI in the gastrointestinal tract (RTI, 1985). For oral administration, 'dried' corn oil was utilized as a vehicle since TDI readily reacts with water. The 2 ppm inhalation concentration represented a reasonable target that would provide adequate analytical sensitivity, while producing minimal acute toxicity in the rat.

Phase II. Two groups of four male rats each were administered single oral doses of 3 or 60 mg ^{14}C-2,4-TDA/kg of body weight. An additional group of 4 male rats was given a single intravenous (iv) dose of 3 mg/kg via a jugular vein cannula. The oral dose solutions were prepared in distilled water while the iv dose was administered in normal sterile saline. Following oral and iv administration of ^{14}C-2,4-TDA, four rats/group were placed in glass Roth-type metabolism cages, and urine and feces were collected for up to 48 hr and analyzed for radioactivity. At 48 hr post-dosing the rats were sacrificed and selected tissues were collected and analyzed. Pooled urine specimens were analyzed by GC/MS and GC/MS/MS.

An oral ^{14}C-2,4-TDA dose of 60 mg/kg was chosen since it would be comparable to the oral 2,4-TDI dose. The 3 mg/kg ^{14}C-2,4-TDA dose was selected to be comparable to the 2,4-TDI inhalation dose and the iv exposure route was used to eliminate any potential absorption effects.

MATERIALS AND METHODS

Test Materials. 2,4-Toluene diisocyanate (Lot No. 28F0167) with a purity of 97.72% by weight and an isomer ratio of 200:1 (2,4-TDI : 2,6-TDI) as determined by a titration procedure and nuclear magnetic resonance spectroscopy, respectively (Hardas, 1989) was obtained from Sigma Chemical Company, St.

Louis, MO. Uniformly ring labeled ^{14}C-2,4-TDI was obtained from Sigma Chemical Company, St. Louis, MO. The radiotracer (Lot # 089F9246) had a specific activity of 23.7 mCi/mmol and was reported to be >99% radiochemically pure. The radiochemical purity was evaluated repeatedly throughout the study and ranged from 94.6% to 100% (Tables 1 and 3).

2,4-Toluene diamine (Lot No. AX13004AX) was obtained from Aldrich Chemical Company, Milwaukee, WI. This test material was found to be >99% pure. Uniformly ring labeled ^{14}C-2,4-TDA was obtained from Sigma Chemical Company, St. Louis, MO. The radiotracer (Lot # 030H9222) had a specific activity of 20.7 mCi/mmol and was reported to be >98% radiochemically pure. The radiochemical purity was determined to be 97.7% (Table 1).

2,4-Diacetylaminotoluene (2,4-Ac_2-TDA), 2-acetyl-4-aminotoluene (2-Ac-TDA) and 4-acetyl-2-aminotoluene (4-Ac-TDA) were prepared as described previously (Glinsukon et al., 1975a). D_9-2,4-Diacetylaminotoluene (D_9-2,4-Ac_2-TDA), used as an internal standard in the quantitation of acetylated TDA metabolites, was prepared in the same manner as 2,4-Ac_2-TDA using D_6-acetic anhydride and D_3-2,4-TDA (ring label; prepared from 2,4-TDA via acid-catalyzed deuterium exchange). 2,4-Di((N-piperidylformyl)amino)toluene, used in the quantitation of 2,4-TDI in air samples, was prepared via addition of 2,4-TDI to piperidine at room temperature. Identification and purity analysis of the synthesized standards was performed via mass spectral and/or NMR analysis.

D_6-Acetic anhydride was obtained from Aldrich Chemical Company. Heptafluorobutyric anhydride was obtained from the Pierce Chemical Company (Rockville, IL) or Alltech Associates, Inc. (Deerfield, IL). All other reagents and solvents were reagent grade or better.

Test Species. Male Fischer 344 rats (~200g) were purchased from the Charles River Breeding Laboratory (Kingston, NY) and used throughout this experiment. Care and husbandry of animals was in accordance with the Standard Operating Procedures of the Laboratory[1]. Upon arrival in the laboratory, the rats were examined by a veterinarian and found to be in good health. The rats were then acclimated to the laboratory environment for at least one week prior to use. The rooms in which the animals were housed had a 12-hr photocycle and were designed to maintain adequate environmental conditions concerning temperature and relative humidity for rats. Prior to the start of each experiment, rats were randomly assigned to treatment groups utilizing a computer driven randomization procedure and were then individually identified by a numbered ear tag. All rats were allowed to acclimate to glass Roth-type metabolism cages for 2 - 3 days prior to the administration of the radiotracer. Rats utilized for the iv 2,4-TDA administration were anesthetised with methoxyflurane and surgically implanted with an indwelling jugular vein cannula (modified method of Harms and Ojeda, 1974). These rats were allowed approximately one day to recover from surgery before dosing. The rats which were utilized for the inhalation study, were acclimated to the head-only chamber for approximately one week prior to the initiation of the study. Purina Certified Rodent Chow (#5002) and municipal drinking water were provided ad libitum, except during the inhalation exposure. Feed and water were analyzed in accordance with the Standard Operating Procedures of The Toxicology Research Laboratory.

Preparation and Administration of the Dose Solutions.

Oral and Intravenous Dose Solutions. The oral ^{14}C-2,4-TDI dosing solution was prepared in 'dried' corn oil (Allworld Scientific, Lynwood, CA.) at a target

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[1]Fully Accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC).

dose of 60 mg/kg with a volume of administration of 3 ml/kg of body weight. The radiotracer was diluted with non-radiolabeled 2,4-TDI to obtain a target radioactivity and concentration of 72.5 µCi and 21.7 mg/g of dosing solution, respectively. The dosing solution was prepared in a controlled atmosphere glove box under a dry N_2 atmosphere. The dosing solution was analyzed for radioactivity via liquid scintillation counting. In addition, weighed aliquots (~ 200 µl) of the dose solution were derivatized with 0.5 ml piperidine in 2 ml toluene (1 hr, room temperature). The resulting ^{14}C-2,4-TDI-dipiperdyl derivative was then isolated by evaporation of the excess reagents and solvent (N_2 stream) and reconstituted in acetone:acetonitrile:water. The samples were then analyzed by HPLC. The analysis of the tracer for radiochemical purity as well as the oral dose solution for 2,4-TDI concentration and radioactivity are presented in Table 1. The ^{14}C-2,4-TDI dose solution contained 111% and 98% of target radioactivity and concentration, respectively.

The oral ^{14}C-2,4-TDA dosing solutions were prepared in distilled water at target doses of 3 and 60 mg/kg with a volume of administration of 3 ml dosing solution/kg of body weight. The radiotracer was diluted with non-radiolabeled compound to obtain a target radioactivity of 67 µCi/g of dosing solution. Adequate non-radiolabeled 2,4-TDA was added to the 3 and 60 mg/kg dose solutions to obtain target concentrations of 1 and 20 mg 2,4-TDA/g dosing solution, respectively. The iv ^{14}C-2,4-TDA dosing solution was prepared in sterile normal saline at a target dose of 3 mg/kg with a volume of administration of 1 ml dosing solution/kg of body weight. The radiotracer was also diluted with non-radiolabeled 2,4-TDA to obtain a target radioactivity and concentration of 200 µCi and 3 mg/g dosing solution, respectively. The dosing solutions were analyzed for radioactivity and 2,4-TDA as previously described for 2,4-TDI and the results are presented in Table 1. The amount of radioactivity in the TDA dose solutions ranged from 83% to 148% of target, and all solutions were within 10% of their target concentration. The differences between targeted and measured

concentrations and radioactivity had no effect on the results obtained in this study.

The rats were weighed prior to dosing and based on their body weights a measured volume of the dose solution was administered. Oral dose solutions were administered by gavage using a glass syringe and stainless steel feeding needle (Popper & Sons, Inc., Hyde Park, NY). The iv dosing solution was slowly infused (~1-2 min) via the jugular vien cannula using a glass syringe, and a Intramedic® Luer Stub Adapter (Becton Dickinson, Franklin Lakes, NJ) and the cannula was then flushed with normal sterile saline (~1 ml). In all cases, the quantity of dose solution actually administered was determined by weighing the syringe prior to and following dosing. At the time of dosing the body weights of male rats ranged from 0.214 to 0.259 kg. The doses of 2,4-TDI actually administered ranged from 87% to 113% of target and are presented in Table 2. For individual animal data see Appendix Tables 1-4.

Inhalation Exposure. Rats were exposed via a head-only chamber to a target concentration of 2 ppm ^{14}C-labeled 2,4-TDI for a 4 hr period under dynamic flow-through conditions. The inhalation chamber was constructed of Teflon-lined plastic and was designed for the simultaneous exposure of 4 rats to radioactive vapors. The ^{14}C-2,4-TDI (23.7 mCi/mmole) was diluted approximately 10 fold with non-radiolabeled 2,4-TDI and the ^{14}C-2,4-TDI vapors were generated by gently heating a U-tube containing the radiotracer (~ 25-50 µl) to approximately 90°C with an air-flow through the tube of 0.5 liters/min. The ^{14}C-2,4-TDI vapors were then diluted with approximately 1.5 liters/min of dry room air so that the total flow through the chamber was approximately 2.0 liters/min, and the target radioactivity of the vapors generated was 432 dpm/ml (~ 0.2 µCi/liter). In order to accurately determine 2,4-TDI exposure concentration a sampling port was positioned to sample chamber atmosphere in the animal's breathing zone. The 2,4-TDI concentration and radioactivity were determined by bubbling 0.2 liters/min of

the chamber atmosphere through ~15 ml of toluene:piperidine (95:5, v:v) for 10 min. The resulting ^{14}C-2,4-TDI-dipiperidyl derivative was then isolated by evaporation of the excess reagents and solvent (N_2 stream) and reconstituted in acetonitrile:water. These samples were then analyzed via HPLC and liquid scintillation counting to determine the concentration of 2,4-TDI and radioactivity in the chamber. Samples of the chamber atmosphere were taken at ~20 min intervals for a total of 8 samples per exposure. TWA chamber 2,4-TDI concentration, ^{14}C concentration and tracer radiochemical purity are presented in Table 3. The test atmospheres ranged from 78% to 83% and 63% to 83% of their target 2,4-TDI and radioactivity concentration, respectively. The differences between targeted and measured concentrations and radioactivity had no effect on the results obtained in this study. At the time of exposure, body weights ranged from 0.238 - 0.258 kg (Appendix Table 5).

Specimen Collection and Analysis. Following administration of ^{14}C-2,4-TDI or ^{14}C-2,4-TDA, rats were housed in glass Roth-type metabolism cages designed for the separate collection of urine, feces and expired air (^{14}C volatile organics and $^{14}CO_2$). All urine voided during successive 12 hr intervals was collected in dry-ice chilled containers. Following collection of each urine specimen the cage was rinsed with distilled water. The weight of each 12 hr urine and cage rinse was determined, and weighed aliquots of each radiolabeled specimen were mixed with either Aqueous Counting Scintillant® (ACS, Amersham Corp., Arlington Heights, IL) or Aquasol® Counting Scintillant (New England Nuclear, Boston, MA) and analyzed for radioactivity by liquid scintillation counting (LSC). In addition, aliquots of urine were taken from the 0-12 hr and 12-24 hr collections and pooled into their respective time and dose group. The pooled urine specimens were then profiled via HPLC to obtain a qualitative picture of the extent of 2,4-TDI and 2,4-TDA metabolism. The presence of possible acid-labile metabolic conjugates was evaluated by performing acid hydrolysis (10% v:v conc. HCL; 80°C x 1 hr) and reanalysis of selected urine specimens.

Pooled urine specimens from the five 2,4-TDI and 2,4-TDA experiments were prepared for analysis of free 2,4-TDA as follows: weighed aliquots (0.25 ml; n=2) were fortified with 0.13 or 1.30 µg 2,6-TDA (used as internal standard), adjusted to pH 13-14 (4 ml 1N KOH) and extracted with toluene (1 x 4 ml). A 2 ml aliquot of each extract was derivatized with 0.1 ml heptafluorobutyric anhydride (HFBA) at room temperature with continuous vortexing. The derivatized extracts were washed with 1 ml 1M NaH_2PO_4 (pH 7.0), taken to dryness (N_2 stream) and reconstituted in 0.1 ml or 1 ml toluene for subsequent GC/MS analysis. Aliquots (0.25 ml) of control urine from male rats were fortified with 0-10.5 µg 2,4-TDA and 0.13 or 1.30 µg 2,6-TDA, extracted and derivatized as above for use as standards in the GC/MS analysis.

The pooled urine specimens from the five 2,4-TDI and 2,4-TDA experiments were analyzed for total acid-labile conjugates of 2,4-TDI/2,4-TDA in a similar manner. Weighed aliquots (0.5 ml; n=2) of each pooled urine specimen were fortified with 0.56 or 5.60 µg 2,6-TDA (used as internal standard), acidified (1.5 ml 4N HCl) and heated at 100°C for 2 hr. The samples were then adjusted to pH 13-14 (2 ml 5N KOH) and extracted and derivatized with HFBA as above, for subsequent GC/MS analysis. Standards were prepared via fortification of control urine (0.5 ml) with 0-56.3 µg 2,4-TDA and 0.56 or 5.60 µg 2,6-TDA, followed by extraction and derivatization with HFBA.

Pooled urine specimens from the five 2,4-TDI and 2,4-TDA experiments were prepared for analysis of mono- and diacetyl-TDA as follows: weighed aliquots (1.0 ml; n=2) were diluted with 0.5 ml distilled water (final pH 6.5-7.0), fortified with 9.4 µg D_9-2,4-Ac_2-TDA (used as internal standard) and extracted with ethyl acetate (2 x 2 ml). The combined ethyl acetate extracts were taken to dryness (N_2 stream) and the residues derivatized with 50 µl D_6-acetic anhydride and 50 µl pyridine (room temperature x 30 min). The excess reagents were then removed by evaporation (N_2 stream) and the derivatized residues taken up in 100 µl ethyl acetate for subsequent GC/MS/MS [illegible]s. Aliquots (1.0 ml) of control urine from male rats were fortified with [illegible] µg

4-Ac-TDA, 0-216 µg 2,4-Ac_2-TDA and 9.4 µg D_9-2,4-Ac_2-TDA, extracted and derivatized as above for use as standards in the GC/MS/MS analysis.

The structural identification of the monoacetyl-TDA metabolite was performed via GC/MS analysis of the HFBA derivative. Weighed aliquots (0.25 ml; n=2) of each pooled urine specimen were diluted with 0.25 ml distilled water and extracted with ethyl acetate (1 x 2 ml). The ethyl acetate extracts were taken to dryness (N_2 stream) and the residues derivatized with 100 µl HFBA (room temperature x 30 min). The excess reagent was removed by evaporation (N_2 stream). The derivatized residues were then taken up in 1 ml toluene, washed with 0.5 ml 1M NaH_2PO_4 (pH 7.0) and again taken to dryness (N_2 stream). The final residues were then dissolved in 50 µl toluene for subsequent GC/MS analysis. Aliquots (0.25 ml) of control urine from male rats were fortified with 9.5 µg 2-Ac-TDA and/or 9.5 µg 4-Ac-TDA, extracted and derivatized as above for use as standards in the GC/MS analysis.

Feces were collected at 24 hr intervals in dry-ice chilled containers and stored frozen (-20°C). An aqueous homogenate (33-50% w:w) was prepared for each sample and weighed aliquots were oxidized in an OX-300 Biological Material Oxidizer (R.J. Harvey Instrument Corporation, Hillsdale, NJ). The $^{14}CO_2$ released upon oxidation was trapped in a solution of 1-methoxy-2-propanol:monoethanolamine (7:3, v:v), mixed with liquid scintillation fluid (spectrafluor:1-methoxy-2-propanol:toluene, 12:22:66, v:v:v) and analyzed for radioactivity by LSC. Pooled fecal specimens (0 - 24 hr collection interval) obtained from rats administered a 60 mg/kg oral ^{14}C-2,4-TDI dose or fecal specimens (0 - 48 hr collection interval) obtained from rats exposed to a 2 ppm ^{14}C-2,4-TDI concentration were extracted for HPLC analysis. Weighed aliquots (approx. 0.25 g) of the fecal homogenates were extracted sequentially with methanol (2 x 2 ml) and then distilled water (2 x 1 ml). The remaining fecal specimens were then hydrolyzed (1 ml 4N KOH, 2 hr x 80°C). The hydrolyzed extracts were then extracted with ethyl acetate (3 x 1 ml). The methanol and ethyl acetate extracts were taken to dryness (N_2 stream) and reconstituted in

0.5 ml 50:50 acetonitrile:water (v:v). These reconstituted organic extracts and the aqueous extracts were then analyzed individually via reverse phase HPLC.

The air exiting the metabolism cages (flow rate approximately 500 ml/min) from the 60 mg/kg and 2 ppm ^{14}C-2,4-TDI exposure groups was passed through a ^{14}C-volatile organic trap (activated charcoal) and $^{14}CO_2$ trap and collected at 24 hr intervals. The charcoal was desorbed overnight with toluene and an aliquot removed for ^{14}C-analysis. Expired $^{14}CO_2$ was trapped in a solution of 1-methoxy-2-propanol:monoethanolamine (7:3, v:v); aliquots of the trapping solution were mixed directly with liquid scintillation fluid and the radioactivity quantified by LSC.

The animals in the 60 mg/kg ^{14}C-2,4-TDI and 2 ppm inhalation treatment groups used for the collection of excreta were sacrificed with CO_2 and exsanguinated 48 hr post-dosing. Additional groups of animals were sacrificed 2 hr following a 60 mg/kg oral dose and immediately upon termination of a 4 hr 2 ppm inhalation exposure. The following tissues were collected and analyzed for radioactivity: blood, fat, gastrointestinal tract, gastrointestinal tract contents, fat, kidney, liver, lung, skin and the remaining carcass. In addition, the groups of animals dosed with ^{14}C-2,4-TDA were likewise sacrificed with CO_2 and exsanguinated 48 hr post-dosing, however, only the whole carcass (including all tissues) and skin were analyzed for radioactivity. Fat and skin were oxidized directly. Aqueous homogenates (33-50% w:w) of all other tissues were prepared, and aliquots of these homogenates were oxidized in a biological material oxidizer. The $^{14}CO_2$ released on oxidation of these tissues was trapped and quantified as described for the feces.

^{14}C Analysis. Radioactivity was quantitated with a Beckmann LS 3801 (Fullerton, CA) liquid scintillation counter and count rates were corrected for background and quench (H# technique) to convert counts per minute (cpm)

to disintegrations per minute (dpm). Samples containing less than two times the average count rate of the concurrently run blanks or backgrounds were considered to contain insufficient radioactivity to reliably quantify (that is net dpm were less than 1.0 times average background).

HPLC Analysis. All of the HPLC analyses done for this study were performed with Waters models 510 and 590 pumps (Millipore Waters Associates, Inc., Milford, MA), a model 680 gradient controller, a C_{18}-precolumn (2.9 cm x 8 mm id, 37 - 50 µM) and a Waters Radial-Pak® reverse phase C_{18}-column (10 cm x 8 mm, 10 µm). A Waters model 481 UV absorbance detector (254 nm) and a Beckman model 171 in-line radiochemical detector (Beckman Instruments, Inc., Fullerton, CA) were employed.

Aliquots of the radiolabeled 2,4-TDI were derivatized with piperidine:toluene (as above) to afford the corresponding dipiperidyl derivative for radiochemical purity determinations. Separation of the resulting radiolabeled components was performed via a 10 min linear gradient (70:30, 0.03M NH_4Ac (pH 6.5):acetonitrile to 100% acetonitrile; flow rate=2.0 ml/min) with a 10 min final hold at 100% acetonitrile. Parent material and radiolabeled impurities were quantified via the in-line radiochemical detector.

Radiochemical purity determination of the ^{14}C-2,4-TDA was performed via a 20 min linear gradient (100% solvent A (0.03M NH_4Ac, pH 6.5) to 50:50 solvent A:acetonitrile (v:v); flow rate=1.5 ml/min). Parent material and radiolabeled impurities were quantified via the in-line radiochemical detector.

Quantitative determination of 2,4-TDI, in the corn oil dose solution or air-monitoring impinger samples was performed on the dipiperidyl derivative via an isocratic HPLC separation (50:50, 0.03M NH_4Ac (pH 6.5):acetonitrile (v:v)) at flow rates of 2.5 ml/min and 1.5 ml/min, respectively.

Quantitative determination of 2,4-TDA in aqueous dose solutions was performed either via isocratic HPLC separation (80:20, 0.03M NH_4Ac (pH 6.5):acetonitrile (v:v); flow rate of 1.5 ml/min) or using the gradient HPLC conditions described for the radiochemical purity determination of ^{14}C-2,4-TDA (above).

HPLC separation of the urinary metabolites of 2,4-TDI and 2,4-TDA was performed via a multistep gradient separation (5 min at 100% solvent A, 15 min linear gradient to 50% solvent A, 10 min at 50% solvent A, 15 min linear gradient to 100% solvent B, 5 min at 100% solvent B; where solvent A is 0.03M NH_4Ac (pH 6.5) and solvent B is 75:25 acetonitrile:solvent A (v:v)) at a flow rate of 1.5 ml/min. The same conditions were used in the HPLC analysis of the 2,4-TDI fecal specimen extracts. The radiolabeled HPLC analyses of metabolite specimens were performed via collection of one minute eluent fractions and subsequent LSC analysis.

<u>GC/MS and GC/MS/MS Analysis.</u> All GC/MS and GC/MS/MS analyses were performed on a Finnigan 4610 GC/MS or a Finnigan TSQ-70 GC/MS/MS (Finnigan MAT Corp., San Jose, CA), both equipped with Hewlett Packard 5890 gas chromatographs and 7673A autosamplers (Hewlett Packard Co., Avondale, PA). Separations were achieved using either DB-1 or DB-5 fused silica capillary GC columns from J&W Scientific, Folsom, CA.

Representative GC conditions for the analysis of free or total urinary 2,4-TDA, as the HFBA derivative, were as follows: a 30-m DB-1 capillary column (0.25 mm id x 0.25 µm film); helium carrier gas (10 psig) at a flow rate of approximately 1 ml/min; gas chromatograph oven temperature programmed from 120°C (1 min initial hold) to 190°C at 20°/min, 6 min hold at 190°C, 30°/min to 280°C; injector and capillary transfer line at 250°C; 2-µl autosampler injection (0.5 min splitless). The mass spectrometer conditions (electron impact) were: ion source temperature, 150°C; ionizing current, 0.2

mamp; electron energy, 70 eV. The ions m/z 345 and 514 were examined (0.1 sec/scan) for the detection and quantitation of the HFBA derivative of 2,4-TDA. Quantitation was performed using the ratio of peak areas (m/z 345) for the 2,4-TDA and 2,6-TDA derivatives. A representative GC/MS chromatogram is presented in Figure 1.

The GC/MS/MS conditions used for the identification and quantitation of acetylated-TDA metabolites were as follows: a 30-m DB-5 capillary column (0.25 mm id x 1.0 µm film); helium carrier gas (11 psig) at a flow rate of approximately 1 ml/min; gas chromatograph oven temperature programmed from 50°C (1 min initial hold) to 300°C at 25°/min; injector and capillary transfer line at 280°C; 1-µl autosampler injection (0.5 min splitless). The mass spectrometer conditions (electron impact-MS/MS) were: ion source temperature, 150°C; ionizing current, 0.2 mamp; electron energy, 70 eV; argon collision cell gas at a pressure of 0.5 torr. Quantitation of the mono- and diacetyl-TDA metabolites was achieved by selected-ion monitoring (0.125 sec/scan) of the unique daughter ions formed from collision-induced dissociation of the diacetyl-TDA parent ions for 4-Ac-TDA (D_3-acetyl derivative), 2,4-Ac_2-TDA and D_9-2,4-Ac_2-TDA (209->167, 206->164 and 215->172, respectively). Quantitation was performed using the ratio of daughter ion peak areas for the metabolites vs the D_9-internal standard. A representative GC/MS/MS chromatogram is presented in Figure 2. Subsequent MS/MS identification work for the monoacetyl-TDA metabolite was performed using the GC/MS/MS conditions listed above, with the exception that a limited-range daughter ion spectrum (m/z 163-172) was obtained for this metabolite.

The GC/MS conditions used for the structural identification of the monoacetyl-TDA metabolite were as follows: a 30-m DB-5 capillary column (0.25 mm id x 1.0 µm film); helium carrier gas (10 psig) at a flow rate of approximately 1 ml/min; gas chromatograph oven temperature programmed from 150°C (1 min initial hold) to 270°C at 20°/min; injector

and capillary transfer line at 280°C; 1-µl autosampler injection (0.5 min splitless). The mass spectrometer conditions (electron impact) were: ion source temperature, 150°C; ionizing current, 0.4 mamp; electron energy, 70 eV. The ions m/z 318 and 360 were examined (0.1 sec/scan) for the detection of the HFBA derivatives of 2-Ac-TDA and 4-Ac-TDA.

RESULTS

Targeted and Actual Concentration(s) of 2,4-TDI/2,4-TDA Administered.
The amounts of 2,4-TDI, 2,4-TDA and radioactivity administered to each group are presented in Table 2. The 2,4-TDI inhalation exposure concentrations and chamber radioactivity are presented in Table 3. For individual animal data and chamber concentration calculations see Appendix Tables 1 - 6. The average ^{14}C-2,4-TDI and ^{14}C-2,4-TDA doses administered to each treatment group ranged from 87% to 113% of target. The time-weighted average ^{14}C-2,4-TDI chamber concentrations were 78% and 83% of target.

No signs of toxicity were observed following oral administration of a 60 mg ^{14}C-2,4-TDI/kg body weight dose or following administration of 3 and 60 mg ^{14}C-2,4-TDA/kg body weight doses. Likewise, no signs of toxicity were observed following the iv ^{14}C-2,4-TDA dose; however, one rat died in the Roth cage due to suffocation, which was the result of a vacuum line failure to the cage. Immediately following the 2 ppm ^{14}C-2,4-TDI inhalation exposure, all rats appeared lethargic and were not drinking water or eating. However, within 12 hr post-exposure these animals appeared normal and were consuming both water and food.

Distribution of Radioactivity Following Oral and Inhalation Exposure to ^{14}C-2,4-TDI.
The distribution of radioactivity recovered in the rat 48 hr after an oral 60 mg ^{14}C-2,4-TDI/kg body weight dose and 48 hr following a 2 ppm 4 hr inhalation exposure to ^{14}C-2,4-TDI are presented in Table 4. Following oral

administration, approximately 94% of the administered radioactivity was recovered in the urine, feces, tissues and carcass and final cage wash. Insufficient radioactivity was eliminated as $^{14}CO_2$ or ^{14}C-volatile organics to quantify. Approximately 81% of the oral ^{14}C-2,4-TDI dose was eliminated in the feces which represented the primary elimination route, whereas 8% of the dose was eliminated in the urine. The tissues and carcass accounted for approximately 4% of the dose and approximately $^{2}/_{3}$ of this radioactivity (2.6% of the dose) was associated with the gastrointestinal tract contents. Less than 1% of the recovered radioactivity was in the final cage wash. Following inhalation exposure, excreta were collected through 48 hr post-exposure and the feces and urine accounted for 47% and 15% of the recovered radioactivity, respectively. The tissues and carcass accounted for 34% of the recovered radioactivity, and approximately $^{1}/_{2}$ of this (17% of the recovered radioactivity) was associated with the gastrointestinal tract contents. Approximately 4% of the radioactivity was recovered in the final cage wash and insufficient radioactivity was eliminated as $^{14}CO_2$ or ^{14}C-volatile organics to quantify.

To facilitate a meaningful comparison between the oral and inhalation exposure routes, the percent recovered radioactivity was expressed as µg eq 2,4-TDI. Rats that were administered a 60 mg/kg oral dose received approximately 15 fold more 2,4-TDI than rats which were exposed to a 2 ppm concentration for 4 hr. In addition, the µg eq 2,4-TDI recovered in the urine and feces following the oral dose were, respectively 9 and 28 fold higher than what was recovered following the inhalation exposure. However, nearly equivalent amounts of ^{14}C activity (552 and 309 µg eq, respectively) remained in the tissues and carcass 48 hr following oral administration or inhalation exposure to ^{14}C-2,4-TDI.

Distribution of Radioactivity Following Oral and iv Administration of ^{14}C-2,4-TDA.

The distribution of radioactivity recovered 48 hr after an oral 60 mg or an oral and iv 3 mg ^{14}C-2,4-TDA/kg body weight doses are presented in Table 5. For all treatment groups, between 93% and 97% of the administered radioactivity was recovered in the urine, feces, carcass/skin and final cage wash. The urine was the primary excretion route accounting for 64 - 72% of the administered radioactivity, while 20 - 31% was recovered in the feces. For all treatment groups the percent radioactivity recovered in the carcass/skin was comparable, accounting for 2 - 5% of the administered dose, and less than 0.5% was recovered in the final cage wash. Comparison of the oral and iv 3 mg/kg treatment groups indicated that a slightly larger percentage of the iv dose (~10%) was recovered in the urine. However, overall the urinary recoveries were quite comparable indicating that ^{14}C-2,4-TDA was well absorbed following oral administration. Comparison of the oral 60 and 3 mg ^{14}C-2,4-TDA/kg body weight treatment groups indicated no quantitative dose-dependent differences in the routes of elimination.

Quantitative differences in the primary routes of elimination following ^{14}C-2,4-TDI (oral or inhalation) and ^{14}C-2,4-TDA (oral or iv) administration were observed (Tables 4 and 5). Whereas the urine was a minor excretion pathway following ^{14}C-2,4-TDI exposure it represented the major excretion pathway following ^{14}C-2,4-TDA administration.

Radioactivity in Tissues Following Oral and Inhalation Exposure to ^{14}C-2,4-TDI.

Radioactivity recovered in the tissues and carcass at 2 and 48 hr following an oral 60 mg/kg dose as well as immediately post-exposure and 48 hr following a 2 ppm inhalation exposure are summarized in Table 6 and 7 and listed for individual animals in Appendix Tables 7 - 10. At 2 hr following the oral ^{14}C-2,4-TDI dose, the concentration of radioactivity detected in tissues and carcass ranged from 0.002% to 1.306% of the dose/g wet weight. The highest activity

was detected in the gastrointestinal tract while the fat contained the lowest amounts of radioactivity. Radioactivity was evenly distributed between the other tissues, which contained between 0.02% and 0.07% of the dose/g wet weight. At 48 hr post-dosing the gastrointestinal tract, liver and kidneys each accounted for approximately 0.01% of the dose/g wet weight, while the remaining tissues contained less than 0.01%. Immediately following a 2 ppm inhalation exposure the radioactivity detected in the tissues and carcass ranged from 0.02% to 2.64% of the recovered radioactivity/g wet weight. The highest activity was in the lungs while the fat contained the lowest amounts of radioactivity. The remaining tissues accounted for between 0.17 and 0.51% of the recovered radioactivity/g wet weight. At 48 hr post-exposure the kidneys and lungs contained the highest amounts of radioactivity accounting for 0.15% and 0.28% of the recovered radioactivity/g wet weight, respectively. The remaining tissues accounted for approximately 0.11% of the radioactivity/g wet weight or less.

The radioactivity detected in the tissues and carcass was also expressed as µg eq TDI/g wet weight to facilitate comparison across exposure routes. Analysis of the tissues and carcass 2 hr after a 60 mg/kg oral dose and immediately following a 2 ppm inhalation exposure indicated that the µg eq of 2,4-TDI detected in the carcass, fat, kidneys, liver and skin following inhalation exposure was 3 - 10 fold lower than levels detected in the oral treatment group. As would be expected, the µg eq TDI detected in the gastrointestinal tract 2 hr after oral administration was much greater (133 fold) than the amount detected following inhalation. The µg eq of 2,4-TDI detected in the lungs were comparable for both treatment groups. By 48 hr post-dosing, the µg eq of 2,4-TDI found in the carcass and kidneys was virtually the same for the oral and inhalation treatment groups. However, the µg eq of 2,4-TDI detected in the lungs and skin following inhalation exposure was 3 - 6 fold greater than the amounts of ^{14}C activity detected in these tissues following oral administration.

Radioactivity in Urine Following Oral and Inhalation Exposure to ^{14}C-2,4-TDI.

The fraction of the radioactivity excreted in the urine during successive 12 hr collection intervals after an oral 60 mg ^{14}C-2,4-TDI/kg body weight dose and following a 4 hr inhalation exposure to 2 ppm ^{14}C-2,4-TDI are presented in Table 8. The individual animal data are listed in Appendix Tables 14 and 15. In addition, plots of the ^{14}C urinary time-course for both treatment groups are presented in Figure 3. Following oral administration and inhalation exposure to ^{14}C-2,4-TDI, peak urinary excretion of radioactivity occurred during the first 12 hr collection interval. However, compared to the oral treatment group, the urinary ^{14}C excretion was slower following inhalation exposure to ^{14}C-2,4-TDI. The half-lives derived from the slope of the urinary ^{14}C excretion time-course following ^{14}C-2,4-TDI oral administration and inhalation exposure were 7.5 hr and 20 hr, respectively.

Radioactivity in Urine Following Oral and iv Administration of ^{14}C-2,4-TDA.

The fraction of the radioactivity excreted in the urine during successive 12 hr collection intervals after an oral 60 mg and oral and iv 3 mg ^{14}C-2,4-TDA/kg body weight doses are presented in Table 9. The individual animal data are listed in Appendix Tables 16 - 18. In addition, plots of the ^{14}C urinary time-course for both treatment groups are presented in Figure 3. Urinary ^{14}C-excretion was comparable following oral and iv 3 mg/kg doses. During the 0 - 12 hr collection interval 60% and 67% of the radioactivity was excreted in the urine for both 3 mg/kg treatment groups and an additional 3 - 5% was recovered during the 12 - 48 hr interval. Following a 60 mg/kg oral administration of ^{14}C-2,4-TDA, 36% and 20% of the dose was recovered in the 0 - 12 hr and 12 - 24 hr collection intervals, respectively; indicating a slightly slower rate of urinary ^{14}C excretion compared to the 3 mg/kg dose groups. The half-lives derived from the slope of the ^{14}C urinary excretion time-course were 8 hr and 4.6 hr for the 60 and 3 mg/kg dose groups, respectively.

Comparison of the ^{14}C urinary time-course following an oral 60 mg/kg ^{14}C-2,4-TDI or 60 and 3 mg/kg ^{14}C-2,4-TDA doses indicated that the ^{14}C urinary excretion rates ($t_{1/2}$ = 5 - 8 hr) were similar. In contrast, inhalation exposure to ^{14}C-2,4-TDI resulted in a much slower clearance of ^{14}C activity in the urine ($t_{1/2}$ = 20 hr).

Radioactivity in Feces Following Oral and Inhalation Exposure to ^{14}C-2,4-TDI. The amount of radioactivity excreted in the feces during successive 24 hr collection intervals after an oral 60 mg ^{14}C-2,4-TDI/kg body weight dose and following an inhalation exposure to 2 ppm ^{14}C-2,4-TDI are presented in Table 10. The individual animal data are listed in Appendix Tables 19 and 20. Following oral administration, 50% and 30% of the administered dose was recovered in the feces during the 0 - 24 hr and 24 - 48 hr collection intervals, respectively. For the inhalation exposure group, the rats did not eliminate enough feces during the first collection interval for analysis, therefore feces were collected and analyzed only through 48 hr post-exposure. The feces accounted for 47% of the recovered radioactivity following inhalation exposure to ^{14}C-2,4-TDI.

Radioactivity in Feces Following Oral and iv Administration of ^{14}C-2,4-TDA. The fraction of the radioactivity excreted in the feces during successive 24 hr collection intervals after an oral 60 mg and oral and iv 3 mg ^{14}C-2,4-TDA/kg body weight dose are presented in Table 11. The individual animal data are listed in Appendix Tables 21 - 23. Fecal ^{14}C excretion was comparable following an oral and iv 3 mg/kg dose. During the 0 - 24 hr collection interval 25% and 15% of the radioactivity was excreted in the feces for the oral and iv treatment groups, respectively, an additional 6% was recovered during the 24 - 48 hr interval. Following a 60 mg/kg oral administration, 8% and 14% of the dose was recovered in the 0 - 24 hr and 24 - 48 hr collection intervals, respectively; indicating a slower rate of fecal ^{14}C excretion compared to the 3 mg/kg dose groups.

Chromatograms of Urine and Fecal Specimens Following Oral and Inhalation Exposure to ^{14}C-2,4-TDI.

HPLC analyses were performed on pooled urine specimens and solvent extracted pooled fecal specimens following a 60 mg/kg oral dose and 2 ppm inhalation exposure to ^{14}C-2,4-TDI. Representative chromatograms are presented in Figures 4 and 5. Recovery from the HPLC system ranged from 89% to 100%. Overall HPLC analyses of the urine and fecal specimens indicated no qualitative differences in metabolite profiles between the oral and inhalation treatment groups. Between 5 and 6 radioactive peaks were observed in the urine and the majority of the radioactivity eluted with retention times ranging from 20 to 30 min. HPLC profiles of the fecal extracts from both the oral and inhalation treatment groups were also comparable. Both contained 3 major peaks with the majority of the radioactivity eluting from the HPLC between 20 and 30 min. HPLC analysis of 2,4-TDA, mono- and diacetylated-TDA standards indicated that these metabolites eluted with retention times of 25, 27 and 26 min, respectively, corresponding to the retention times of the majority of ^{14}C activity in the urine and fecal specimens.

Concentration of Acetylated, Free 2,4-TDA and Acid-Labile 2,4-TDI/TDA Conjugates in Urine.

The concentration of mono- and diacetylated-TDA, free 2,4-TDA, and acid-labile conjugates of 2,4-TDI/2,4-TDA in the urine following exposure of rats to ^{14}C-2,4-TDI and ^{14}C-2,4-TDA are presented in Tables 12 and 13. Mono- and diacetylated TDA were detectable in the urine following an oral 60 mg/kg dose and 2 ppm exposure to ^{14}C-2,4-TDI. MS/MS analysis indicated that the monoacetyl-TDA was primarily the 4-acetyl isomer (Figure 6). These results were confirmed via additional GC/MS analysis of the HFBA derivative of the metabolic monoacetyl-TDA (data not shown). Free 2,4-TDA was only detectable in the urine specimen from the 60 mg/kg dose group. The total amount of free + acetylated TDA metabolites detected in the urine specimens following oral or inhalation exposure to ^{14}C-2,4-TDI were 1.92 and 0.05 µg eq

2,4-TDA/g urine, respectively; indicating that following the oral ^{14}C-2,4-TDI dose a larger amount of 2,4-TDA and acetylated metabolites (38 fold) were detected in the urine compared to the inhalation exposure group.

Analysis of urine specimens for total acid-labile conjugates of 2,4-TDI/2,4-TDA (Table 13), detected 5.56 and 0.49 µg eq 2,4-TDA/g urine following the oral and inhalation exposure to ^{14}C-2,4-TDI, respectively. Comparison of the ratio between the free + acetylated TDA to the total acid-labile metabolites indicated that following an oral 60 mg/kg ^{14}C-2,4-TDI dose approximately 35% of detected metabolites existed as either free or acetylated 2,4-TDA and 65% existed as other 2,4-TDI/2,4-TDA conjugates. In contrast, analysis of the urine specimens from rats exposed to 2 ppm ^{14}C-2,4-TDI indicated that only 10% of the quantitated metabolites were identified as acetylated TDA while the remaining 90% were other conjugated forms of either 2,4-TDI or 2,4-TDA. No free 2,4-TDA was detected.

Mono- and diacetylated-TDA as well as free 2,4-TDA were detectable in the urine specimens following an oral 60 mg and oral and iv 3 mg ^{14}C-2,4-TDA/kg body weight doses (Table 12). There were no quantitative differences in the amount of acetylated metabolites or 2,4-TDA detected in the urine following the oral and iv 3 mg/kg doses. In addition, the amounts of acetylated metabolites and 2,4-TDA detected in the urine from all treatment groups were proportional to the administered dose. Comparison of the ratio between the free + acetylated TDA metabolites with the total acid-labile metabolites indicated that following an oral 60 mg/kg ^{14}C-2,4-TDA dose approximately 61% of detected metabolites existed as either free or acetylated 2,4-TDA while the remaining 39% existed as other conjugates of 2,4-TDA. Whereas, following the 3 mg/kg dose, between 84% and 87% of the detectable metabolites were identified as either acetylated or free 2,4-TDA. This indicates that at lower doses, 2,4-TDA does not undergo much conjugation other than acetylation.

Urine specimens obtained from rats following an oral 60 mg/kg ^{14}C-2,4-TDI dose contained 38 and 20 fold less mono- and diacetyl-TDA and 69 fold less free 2,4-TDA than the urine obtained from rats administered an oral 60 mg/kg ^{14}C-2,4-TDA dose. The amount of free + acetylated TDA metabolites detected in the urine specimens following a 2 ppm inhalation exposure to ^{14}C-2,4-TDI can be compared with the amounts detected following the oral 3 mg/kg ^{14}C-2,4-TDA dose. This comparison was reasonable since 2 ppm 2,4-TDI inhalation exposure and 3 mg/kg oral 2,4-TDA dose were nearly equivalent on a µg eq basis (i.e. 899 vs 729 µg eq). Following ^{14}C-2,4-TDI inhalation exposure no free 2,4-TDA was detectable in the urine. In addition, the ^{14}C-2,4-TDI urine specimens contained 38 and 94 fold less mono- and diacetyl-TDA than the urine obtained from rats administered an oral ^{14}C-2,4-TDA dose. Comparison of the 2 ppm ^{14}C-2,4-TDI inhalation exposure and 3 mg/kg oral ^{14}C-2,4-TDA dose groups indicated that approximately 90% and 16% of the detected metabolites existed as acid-labile conjugates, respectively. Based on these data, the rats exposed via inhalation to ^{14}C-2,4-TDI had a much larger percentage of their TDI metabolites existing in a conjugated form, when compared to the ^{14}C-2,4-TDA treatment group.

DISCUSSION

This study was initiated to provide a qualitative and quantitative assessment of ^{14}C-2,4-TDI absorption, distribution, metabolism and excretion following oral and inhalation exposure in the rat. It was anticipated that a comparative evaluation of the pharmacokinetics following oral and/or inhalation exposure could provide perspective on the route dependent differences in ^{14}C-TDI carcinogenicity (Loeser, 1983; Dieter et al., 1990). In this respect, a major focus of this study was to quantify the formation of 2,4-TDA and acetylated TDA metabolites following ^{14}C-2,4-TDI exposure (oral and inhalation). In addition, comparative ^{14}C-2,4-TDA pharmacokinetic studies were conducted. The rationale for this approach was reasonable based on the

potential chemical or metabolic conversion of 2,4-TDI to 2,4-TDA, a known rodent carcinogen (NCI, 1979).

The data suggest that orally administered ^{14}C-2,4-TDI is not very well absorbed. The rats eliminated approximately 8% of the radioactivity in the urine and cage wash while 4% was recovered in the tissues and carcass. Thus the minimum estimate for absorption was 12%, which assumed that the radioactivity recovered in the feces (~81%) represented unabsorbed material. A more realistic estimate for absorption was obtained by assuming that some of the radioactivity in the feces was absorbed. Saclay (1977) reported that ~8% of an inhaled TDI dose (<1 ppm) was eliminated through the bile into the feces with an elimination half-life of 26 hr. Assuming that Saclay's data on biliary excretion is correct then as much as 20% of the oral dose may have been absorbed. The incomplete absorption following oral administration of ^{14}C-2,4-TDI is consistent with previous studies (RTI, 1985, 1988; Stoltz et al., 1987). RTI (1985) reported that TDI was very unstable in stomach contents in vitro with a half-life of 2 min. In addition, orally administered TDI was reported to undergo a rapid hydrolysis under aqueous conditions to form TDA which reacted with available isocyanate groups (TDI) to form polyureas. This explains why increasing the dose decreases bioavailability and why at relatively high doses (700 and 900 mg/kg) polymerized TDI was observed lining the stomach and intestines (RTI 1985, 1988; Stoltz et al., 1987). Based on this rapid reactivity it is doubtful that TDI was absorbed prior to its hydrolysis to TDA. Therefore, the 12 - 20% of the ^{14}C-2,4-TDI dose that was absorbed, most probably represented ^{14}C-2,4-TDA that had not reacted to form polyureas. Based on the above, it was assumed that the radioactivity that was absorbed and excreted in the urine following the ^{14}C-2,4-TDI oral dose was absorbed primarily as ^{14}C-2,4-TDA. The incomplete oral absorption of 2,4-TDI was also evident when comparing the percent recovered radioactivity in the urine following the 60 mg/kg oral ^{14}C-2,4-TDA and ^{14}C-2,4-TDI treatments. If 100% of the TDI dose was hydrolyzed to TDA and absorbed, then based on the TDA absorption data it was anticipated that 65% of the TDI dose would be

excreted in the urine. However, only 8% of the TDI dose was excreted in the urine suggesting that only 12% (8%/65%) of the dose was absorbed. Additional evidence for incomplete oral absorption of ^{14}C-2,4-TDI was provided by comparing the amount of free + acetylated TDA metabolites in the urine of rats administered 60 mg/kg doses of ^{14}C-2,4-TDI and ^{14}C-2,4-TDA. Again, if 100% of the oral TDI dose was hydrolyzed to TDA and absorbed then approximately 63 µg eq 2,4-TDA/g urine should have been excreted in the urine. However, following the oral TDI dose, 1.9 µg eq 2,4-TDA/g urine was excreted, suggesting that only 3% (1.9/63) of the dose was absorbed. Therefore, it was concluded that in the rat orally administered 2,4-TDI at a dose of 60 mg/kg body weight was incompletely absorbed (~12 - 20%) and primarily eliminated as unabsorbed material in the feces (~80%).

Rats which were exposed to ^{14}C-2,4-TDI vapors retained essentially all the radioactivity that they inhaled. The estimated inhaled ^{14}C-2,4-TDI was calculated to be approximately 709 µg eq 2,4-TDI/ rat. This estimate was based on a 4 hr exposure to a target concentration of 2 ppm ^{14}C-2,4-TDI using a minute volume of 0.264 liter min^{-1} in the rat (Mauderley, 1986). The total recovered radioactivity in the excreta, tissues and carcass averaged 899 µg eq 2,4-TDI/ rat, indicating that the rats retained approximately 1.3 times as much ^{14}C-2,4-TDI as predicted. However, because of stress the minute volume may have been greater than 0.264 liter min^{-1} resulting in a larger exposure to ^{14}C-2,4-TDI. Nonetheless, these data suggest that essentially all the inhaled ^{14}C-2,4-TDI was retained in the animal. These observations were consistent with the findings of Kennedy et al. (1989) who reported that the uptake of TDI into the blood of guinea pigs exposed for 1 hr to concentrations ranging from 0.05 ppb to 146 ppb was linear and the concentration of TDI in the blood was directly related to the atmospheric TDI concentration multiplied by duration of exposure (ppm x hr). These findings of Kennedy et al. (1989) suggest that the fraction of inhaled TDI that was retained in the body was constant for each breath and over a wide range of TDI concentrations.

Although essentially all the inhaled ^{14}C-2,4-TDI appeared to be retained by the rat, the data suggest that not all of this TDI was absorbed through the lungs into the blood. At 48 hr following a ^{14}C-2,4-TDI inhalation exposure, the urine and cage wash accounted for 19% of the recovered radioactivity, the tissues and carcass accounted for 34%, and 47% was recovered in the feces. As previously noted, Saclay (1977) reported that 8% of an inhaled dose (< 1 ppm) was eliminated in the bile of a rat with a half-life of approximately 26 hr. If 8% of the inhaled ^{14}C-2,4-TDI dose was absorbed through the lungs and cleared into the feces by biliary excretion, at a minimum 61% of the inhaled ^{14}C-2,4-TDI dose was absorbed. However, quantitation of the radioactivity detected in the tissues and carcass immediately post-exposure suggested that at least 80% of the radioactivity was absorbed (Appendix Table 25). The remaining 20% of the retained radioactivity may have been rapidly cleared from the respiratory tract and subsequently swallowed. The pulmonary clearance of radioactivity from the lung to the gastrointestinal tract was supported by the fact that approximately 10% of the recovered inhalation dose was detected in the gastrointestinal tract contents of rats immediately post-exposure (Appendix Table 25). Due to the relatively slow and minimal (8% of dose; $t_{1/2} > 52$ hr) biliary clearance of ^{14}C-TDI (Saclay, 1977) it is improbable that all the radioactivity detected in the gastrointestinal tract was eliminated via the bile. These data suggest that between 61 - 80% of the inhaled ^{14}C-2,4-TDI dose was absorbed, and the remaining radioactivity was rapidly cleared from the respiratory tract, ingested and then eliminated in the feces.

The distribution of radioactivity in the tissues following oral and inhalation exposure to ^{14}C-2,4-TDI were consistent with the results of previous studies (Saclay, 1977; Stoltz et al., 1987; RTI, 1988). In general, organs which were in direct contact with the ^{14}C-2,4-TDI vapor or liquid or that were involved in elimination had the highest levels of radioactivity. Comparison of the total amount of radioactivity in the tissues and carcass of rats indicated that a larger fraction of the recovered radioactivity was in the tissues and carcass following the inhalation exposure. These data are consistent with the findings of Stoltz

et al., (1987) and RTI (1988) who reported that, following oral administration of ^{14}C-TDI at doses of 60 and 70 mg/kg, the tissues (including intestinal contents) accounted for only 2% and 0.7% of the recovered dose at 48 hr and 72 hr post-dosing, respectively. Likewise, the higher percentages of the recovered radioactivity observed in the tissues and carcass following inhalation exposure were consistent with a previous study in which 18 -24% of an inhaled ^{14}C-2,4-TDI dose was recovered in the carcass 96 hr post-exposure (Stoltz et al., 1987). These higher levels of radioactivity detected in the tissues and carcass appear to be uniquely associated with inhalation exposure to TDI. This conclusion was supported by the relatively small percentages of the dose recovered in the tissues and carcass following oral ^{14}C-2,4-TDI or ^{14}C-2,4-TDA administration (range 2 - 5% of the dose).

Overall, these data suggest that following inhalation exposure, a large percentage of the ^{14}C-2,4-TDI was absorbed through the lungs and existed in a different form than what was absorbed following oral 2,4-TDI and/or 2,4-TDA doses. One possible explanation for this difference was that inhaled TDI may have interacted with available proteins in the lungs or blood forming TDI-protein adducts. Brown et al. (1987) reported that TDI readily reacts with selected functional groups on proteins such as hydroxyl, sulfhydryl, and imidazole groups under physiological conditions. Plasma obtained from rats exposed to ^{14}C-TDI by inhalation had greater than 90% of the radioactivity associated with proteins (Saclay, 1977). The rate of reaction with these proteins may be quite fast since in vitro the half-life of unchanged TDI in serum was reported to be 30 seconds (RTI, 1985). Furthermore, Kennedy et al. (1990) reported that, following a ^{14}C-TDI inhalation exposure in guinea pigs, 95% of the radioactivity in the blood was covalently associated with a 70 kDa plasma protein, forming a stable ^{14}C-2,4-TDI-protein conjugate. Finally, the possibility of a TDI-protein adduct is supported by analysis of the urinary metabolites which indicated that following inhalation exposure approximately 90% of the quantifiable 2,4-TDI/TDA was excreted in the urine of rats in a conjugated form (other than acetylation).

The urinary excretion of radioactivity by the kidneys was slower following inhalation exposure ($t_{1/2}$= 20 hr) when compared to the oral ^{14}C-2,4-TDI dose group ($t_{1/2}$= 7.5 hr). This slower rate of urinary excretion of inhaled ^{14}C-2,4-TDI appears to be uniquely associated with inhalation exposure to TDI and is not linked to TDA or TDA metabolites. This conclusion is supported by the faster urinary excretion of radioactivity following ^{14}C-2,4-TDA administration (range $t_{1/2}$= 5 - 8 hr). These findings are also consistent with the slower clearance of radioactivity from the tissues and carcass following inhalation exposure to ^{14}C-2,4-TDI and also suggest that inhaled 2,4-TDI was eliminated in the urine in a different form having a longer biological half-life than orally administered 2,4-TDI and/or 2,4-TDA

HPLC analysis of urine specimens obtained following oral and inhalation exposure to ^{14}C-2,4-TDI did not provide adequate resolution for any meaningful interpretation of these HPLC profiles. However, GC/MS and GC/MS/MS were successfully utilized to identify and quantify free and acetylated 2,4-TDA metabolites in the urine following exposure to either ^{14}C-2,4-TDI or ^{14}C-2,4-TDA. Quantifiable levels of mono- and diacetylated TDA were detectable in the urine following oral and inhalation exposure to ^{14}C-2,4-TDI. However, free 2,4-TDA was found only in the urine specimens following oral ^{14}C-2,4-TDI administration. The total amount of free + acetylated TDA metabolites excreted following a 60 mg/kg oral ^{14}C-2,4-TDI dose was comparable to the amount detected in the urine following a 3 mg/kg ^{14}C-2,4-TDA dose suggesting that, these doses were biological equivalent in terms of TDA. In contrast, the amount of free + acetylated TDA detected in the urine following a 4-hr inhalation exposure to 2 ppm of ^{14}C-2,4-TDI was relatively small. This suggests that in the rat, very little 2,4-TDA is formed following inhalation exposure to ^{14}C-2,4-TDI vapors. In addition, 90% of the quantitated metabolites in the urine specimens following inhalation exposure to ^{14}C-2,4-TDI existed as acid-labile conjugates of TDI/TDA while only 10% existing as acetylated TDA. This indicated that following inhalation

exposure, a larger percentage of the ^{14}C-2,4-TDI was excreted in the urine in a conjugated form and not as free or acetylated TDA.

A proposed metabolic scheme is presented in Figure 7. Under appropriate conditions 2,4-TDI readily hydrolyzed to form 2,4-TDA. The 2,4-TDA can react with free 2,4-TDI forming polyureas, or the 2,4-TDA can be absorbed and undergo further metabolism. It is also conceivable that 2,4-TDI will covalently react with available hydroxyl, sulfhydryl and amine groups on macromolecules which can in turn be eliminated in the urine/feces as acid-labile TDI conjugates. Absorbed 2,4-TDA can likewise be excreted in the urine either unchanged or as acid-labile conjugates. Additionally, 2,4-TDA can be N-acetylated forming mono- and diacetylated TDA metabolites which are readily excreted in the urine. The monoacetylated TDA was found to be primarily the 4-acetyl isomer. This agrees with previous in vivo and in vitro studies with 2,4-TDA which showed selective N-acetylation at the 4-position of TDA (Grantham et al., 1980, Glinsukon, et al., 1975b). Although no attempt was made to quantitate ring hydroxylated metabolites it should be noted that a number of aminophenol and aminobenzoic acid metabolites have been detected in the urine from rats, mice, guinea pigs and rabbits following 2,4-TDA administration (Waring and Pheasant, 1976; Grantham et al., 1980).

Overall, these data suggest that the metabolic disposition and carcinogenic potential of 2,4-TDI are dependent upon the route of exposure. Oral administration enhances the hydrolysis of 2,4-TDI forming 2,4-TDA which is readily absorbed. Whereas, inhalation exposure to 2,4-TDI primarily results in the formation of 2,4-TDI-protein adducts and only small amounts of free 2,4-TDA are produced. These findings are consistent with the chronic bioassay data which indicated that 2,4-TDI was not carcinogenic following inhalation exposure, but did result in tumor formation following oral administration in corn oil. Considering that the primary route of occupational exposure to TDI is via the inhalation route, then the metabolic

and bioassay data would suggested that the carcinogenic potential of TDI is low.

In summary, these data indicate that there are clear qualitative and quantitative differences in the absorption, distribution, metabolism and excretion of ^{14}C-2,4-TDI following oral and inhalation exposure in the rat. Following both oral and inhalation exposure to ^{14}C-2,4-TDI the majority of the recovered radioactivity was eliminated in the feces while the urine represented the minor excretory route. Following a 60 mg/kg oral dose, ^{14}C-2,4-TDI is incompletely absorbed (12 - 20%) with the majority of the dose (~80%) excreted in the feces. Whereas, following a 2 ppm inhalation exposure, essentially all the inhaled ^{14}C-2,4-TDI was retained by the animal and between 61 - 80% of this retained radioactivity was absorbed through the lungs, while the remaining radioactivity was cleared from the lung, swallowed and eliminated in the feces. The overall percent recovery of radioactivity in the tissues and carcass was greater following inhalation exposure when compared to the orally administered ^{14}C-2,4-TDI or ^{14}C-2,4-TDA. Likewise, the time-course of ^{14}C-2,4-TDI excretion in the urine was slower when compared to the oral ^{14}C-2,4-TDI and ^{14}C-2,4-TDA dose groups. This higher percent radioactivity in the tissues and carcass and the slower urinary ^{14}C excretion are uniquely associated with inhalation exposure to TDI. Quantifiable levels of mono- and diacetylated TDA were detectable in the urine following oral and inhalation exposure to ^{14}C-2,4-TDI, however free 2,4-TDA was only detectable in the urine specimen following oral administration of ^{14}C-2,4-TDI or ^{14}C-2,4-TDA. Quantitation of the urinary metabolites from the 60 mg/kg ^{14}C-2,4-TDI oral dose indicated that this dose was equivalent, in terms of systemic exposure, to a 3 mg/kg ^{14}C-2,4-TDA dose. Following inhalation exposure to ^{14}C-2,4-TDI small amounts of acetylated TDA were detectable in the urine. However, approximately 90% of the quantitated urinary metabolites existed as acid-labile conjugates. These findings suggest that following inhalation exposure a large percentage of the

^{14}C-2,4-TDI was absorbed and excreted in a different form than what was observed following an oral 2,4-TDI and/or 2,4-TDA doses.

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METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Table 1. Radioactivity, Concentration and Radiochemical Purity of the 2,4-TDI and 2,4-TDA Dose Solutions.

	Target[a] Radioactivity (µCi/g)	Target Concentration (mg/g)	Actual Radioactivity (µCi/g)	Actual Concentration (mg/g)	Radiochemical Purity (Percent)
Oral					
60 mg/kg 2,4-TDI	72.5[a]	21.7[a]	80.4 (111%)	21.3 (98%)	100.0±0.1
60 mg/kg 2,4-TDA	66.7	20.0	98.8 (148%)	21.4 (107%)	97.7±0.2
3 mg/kg 2,4-TDA	66.7	1.0	60.4 (91%)	1.1 (110%)	97.7±0.2
iv					
3 mg/kg 2,4-TDA	200.0	3.0	166.7 (83%)	2.8 (93%)	97.7±0.2

[a]Corrected for the specific gravity of corn oil (0.92 g/ml).
Values in ( ) represent the percent of target concentration.

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Table 2. Average Amount of Radioactivity and 2,4-TDI or 2,4-TDA Administered to Male Fischer 344 Rats.

	Body Wt. (kg)	Radioactivity (µCi)	Dose (mg)	Dose (mg/kg)
48 hr Post-Dosing Groups				
Oral				
60 mg/kg 2,4-TDI[a]	0.249±0.003	55.19±0.28	14.63±0.08	58.80±0.30 (98%)
60 mg/kg 2,4-TDA	0.226±0.003	66.65±0.76	14.30±0.16	63.42±0.33 (106%)
3 mg/kg 2,4-TDA	0.229±0.003	43.13± 0.99	0.779±0.02	3.40±0.12 (113%)
iv				
3 mg/kg 2,4-TDA	0.224±0.009	34.85±1.05	0.584±0.02	2.61±0.05 (87%)
2 hr Post-Dosing Group				
Oral				
60 mg/kg 2,4-TDI	0.253±0.006	56.66±1.42	15.01±0.40	59.37±0.22 (99%)

Values represent Mean ± SD for 4 animals
[a]Values represent Mean ± SD for 3 animals.
Values in ( ) represent the percent of target dose.

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Table 3. Targeted and Actual Time-Weighted Average (TWA) 2,4-TDI Concentration, Radioactivity, ^{14}C-2,4-TDI and Radiochemical Purity, for Groups of Fischer 344 Male Rats Exposed to ^{14}C-2,4-TDI via Inhalation for a 4 hr Period.

Groups	Body wt.[a] (kg)	Target Conc. (ppm)	TWA Conc. (ppm)	Target Radioactivity (dpm/ml)	Actual Radio-activity (dpm/ml)	Radiochemical Purity Percent
48 hr Post-Dosing	0.250±0.008	2	1.55 (78%)	432	272.4 (63%)	} 94.6±0.1%
Immediately Post-Dosing	0.247±0.003	2	1.65 (83%)	432	358.0 (83%)	

[a]Values represent Mean ± SD for 4 animals.
Values in ( ) represent the percent of target concentration.

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Table 4. Distribution of Radioactivity 48 hr After Male Fischer 344 Rats were Given an Oral Dose of 60 mg ^{14}C-2,4-TDI/kg of Body Weight or Exposed to 2 ppm ^{14}C-2,4-TDI Vapors for a 4 hr Period.

	60 mg/kg 2,4-TDI[a]		2 ppm 2,4-TDI[b]	
	Percent of Administered Dose	µg eq 2,4-TDI	Percent of Recovered Radioactivity	µg eq 2,4-TDI
Urine	8.38±0.41	1225±59	14.85±1.39	133±10
Feces	80.67±5.30	11795±718	47.29±11.98	424±102
Tissues & Carcass	3.77±0.87 (2.56±0.84)	552±129 (375±124)	34.14±11.53 (16.63±9.18)	309±113 (150±86)
$^{14}CO_2$	NQ	NQ	NQ	NQ
^{14}C Volatile Organics	NQ	NQ	NQ	NQ
Cage Wash	0.99±0.52	145±77	3.73±1.95	33±17
TOTAL	93.80±4.32	13717±573	NA	899±35

[a]Values represent Mean ± SD for 3 animals.
[b]Values represent Mean ± SD for 4 animals.
Values in ( ) represent ^{14}C activity found in the gastrointestinal tract contents.
NA - Not Applicable.
NQ - Not Quantifiable.

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Table 5. Distribution of Radioactivity 48 hr After Male Fischer 344 Rats were Given Doses of 60 (Oral) or 3 (Oral and iv) mg ^{14}C-2,4-TDA/kg of Body Weight.

	60 mg/kg 2,4-TDA, Oral		3 mg/kg 2,4-TDA, Oral		3 mg/kg 2,4-TDA, iv[a]	
	Percent of Administered Dose	µg eq 2,4-TDA	Percent of Administered Dose	µg eq 2,4-TDA	Percent of Administered Dose	µg eq 2,4-TDA
Urine	64.96±2.47	9287±300	63.67±15.0	472±128	72.49±0.74	427±13
Feces	22.57±1.54	3228±229	30.70±3.78	239±27	20.06±0.90	118±8
Carcass/Skin	4.62±1.67	662±245	2.02±0.36	16±3	2.13±0.12	13±1
Cage Wash	0.49±0.12	70±18	0.24±0.11	2±1	0.37±0.36	5±5
TOTAL	92.63±0.67	13246±173	96.62±12.08	729±110	95.05±0.43	563±24

Values represent Mean ± SD for 4 animals.
[a]Values represent Mean ± SD for 3 animals for 24 - 48 hr post-dosing interval.

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Table 6. Concentration of radioactivity and in the Tissues 2 hr After Male Fischer 344 Rats Were Given an Oral Dose of 60 mg ^{14}C-2,4-TDI/kg of Body Weight or Immediately Following a 4 hr to 2 ppm ^{14}C 2,4-TDI Inhalation Exposure.

	60 mg 2,4-TDI/kg		2 ppm 2,4-TDI	
	Percent of Administered Dose/g Wet Tissue	µg eq 2,4-TDI/g Wet Tissue	Percent of Recovered Radioactivity/g Wet Tissue	µg eq 2,4-TDI/g Wet Tissue
Blood	NA	NA	NA	NA
Carcass	0.038±0.024	5.69±3.78	0.508±0.018	2.02±0.30
Fat	0.002±0.002	0.34±0.29	0.019±0.004	0.07±0.03
Gastrointestinal Tract	1.306±0.450	195.86±66.27	0.375±0.109	1.51±0.62
Kidney	0.044±0.009	6.54±1.46	0.420±0.076	1.65±0.23
Liver	0.057±0.017	8.48±2.33	0.220±0.027	0.87±0.13
Lung	0.071±0.114	10.73±17.46	2.639±1.198	10.62±5.73
Skin	0.019±0.009	2.58±1.94	0.169±0.061	0.65±0.26

Values represent Mean ± SD for 4 animals.
NA- Not analyzed.

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Table 7. Concentration of Radioactivity in the Tissues 48 hr After Male Fischer 344 Rats Were Given an Oral Dose of 60 mg ^{14}C-2,4-TDI/kg of Body Weight or Following a 4 hr 2 ppm ^{14}C-2,4-TDI Inhalation Exposure.

	60 mg 2,4-TDI/kg[a]		2 ppm 2,4-TDI[b]	
	Percent of Administered Dose/g Wet Tissue	µg eq 2,4-TDI/g Wet Tissue	Percent of Recovered Radioactivity/g Wet Tissue	µg eq 2,4-TDI/g Wet Tissue
Blood	0.008±0.001	1.22±0.16	0.066±0.012	0.59±0.10
Carcass	0.005±0.002	0.78±0.22	0.075±0.019	0.68±0.20
Fat	0.001±0.001	0.20±0.08	0.008±0.005	0.07±0.04
Gastrointestinal Tract	0.011±0.008	1.56±1.19	0.082±0.028	0.73±0.24
Kidney	0.013±0.001	1.85±0.17	0.147±0.034	1.32±0.30
Liver	0.011±0.001	1.66±0.09	0.055±0.006	0.49±0.06
Lung	0.003±0.001	0.44±0.15	0.283±0.124	2.53±1.07
Skin	0.002±0.001	0.34±0.09	0.108±0.032	0.98±0.32

[a]Values represent Mean ± SD for 3 animals.
[b]Values represent Mean ± SD for 4 animals.

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Table 8. Concentration of Radioactivity Excreted in the Urine During the Indicated Intervals by Male Fischer 344 Rats Given an Oral 60 mg ^{14}C-2,4-TDI/Kg of Body Weight or Exposed to 2 ppm ^{14}C-2,4-TDI Vapors for a 4 hr Period.

	60 mg 2,4-TDI/kg[a]		2 ppm 2,4-TDI[b]	
Collection Interval (Hr Post-Dosing)	Percent of Administered Dose	µg eq 2,4-TDI	Percent of Recovered Radioactivity	µg eq 2,4-TDI
0-12	6.64±0.23	971±34	7.96±1.46	71±10
12-24	1.09±0.24	159±35	2.31±1.37	21±12
24-36	0.43±0.11	63±17	2.68±0.33	24±3
36-48	0.22±0.06	32±9	1.89±0.71	17±7
TOTAL	8.38±0.41	1225±59	14.85±1.39	133±10

[a]Values represent Mean ± SD for 3 animals.
[b]Values represent Mean ± SD for 4 animals.

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Table 9. Radioactivity Excreted in the Urine During the Indicated Intervals by Male Fischer 344 Rats Given Doses of 60 (Oral) or 3 (Oral and iv) mg ^{14}C-2,4-TDA/kg of Body Weight.

	60 mg 2,4-TDA/kg, Oral		3 mg 2,4-TDA/kg, Oral		3 mg 2,4-TDA/kg, iv	
Collection Interval (Hr Post-Dosing)	Percent of Administered Dose	µg eq 2,4-TDA	Percent of Administered Dose	µg eq 2,4-TDA	Percent of Administered Dose	µg eq 2,4-TDA
0-12	35.73±2.20	5154±287	60.42±15.07	472±128	66.96±1.18	391±11
12-24	20.20±6.20	2685±982	2.51±0.41	20±3	3.60±0.65	21±3
24-36	7.86±4.11	1211±673	0.47±0.04	4±0.3	0.89±0.21[a]	5±1[a]
36-48	1.17±0.41	179±67	0.27±0.04	2±0.3	0.36±0.09[a]	2±0.5[a]
TOTAL	64.96±2.47	9287±300	63.67±15.00	498±127	72.49±0.74[a]	427±13[a]

Values represent Mean ± SD for 4 animals.
[a]Values represent Mean ± SD for 3 animals for 24-48 hr post-dosing interval.

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Table 10. Radioactivity Excreted in the Feces During the Indicated Intervals by Male Fischer 344 Rats Given an Oral Dose of 60 mg ^{14}C-2,4-TDI/kg of Body Weight or Exposed to 2 ppm ^{14}C-2,4-TDI Vapors for a 4 hr Period

	60 mg 2,4-TDI/kg[a]		2 ppm 2,4-TDI[b]	
Collection Interval (Hr Post-Dosing)	Percent of Administered Dose	µg eq 2,4-TDI	Percent of Recovered Radioactivity	µg eq TDI
0-24	50.28±4.81	7352±683	} 47.29±11.98[c]	} 424±102[c]
24-48	30.38±2.19	4443±309		
TOTAL	80.67±5.30	11795±718		

[a]Values represent Mean ± SD for 3 animals.
[b]Values represent Mean ± SD for 4 animals.
[c]Collection interval is 0 - 48 hr.

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Table 11. Radioactivity Excreted in the Feces During the Indicated Intervals by Male Fischer 344 Rats Given Doses of 60 (Oral) or 3 (Oral and iv) mg ^{14}C-2,4-TDA/kg of Body Weight

	60 mg 2,4-TDA/kg, Oral		3 mg 2,4-TDA/kg, Oral		3 mg 2,4-TDA/kg, iv	
Collection Interval (Hr Post-Dosing)	Percent of Administered Dose	µg eq 2,4-TDA	Percent of Administered Dose	µg eq 2,4-TDA	Percent of Administered Dose	µg eq 2,4-TDA
0-24	8.31±0.65	1187±79	24.77±5.30	192±40	15.44±3.58	90±19
24-48	14.26±1.74	2040±262	5.93±2.67	46±20	6.22±2.66[a]	37±16[a]
TOTAL	22.57±1.54	3228±229	30.69±3.78	239±27	20.06±0.90[a]	118±8[a]

Values represent Mean ± SD for 4 animals.
[a]Values represent Mean ± SD for 3 animals for 24-48 hr post-dosing interval.

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Table 12. Concentration of Monoacetyl, Diacetyl and Free 2,4-TDA Expressed as 2,4-TDA Equivalents in the 0-12 hr Urine Specimen following Oral and Inhalation Exposure to ^{14}C-2,4-TDI and Oral and iv Administration of ^{14}C-2,4-TDA in Male Fischer 344 Rats.

	µg eq 2,4-TDA / g Urine[a]			
Treatment Group	Monoacetyl	Diacetyl	Free	Free + Acetylated
2,4-TDI				
Oral 60 mg/kg	0.64 (0.62, 0.65)	1.02 (0.99, 1.05)	0.26 (0.25, 0.28)	1.92
Inhalation 2 ppm	0.03 (0.03, 0.03)	0.02 (0.02, 0.02)	ND	0.05
2,4-TDA				
Oral 60 mg/kg	24.36 (23.60, 25.13)	20.34 (19.11, 21.58)	18.03 (16.01, 20.05)	62.73
Oral 3 mg/kg	1.13 (1.09, 1.16)	1.88 (1.87, 1.89)	0.73 (0.70, 0.76)	3.74
iv 3 mg/kg	1.12 (1.07, 1.18)	1.77 (1.70, 1.84)	0.20 (0.19, 0.21)	3.09

[a]Values are the means of two determinations, numbers in ( ) are the individual determinations.
Acetylated - Sum of the Monacetyl and Diacetyl-2,4-TDA.
ND - Not Detected. Detection Limit 100 ngeq 2,4-TDA/g Urine.

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

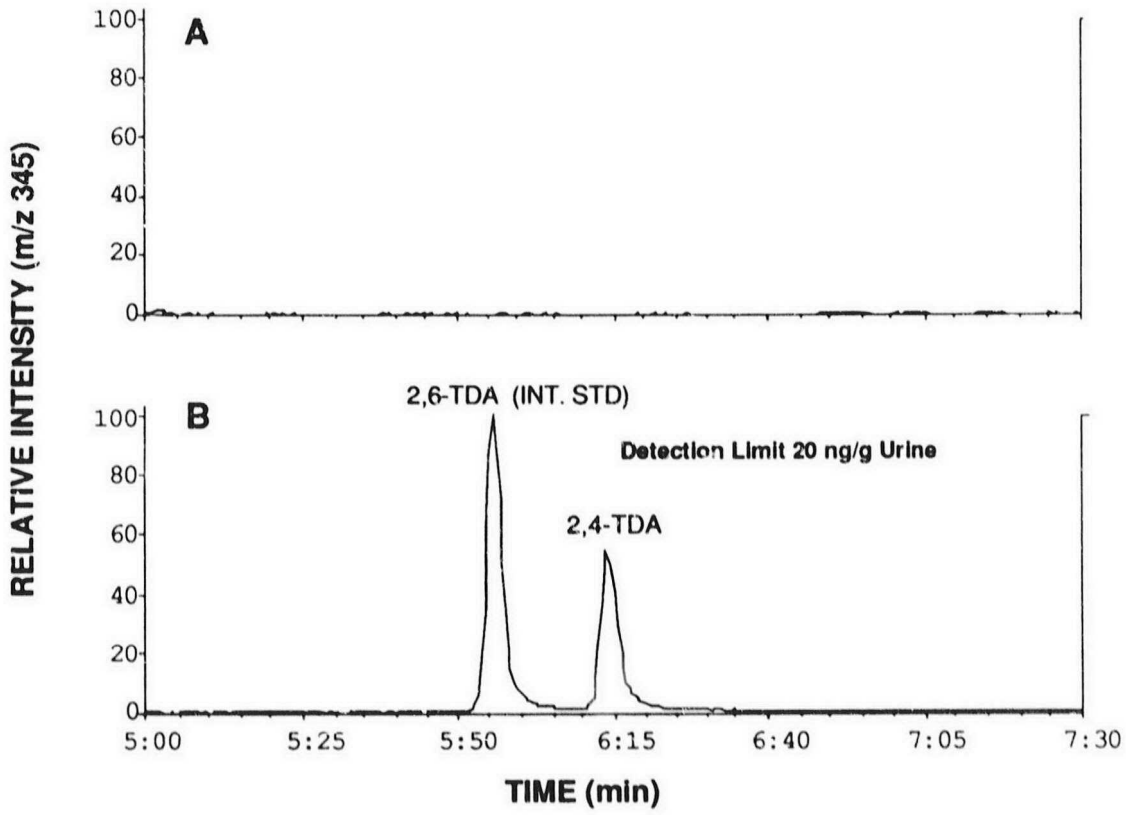
Table 13. Concentration of Free + Acetylated 2,4-TDA and Acid-Labile 2,4-TDI/2,4-TDA Conjugates Expressed as 2,4-TDA Equivalents in the 0-12 hr Urine Specimen following Oral and Inhalation Exposure to ^{14}C-2,4-TDI and Oral and iv Administration of ^{14}C-2,4-TDA in Male Fischer 344 Rats.

	µg eq 2,4-TDA / g Urine		
Treatment Group	Free + Acetylated	2,4-TDI/2,4-TDA[a] Acid-Labile Conjugate	Free + Acetylated[b] / Acid-Labile
2,4-TDI			
Oral 60 mg/kg	1.92	5.56 (5.32, 5.79)	0.35
Inhalation 2 ppm	0.05	0.49 (0.48, 0.50)	0.10
2,4-TDA			
Oral 60 mg/kg	62.73	102.41 (100.77, 104.06)	0.61
Oral 3 mg/kg	3.74	4.47 (4.34, 4.59)	0.84
iv 3 mg/kg	3.09	3.54 (3.51, 3.57)	0.87

[a]Values are the means of two determinations, numbers in ( ) are the individual determinations.
[b] Ratio of Free + Acetylated to Acid-Labile Conjugates.

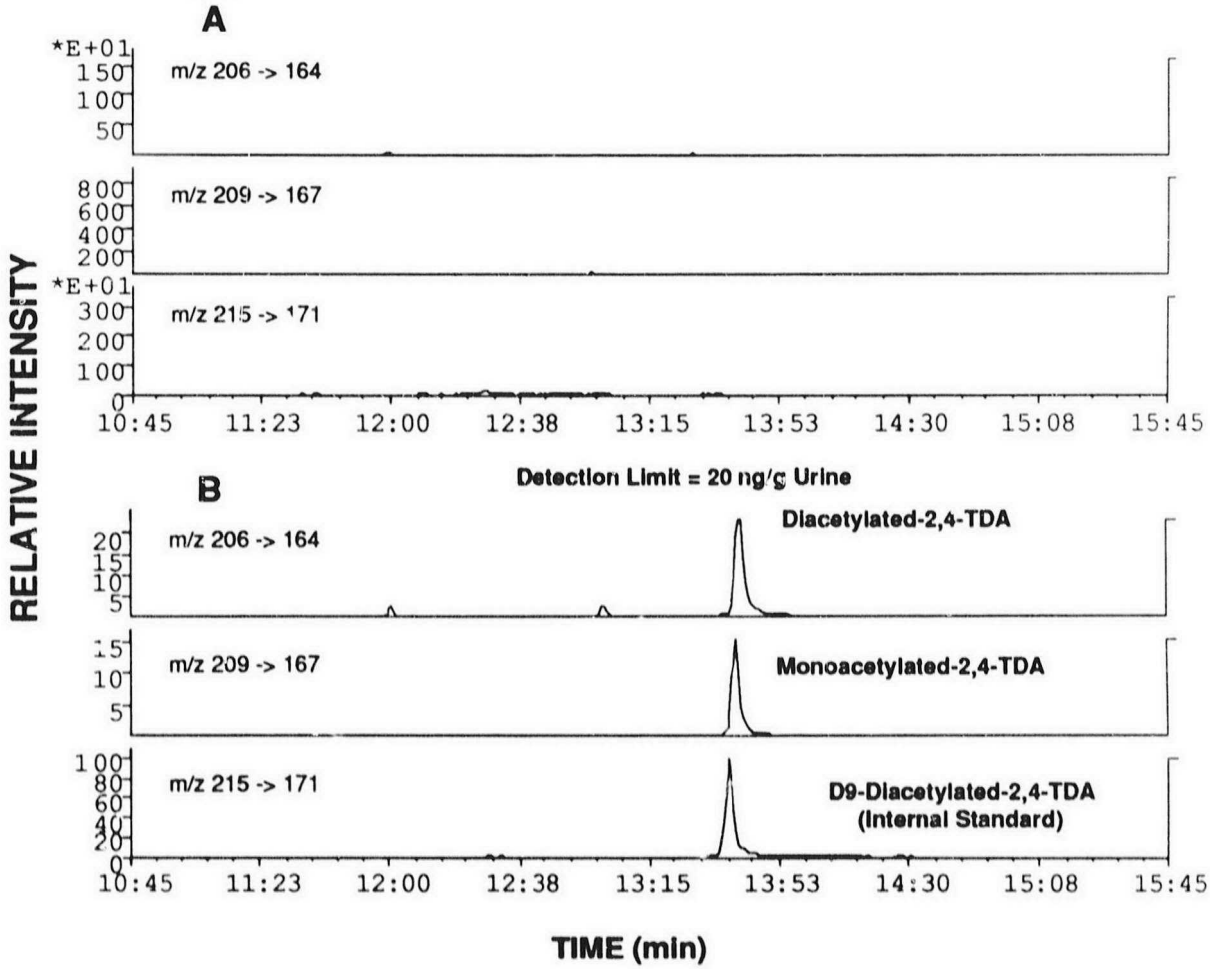
METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Figure 1. **Representative EI-GC/MS Chromatograms for (A) Hydrolyzed Control Urine Extract ; and (B) Hydrolyzed 0-12 hr Urine Specimens Obtained from Male Fischer 344 Rats Exposed to 2 ppm ^{14}C-2,4-TDI via Inhalation for 4 hr (as Heptafluorobutyric Anhydride Derivative).**



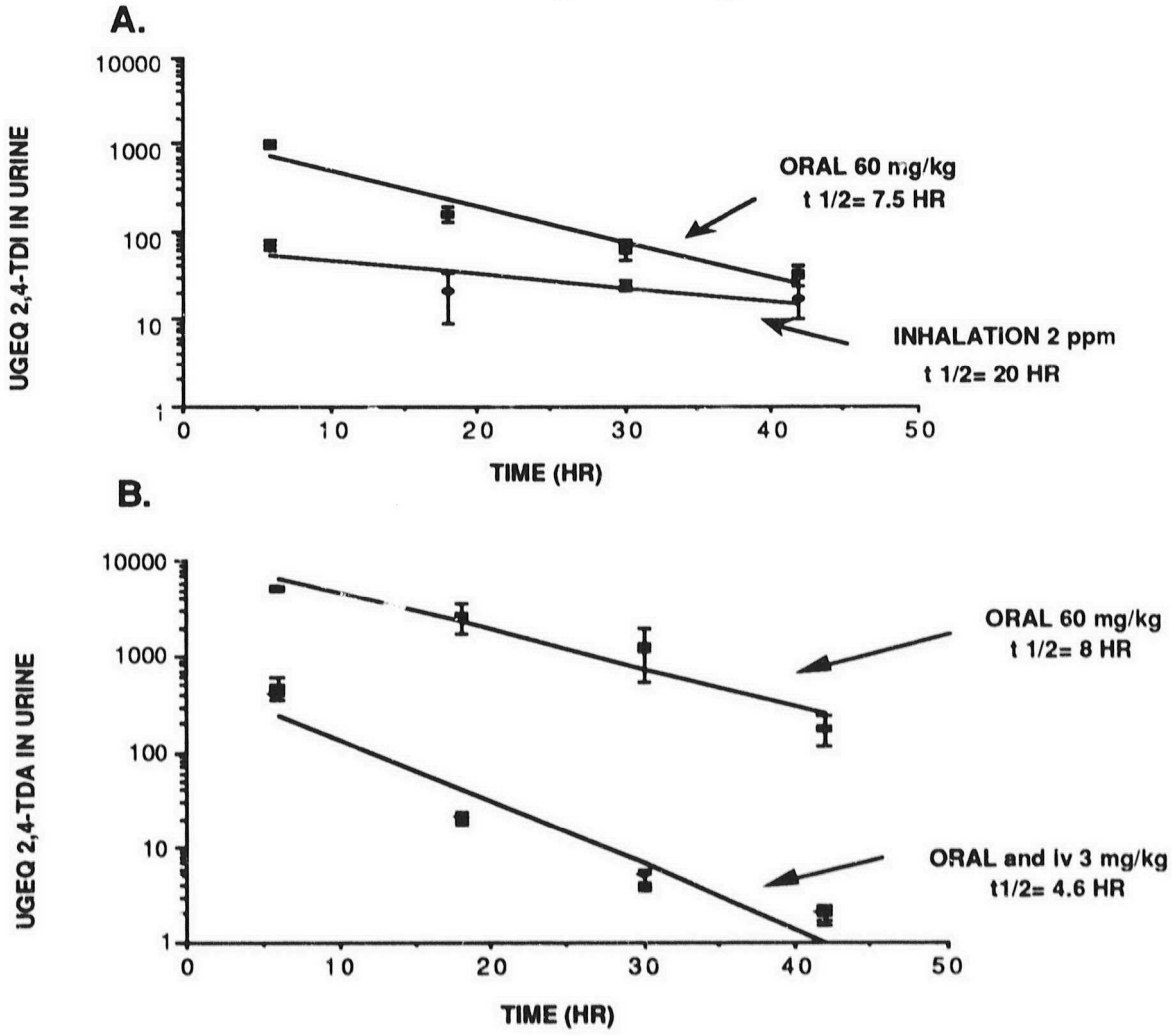
METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Figure 2. **Representative EI-GC/MS/MS Chromatograms for Monoacetyl-2,4-TDA (m/z 209) and Diacetyl-2,4-TDA (m/z 206) as D_6-Diacetyl Derivatives and D_9-Diacetyl 2,4-TDA Internal Standard, (m/z 215). A) Control Urine Extract; B) Extract of Urine Specimen from 0 -12 hr Interval following an Oral 60 mg/kg ^{14}C-2,4-TDA Dose.**



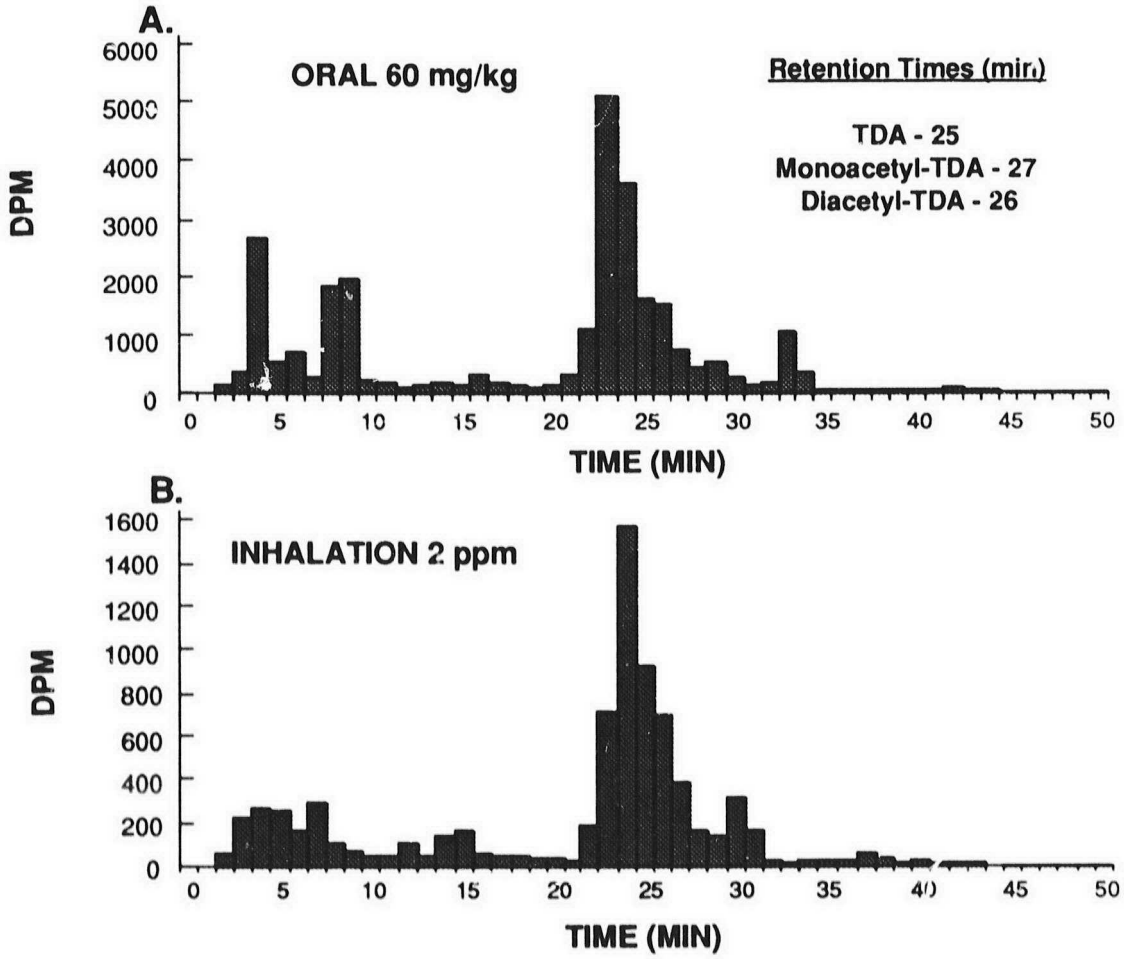
METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Figure 3. **^{14}C Urinary Time Course for 2,4-TDI (A) and 2,4-TDA (B) Excreted in the Urine by Male Fischer 344 Rats. The Half-Lives Were Derived from the Log-Linear Regression.**



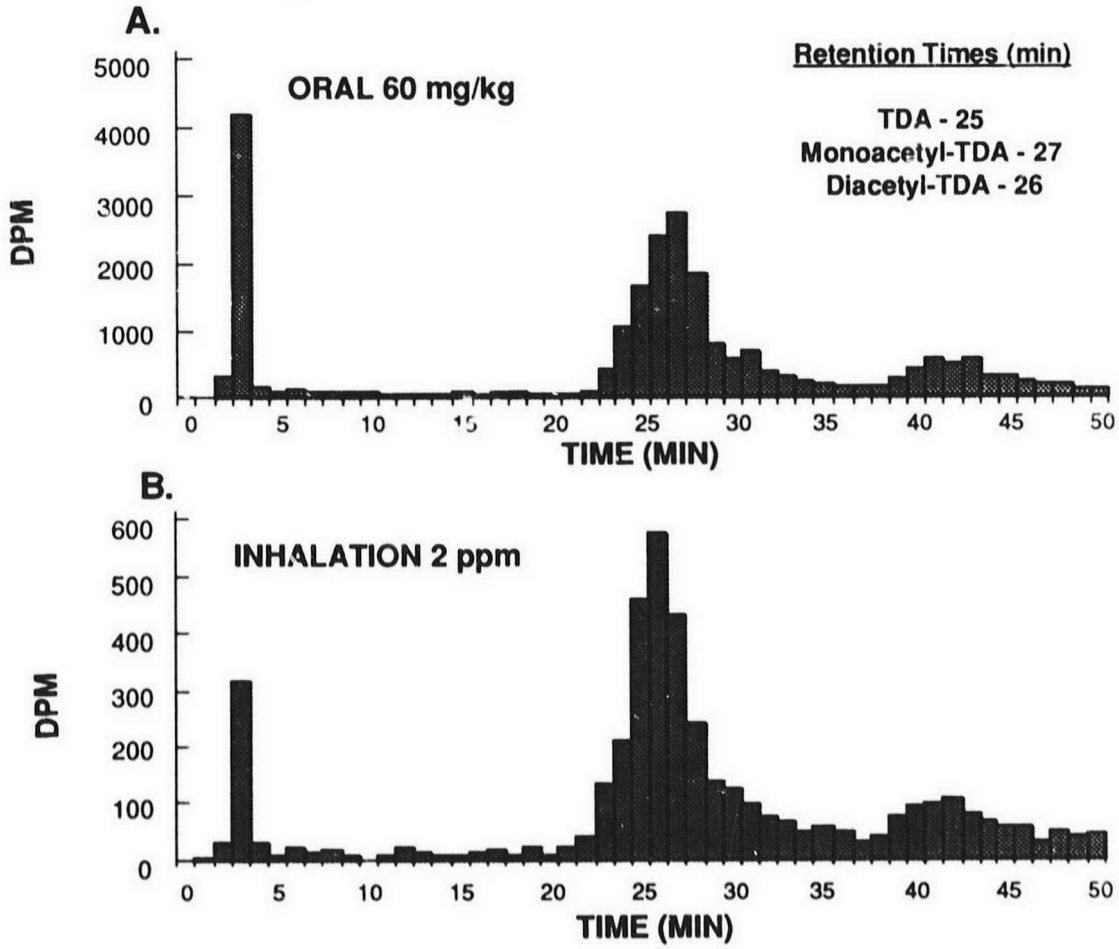
METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Figure 4. HPLC Radiochromatogram of Urine Specimens (0 - 12 hr Post-Dosing) from Male Fischer 344 Rats Given (A) an Oral 60 mg ^{14}C-2,4-TDI/Kg of Body Weight Dose or (B) Exposed to 2 ppm ^{14}C-2,4-TDI Vapors for a 4 hr Period .



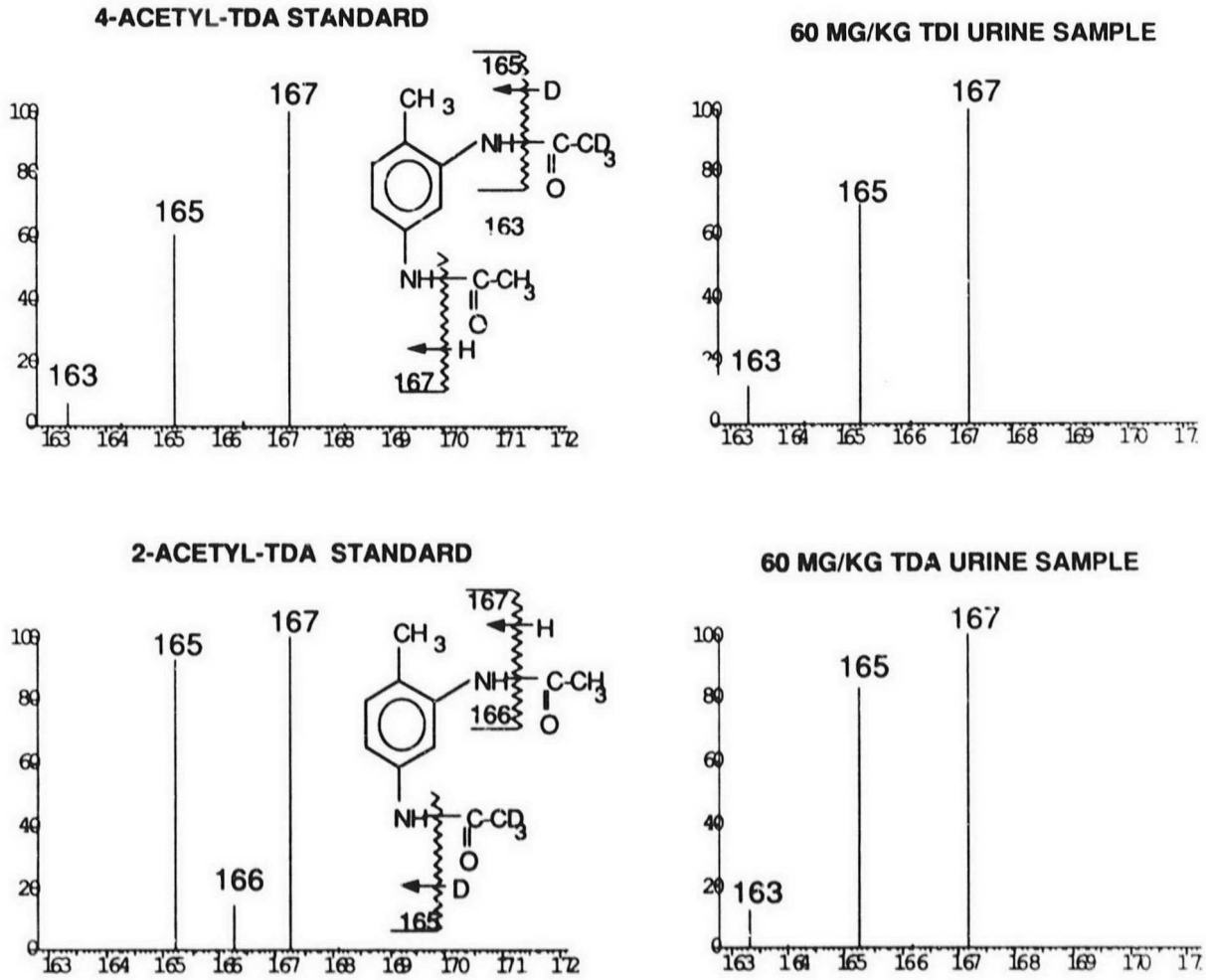
METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Figure 5. **HPLC Radiochromatogram of Fecal Specimens (0 - 24 hr Post-Dosing) from Male Fischer 344 Rats Given (A) an Oral 60 mg ^{14}C-2,4-TDI/Kg of Body Weight Dose or (B) Exposed to 2 ppm ^{14}C-2,4-TDI Vapors for a 4 hr Period.**



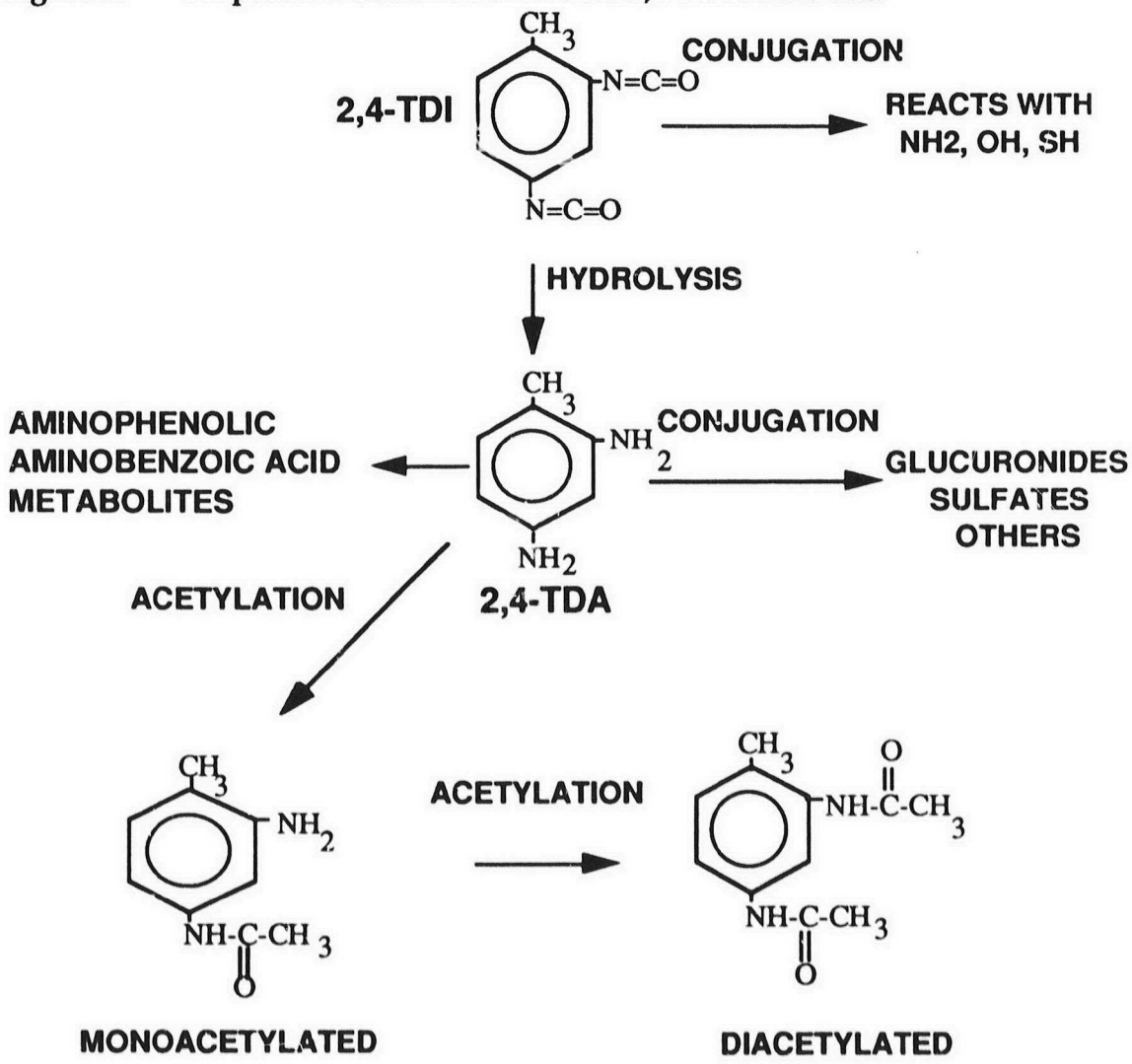
METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Figure 6. MS/MS Spectra (Daughters of 209) of the D_6 -Acetyl Derivatives of 4-Acetyl-TDA and 2-Acetyl-TDA.



METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Figure 7. Proposed Metabolic Scheme for 2,4-TDI in the Rat.



METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Appendix Table 1: Dose Calculations for Individual Male Rats Given the Single Oral 60 mg/kg ^{14}C-2,4-TDI Dose.

ANIMAL #S MALES	BODY WT (KG)	SYRINGE WT FULL (G)	SYRINGE WT EMPTY (G)	NET (G)	DOSE ADMINISTERED DPM	MG	MG/KG
48 HR							
89A-4841	0.246	10.1850	9.5023	0.6827	121824436	14.542	59.112
89A-4842	0.251	10.1948	9.5052	0.6896	123055706	14.688	58.520
89A-4843	0.249	10.1952	9.5077	0.6875	122680972	14.644	58.777
MEAN	0.249				122520371	14.625	58.803
SD	0.003				631151	0.075	0.297
2 HR							
89A-4845	0.246	10.1493	9.4651	0.6842	122092103	14.573	59.242
89A-4846	0.259	10.1871	9.4644	0.7227	128962238	15.394	59.428
89A-4847	0.250	10.1606	9.4638	0.6968	124340511	14.842	59.263
89A-4848	0.256	10.1799	9.4640	0.7159	127748811	15.249	59.644
MEAN	0.253				125785916	15.014	59.371
SD	0.006				3145125	0.375	0.215

Rats 89A-4841 to 89A-4843 and 89A-4845 to 89A-4848 were sacrificed 48 hr and 2 hr after dosing, respectively. Specific act. =8377702 dpm/mg TDI

Concentrations of ^{14}C and 2,4-TDI in the dose solution were 172,445,050 dpm/g and 21.3 mg/g, respectively.

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Appendix Table 2: Dose Calculations for Individual Male Rats Given the Single Oral 60 mg/kg ^{14}C-2,4-TDA Dose.

ANIMAL #S	BODY WT (KG)	SYRINGE WT FULL (G)	SYRINGE WT EMPTY (G)	NET (G)	DOSE ADMINISTERED DPM	MG	MG/KG
90A-2286	0.227	10.0498	9.3761	0.6737	147836068	14.417	63.512
90A-2287	0.221	10.0330	9.3761	0.6569	144149492	14.058	63.609
90A-2288	0.228	10.0466	9.3761	0.6705	147133863	14.349	62.933
90A-2289	0.226	10.0480	9.3762	0.6718	147419134	14.377	63.613
MEAN	0.226				146634639	14.300	63.417
SD	0.003				1681670	0.164	0.326

Concentrations of ^{14}C and 2,4-TDA in the dose solution were 219,439,020 dpm/g and 21.4 mg/g, respectively.
Specific activity = 10,254,160 dpm/mg TDA.

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Appendix Table 3: Dose Calculations for Individual Male Rats Given the Single Oral 3 mg/kg ^{14}C-2,4-TDA Dose.

ANIMAL #S	BODY WT. (KG)	SYRINGE WT FULL (G)	SYRINGE WT EMPTY (G)	NET (G)	DOSE ADMINISTERED DPM	MG	MG/KG
90A-2675	0.231	10.1692	9.4716	0.6976	93501232	0.767	3.322
90A-2676	0.225	10.2020	9.4700	0.7320	98111958	0.805	3.579
90A-2677	0.229	10.1666	9.4683	0.6983	93595055	0.768	3.354
90A-2678	0.232	10.1701	9.4660	0.7041	94372445	0.775	3.338
MEAN	0.229				94895173	0.779	3.398
SD	0.003				2179780	0.018	0.121

Concentrations of ^{14}C and 2,4-TDA in the dose solution were 134,032,730 dpm/g and 1.1 mg/g, respectively. Specific activity 121,847,000 dpm/mg TDA.

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Appendix Table 4: Dose Calculations for Individual Male Rats Given the Single iv 3 mg/kg ^{14}C-2,4-TDA Dose.

ANIMAL #S	BODY WT (KG)	SYRINGE WT FULL (G)	SYRINGE WT EMPTY (G)	NET (G)	DOSE ADMINISTERED DPM	MG	MG/KG
90A-4024	0.217	4.4178	4.2132	0.2046	75261840	0.573	2.640
90A-4025	0.230	4.4245	4.2152	0.2093	76990729	0.586	2.548
90A-4026	0.233	4.4293	4.2124	0.2169	79786379	0.607	2.607
90A-4028	0.214	4.415[illegible]	4.2122	0.2029	74636497	0.568	2.655
MEAN	0.224				76668861	0.584	2.612
SD	0.009				2304541	0.018	0.047

Concentrations of 14C and 2,4-TDA in the dose solution were 367,848,680 dpm/g and 2.8 mg/g, respectively.
Specific activity 131,374,529 DPM/ mg TDA.

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Appendix Table 5: Body Weights of Individual Male Rats Exposed to 2 ppm ^{14}C-2,4-TDI Vapors for a 4 hr Period.

48 Hr POST-EXPOSURE		IMMEDIATELY POST-EXPOSURE	
ANIMAL #S	BODY WT. (KG)	ANIMAL #S	BODY WT. (KG)
89A-7450	0.238	89A-7454	0.253
89A-7451	0.250	89A-7455	0.247
89A-7452	0.258	89A-7456	0.251
89A-7458	0.253	89A-7457	0.238
MEAN	0.250	MEAN	0.247
SD	0.008	SD	0.003

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Appendix Table 6: Calculated Time Weight Average Concentration for 4 hr 2 ppm ^{14}C-2,4-TDI Inhalation Exposure.

48 HR POST EXPOSURE Sample #	Time (hr)	Conc (ppm)	Delta time (hr)	Conc * Time (hr*ppm)	*24 hr PPM*24 HR
	0:00				
1	0:21:00	1.39	0:21:00	0.0203	0.4865
2	0:50:00	1.94	0:29:00	0.0391	0.9377
3	1:20:00	1.85	0:30:00	0.0385	0.9255
4	1:50:00	1.16	0:30:00	0.0242	0.5800
5	2:20:00	2.29	0:30:00	0.0477	1.1450
6	2:50:00	2.08	0:30:00	0.0433	1.0400
7	3:20:00	1.38	0:30:00	0.0288	0.6900
8	3:50:00	0.79	0:30:00	0.0166	0.3950
				Sum	**6.1992**
				Sum/4 hr	**1.5498**
IMMEDIATE POST-EXPOSURE					
9	0:21:00	1.76	0:21:00	0.0257	0.6160
10	0:50:00	1.41	0:29:00	0.0284	0.6815
11	1:20:00	1.90	0:30:00	0.0396	0.9500
12	1:52:00	1.94	0:32:00	0.0431	1.0347
13	2:20:00	1.52	0:28:00	0.0296	0.793
14	2:50:00	2.16	0:30:00	0.0450	1.0800
15	3:20:00	1.51	0:30:00	0.0315	0.7550
16*	3:50:00	1.51	0:30:00	0.0315	0.7550
				Sum	**6.5815**
				Sum/4 hr	**1.6454**

*Sample was lost, assumed same concentration as in sample #7.

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Appendix Table 7: Concentration of Radioactivity in Tissues of Male Rats 2 hr after a Single Oral Dose of 60 mg ^{14}C-2,4-TDI/kg of Body Weight.

PERCENT OF DOSE PER GRAM TISSUE

TISSUE	89A-4845	89A-4846	89A-4847	89A-4848	MEAN	SD
BLOOD	NA	NA	NA	NA	NA	NA
CARCASS	0.017%	0.056%	0.016%	0.061%	0.038%	0.024%
FAT	0.001%	0.004%	0.001%	0.001%	0.002%	0.002%
GI TRACT*	0.920%	1.114%	1.938%	1.174%	1.306%	0.450%
	0.945%	1.154%	2.093%	1.106%		
KIDNEY	0.035%	0.054%	0.048%	0.037%	0.044%	0.009%
LIVER	0.082%	0.046%	0.052%	0.047%	0.057%	0.017%
LUNG	0.008%	0.016%	0.016%	0.242%	0.071%	0.114%
SKIN	0.013%	0.026%	0.010%	0.028%	0.019%	0.009%

µg eq 2,4-TDI PER GRAM TISSUE

TISSUE	89A-4845	89A-4846	89A-4847	89A-4848	MEAN	SD
BLOOD	NA	NA	NA	NA	NA	NA
CARCASS	2.48	8.62	2.37	9.30	5.69	3.78
FAT	0.15	0.77	0.30	0.15	0.34	0.29
GI TRACT*	134.08	171.48	287.63	179.02	195.86	66.27
	137.72	177.64	310.64	168.65		
KIDNEY	5.10	8.31	7.12	5.64	6.54	1.46
LIVER	11.95	7.08	7.72	7.17	8.48	2.33
LUNG	1.17	2.46	2.37	36.90	10.73	17.46
SKIN	1.89	4.00	0.15	4.27	2.58	1.94

NA= Not Analyzed
*Two Determinations.

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Appendix Table 8: Concentration of Radioactvity in Tissues Immediately after Male Rats were Exposed to 2 ppm ^{14}C-2,4-TDI Vapors for a 4 hr Period.

PERCENT OF RECOVERED DOSE PER GRAM TISSUE

TISSUE	89A-7454	89A-7455	89A-7456	89A-7457	MEAN	SD
BLOOD	NA	NA	NA	NA	ND	ND
CARCASS	0.499%	0.487%	0.523%	0.523%	0.508%	0.018%
FAT	0.024%	0.016%	0.018%	0.016%	0.019%	0.004%
GI TRACT	0.516%	0.393%	0.258%	0.332%	0.375%	0.109%
KIDNEY	0.338%	0.481%	0.372%	0.487%	0.420%	0.076%
LIVER	0.215%	0.247%	0.185%	0.233%	0.220%	0.027%
LUNG	3.950%	3.355%	1.529%	1.720%	2.639%	1.198%
SKIN	0.133%	0.171%	0.116%	0.254%	0.169%	0.061%

µg eq 2,4-TDI PER GRAM TISSUE

TISSUE	89A-7454	89A-7455	89A-7456	89A-7457	MEAN	SD
BLOOD	NA	NA	NA	NA	NA	NA
CARCASS	2.34	1.62	2.01	2.11	2.02	0.30
FAT	0.11	0.05	0.07	0.06	0.07	0.03
GI TRACT	2.42	1.30	0.99	1.34	1.51	0.62
KIDNEY	1.58	1.60	1.43	1.97	1.65	0.23
LIVER	1.01	0.82	0.71	0.94	0.87	0.13
LUNG	18.52	11.13	5.89	6.95	10.62	5.73
SKIN	0.53	0.57	0.45	1.03	0.65	0.26

NA=Not Analyzed

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Appendix Table 9: Concentration of Radioactivity in Tissues of Male Rats 48 hr After a Single Oral Dose of 60 mg ^{14}C-2,4-TDi/kg of Body Weight.

	PERCENT OF DOSE PER GRAM TISSUE				
TISSUE	89A-4841	89A-4842	89A-4843	MEAN	SD
BLOOD	0.009%	0.007%	0.009%	0.008%	0.001%
CARCASS	0.005%	0.007%	0.004%	0.005%	0.002%
FAT	0.002%	0.001%	0.001%	0.001%	0.001%
GI TRACT	0.006%	0.006%	0.020%	0.011%	0.008%
KIDNEY	0.012%	0.012%	0.014%	0.013%	0.001%
LIVER	0.011%	0.011%	0.012%	0.011%	0.001%
LUNG	0.003%	0.002%	0.004%	0.003%	0.001%
SKIN	0.003%	0.002%	0.002%	0.002%	0.001%

	µg eq 2,4-TDI PER GRAM TISSUE				
TISSUE	89A-4841	89A-4842	89A-4843	MEAN	SD
BLOOD	1.31	1.03	1.32	1.22	0.16
CARCASS	0.73	1.03	0.59	0.78	0.22
FAT	0.29	0.15	0.15	0.20	0.08
GI TRACT	0.87	0.88	2.93	1.56	1.19
KIDNEY	1.74	1.76	2.05	1.85	0.17
LIVER	1.60	1.62	1.76	1.66	0.09
LUNG	0.44	0.29	0.59	0.44	0.15
SKIN	0.44	0.29	0.29	0.34	0.09

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Appendix Table 10: Concentration of Radioactvity in Tissues 48 hr After Male Rats were Exposed to 2 ppm ^{14}C-2,4-TDI Vapors for a 4 hr Period.

	PERCENT OF RECOVERED DOSE PER GRAM TISSUE					
TISSUE	89A-7450	89A-7451	89A-7452	89A-7458	MEAN	SD
BLOOD	0.083%	0.063%	0.057%	0.058%	0.066%	0.012%
CARCASS	0.063%	0.100%	0.076%	0.059%	0.075%	0.019%
FAT	0.009%	0.007%	0.003%	0.004%	0.008%	0.005%
GI TRACT	0.113%	0.090%	0.047%	0.076%	0.082%	0.028%
KIDNEY	0.190%	0.158%	0.129%	0.111%	0.147%	0.034%
LIVER	0.060%	0.059%	0.048%	0.050%	0.055%	0.006%
LUNG	0.231%	0.314%	0.147%	0.436%	0.283%	0.124%
SKIN	0.065%	0.142%	0.115%	0.108%	0.108%	0.032%

	µg eq 2,4-TDI PER GRAM TISSUE					
TISSUE	89A-7450	89A-7451	89A-7452	89A-7458	MEAN	SD
BLOOD	0.72	0.59	0.53	0.51	0.59	0.10
CARCASS	0.55	0.94	0.70	0.52	0.68	0.20
FAT	0.08	0.07	0.03	0.03	0.05	0.03
GI TRACT	0.98	0.84	0.43	0.66	0.73	0.24
KIDNEY	1.65	1.48	1.19	0.97	1.32	0.30
LIVER	0.52	0.55	0.44	0.44	0.49	0.06
LUNG	2.01	2.94	1.36	3.82	2.54	1.07
SKIN	0.56	1.33	1.06	0.94	0.98	0.32

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Appendix Table 11: Concentration of Radioactivity in Tissues of Male Rats 48 hr After a Single Oral Dose of 60 mg ^{14}C-2,4-TDA/kg of Body Weight.

PERCENT OF DOSE PER GRAM TISSUE

TISSUE	90A-2286	90A-2287	90A-2288	90A-2289	MEAN	SD
CARCASS	0.038%	0.017%	0.027%	0.021%	0.026%	0.009%
SKIN	0.008%	0.004%	0.004%	0.008%	0.006%	0.002%

µg eq 2,4-TDA PER GRAM TISSUE

TISSUE	90A-2286	90A-2287	90A-2288	90A-2289	MEAN	SD
CARCASS	5.48	2.39	3.87	3.02	3.69	1.34
SKIN	1.15	0.56	0.57	1.15	0.86	0.34

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Appendix Table 12: Concentration of Radioactivity in Tissues of Male Rats 48 hr After a Single Oral Dose of 3 mg ^{14}C-2,4-TDA/kg of Body Weight.

	PERCENT OF DOSE PER GRAM TISSUE					
TISSUE	90A-2675	90A-2676	90A-2677	90A-2678	MEAN	SD
CARCASS	0.014%	0.011%	0.010%	0.009%	0.011%	0.002%
SKIN	0.003%	0.003%	0.002%	0.002%	0.003%	0.001%

	µg eq 2,4-TDA PER GRAM TISSUE					
TISSUE	90A-2675	90A-2676	90A-2677	90A-2678	MEAN	SD
CARCASS	0.11	0.09	0.08	0.07	0.09	0.02
SKIN	0.02	0.02	0.02	0.02	0.02	0.00

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Appendix Table 13: Concentration of Radioactivity in Tissues of Male Rats 48 hr After a Single iv Dose of 3 mg ^{14}C-2,4-TDA/kg of Body Weight.

	PERCENT OF DOSE PER GRAM TISSUE					
TISSUE	90A-4024	90A-4025	90A-4026	90A-4028*	MEAN**	SD**
CARCASS	0.012%	0.011%	0.011%	0.035%	0.011%	0.001%
SKIN	0.003%	0.004%	0.006%	0.007%	0.004%	0.002%

	µg eq 2,4-TDA PER GRAM TISSUE					
TISSUE	90A-4024	90A-4025	90A-4026	90A-4028*	MEAN**	SD**
CARCASS	0.07	0.06	0.07	0.20	0.07	0.01
SKIN	0.02	0.02	0.04	0.04	0.03	0.01

* Animal 90A-4028: Died by 24 Hr Post-Dosing.
** Animal 90A-4028 Not Included

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Appendix Table14: Radioactivity Excreted in the Urine of Male Rats During the Indicated Intervals After a Single Oral Dose of 60 mg ^{14}C-2,4-TDI/kg of Body Weight.

PERCENT OF DOSE

COLLECTION INTERVAL (HR)	89A-4841	89A-4842	89A-4843	MEAN	SD
0-12 HR	6.615%	6.414%	6.881%	6.637%	0.234%
12-24 HR	1.107%	0.844%	1.320%	1.090%	0.238%
24-36 HR	0.371%	0.562%	0.368%	0.434%	0.111%
36-48 HR	0.273%	0.152%	0.226%	0.217%	0.061%
TOTAL	8.366%	7.972%	8.795%	8.378%	0.412%

µg eq 2,4-TDI

COLLECTION INTERVAL (HR)	89A-4841	89A-4842	89A-4843	MEAN	SD
0-12 HR	961.92	942.12	1007.64	970.56	33.60
12-24 HR	160.97	123.97	193.30	159.41	34.69
24-36 HR	53.95	82.55	53.89	63.46	16.53
36-48 HR	39.70	22.33	33.09	31.71	8.77
TOTAL	1216.54	1170.97	1287.92	1225.14	58.95

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Appendix Table 15: Radioactvity Excreted in the Urine of Male Rats During the Indicated Intervals After an Exposure to 2 ppm ^{14}C-2,4-TDI Vapors for a 4 hr Period.

PERCENT OF RECOVERED DOSE

COLLECTION INTERVAL (HR)	89A-7450	89A-7451	89A-7452	89A-7458	MEAN	SD
0-12 HR	9.133%	6.283%	7.186%	9.243%	7.961%	1.464%
12-24 HR	2.915%	2.024%	3.757%	0.559%	2.314%	1.367%
24-36 HR	2.407%	2.404%	2.859%	3.065%	2.684%	0.332%
36-48 HR	2.245%	2.682%	1.110%	1.518%	1.889%	0.707%
TOTAL	16.700%	13.393%	14.912%	14.385%	14.848%	1.386%

µg eq 2,4-TDI

COLLECTION INTERVAL (HR)	89A-7450	89A-7451	89A-7452	89A-7458	MEAN	SD
0-12 HR	79.41	58.89	66.10	80.36	71.19	10.47
12-24 HR	25.35	18.97	34.56	4.86	20.94	12.48
24-36 HR	20.93	22.53	26.30	26.65	24.10	2.82
36-48 HR	19.51	25.14	10.21	13.20	17.02	6.66
TOTAL	145.21	125.52	137.16	125.07	133.24	9.75

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Appendix Table 16: Radioactivity Excreted in the Urine of Male Rats During the Indicated Intervals After a Sngle Oral Dose of 60 mg ^{14}C-2,4-TDA/kg of Body Weight.

PERCENT OF DOSE

COLLECTION INTERVAL (HR)	90A-2286	90A-2287	90A-2288	90A-2289	MEAN	SD
0-12 HR	34.047%	38.910%	35.419%	34.524%	35.725%	2.198%
12-24 HR	15.567%	14.235%	26.557%	24.439%	20.200%	6.203%
24-36 HR	10.006%	12.355%	3.156%	5.926%	7.861%	4.110%
36-48 HR	1.689%	1.310%	0.767%	0.924%	1.173%	0.413%
TOTAL	61.309%	66.810%	65.899%	65.813%	64.958%	2.474%

µg eq 2,4-TDA

COLLECTION INTERVAL (HRS)	90A-2286	90A-2287	90A-2288	90A-2289	MEAN	SD
0-12 HR	4908.62	5469.84	5082.17	4963.35	5153.54	287.34
12-24 HR	2244.32	2001.11	3810.58	3513.48	2685.34	982.05
24-36 HR	1442.58	1736.82	452.84	851.95	1210.75	672.65
36-48 HR	243.51	184.16	110.05	132.84	179.24	66.87
TOTAL	8839.03	9391.93	9455.64	9461.62	9287.06	300.34

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Appendix Table 17: Radioactivity Excreted in the Urine of Male Rats During the Indicated Intervals After a Single Oral Dose of 3 mg ^{14}C-2,4-TDA/kg of Body Weight.

PERCENT OF DOSE

COLLECTION INTERVAL (HR)	90A-2675	90A-2676	90A-2677	90A-2678	MEAN	SD
0-12 HR	52.714%	77.623%	43.825%	67.499%	60.415%	15.065%
12-24 HR	2.320%	2.114%	2.565%	3.054%	2.513%	0.405%
24-36 HR	0.516%	0.472%	0.460%	0.419%	0.467%	0.040%
36-48 HR	0.324%	0.249%	0.246%	0.273%	0.273%	0.036%
TOTAL	55.874%	80.458%	47.096%	71.245%	63.668%	14.997%

µg eq 2,4-TDA

COLLECTION INTERVAL (HR)	90A-2675	90A-2676	90A-2677	90A-2678	MEAN	SD
0-12 HR	404.51	625.03	336.64	522.79	472.24	127.64
12-24 HR	17.80	17.02	19.70	23.65	19.54	2.96
24-36 HR	3.96	3.80	3.53	3.25	3.64	0.31
36-48 HR	2.49	2.00	1.89	2.11	2.12	0.26
TOTAL	428.76	647.85	361.76	551.80	497.54	127.42

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Appendix Table 18: Radioactivity Excreted in the Urine of Male Rats During the Indicated Intervals After a Single iv Dose of 3 mg ^{14}C-2,4-TDA/kg of Body Weight.

PERCENT OF DOSE

COLLECTION INTERVAL (HRS)	90A-4024	90A-4025	90A-4026	90A-4028*	MEAN	SD
0-12 HR	66.969%	68.584%	66.507%	65.795%	66.964%	1.183%
12-24 HR	3.552%	3.802%	4.290%	2.738%	3.596%	0.649%
24-36 HR	1.037%	0.646%	0.986%	NS	0.890%	0.213%
36-48 HR	0.469%	0.308%	0.314%	NS	0.364%	0.091%
TOTAL	72.027%	73.340%	72.097%	- -	72.488%	0.739%

µg eq 2,4-TDA

COLLECTION INTERVAL (HRS)	90A-4024	90A-4025	90A-4026	90A-4028*	MEAN	SD
0-12 HR	383.65	401.93	403.91	373.80	390.82	11.17
12-24 HR	20.35	22.28	26.05	15.56	21.06	2.90
24-36 HR	5.94	3.79	5.99	NS	5.24	1.26
36-48 HR	2.69	1.81	1.91	NS	2.14	0.43
TOTAL	412.63	429.81	437.86	- -	426.77	12.63

* No 24-48 hr sample.

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Appendix Table 19: Radioactivity Excreted in the Feces of Male Rats During the Indicated Intervals After a Single Oral Dose of 60 mg ^{14}C-2,4-TDI/kg of Body Weight.

PERCENT OF DOSE

COLLECTION INTERVAL (HR)	89A-4841	89A-4842	89A-4843	MEAN*	SD*
0-24 HR	53.873%	44.402%	55.076%		
	51.979%	44.079%	52.287%	50.283%	4.813%
24-48 HR	32.788%	31.127%	28.035%		
	32.400%	30.423%	27.529%	30.384%	2.194%
TOTAL	85.520%	75.016%	81.464%	80.667%	5.297%

µg eq 2,4-TDI

COLLECTION INTERVAL (HR)	89A-4841	89A-4842	89A-4843	MEAN*	SD*
0-24 HR	7833.95	6521.98	8065.19		
	7558.53	6474.53	7656.78	7351.83	683.42
24-48 HR	4767.87	4572.08	4105.38		
	4711.45	4468.68	4031.28	4442.79	309.33
TOTAL	12435.90	11018.64	11929.31	11794.62	718.17

Two determinations were made per collection interval.
* calculated over 6 determinations.

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Appendix Table 20: Radioactvity Excreted in the Feces of Male Rats During the Indicated Interval After an Exposure to 2 ppm ^{14}C-2,4-TDI Vapors for a 4 hr Period.

PERCENT OF RECOVERED DOSE

COLLECTION INTERVAL (HR)	89A-7450	89A-7451	89A-7452	89A-7458	MEAN	SD
0-48 HR	41.703%	33.245%	54.945%	58.282%	47.294%	11.984%

µg eq 2,4-TDI

COLLECTION INTERVAL (HR)	89A-7450	89A-7451	89A-7452	89A-7458	MEAN	SD
0-48 HR	362.62	311.57	505.43	515.42	423.76	102.30

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Appendix Table 21: Radioactivity Excreted in the Feces of Male Rats During the Indicated Intervals After a Single Oral Dose of 60 mg ^{14}C-2,4-TDA/kg of Body Weight.

PERCENT OF DOSE

COLLECTION INTERVAL (HR)	90A-2286	90A-2287	90A-2288	90A-2289	MEAN	SD
0-24 HR	7.959%	9.254%	8.207%	7.817%	8.309%	0.650%
24-48 HR	16.376%	13.440%	12.365%	14.857%	14.260%	1.741%
TOTAL	24.335%	22.694%	20.572%	22.674%	22.569%	1.542%

µg eq 2,4-TDA

COLLECTION INTERVAL (HR)	90A-2286	90A-2287	90A-2288	90A-2289	MEAN	SD
0-24 HR	1147.46	1300.90	1177.60	1123.81	1187.44	78.78
24-48 HR	2360.96	1889.35	1774.22	2135.92	2040.11	261.76
TOTAL	3508.42	3190.25	2951.82	3259.73	3227.56	229.01

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Appendix Table 22: Radioactivity Excreted in the Feces of Rats During the Indicated Intervals After a Single Oral Dose of 3 mg ^{14}C-2,4-TDA/kg of Body Weight.

COLLECTION INTERVAL (HR)	PERCENT OF DOSE					
	90A-2675	90A-2676	90A-2677	90A-2678	MEAN	SD
0-24 HR	19.071%	23.145%	31.775%	25.067%	24.765%	5.300%
24-48 HR	9.923%	4.764%	4.493%	4.524%	5.926%	2.667%
TOTAL	28.994%	27.909%	36.268%	29.591%	30.691%	3.783%

COLLECTION INTERVAL (HR)	µg eq 2,4-TDA					
	90A-2675	90A-2676	90A-2677	90A-2678	MEAN	SD
0-24 HR	146.34	186.36	244.08	194.15	192.73	40.13
24-48 HR	76.15	38.36	34.51	35.04	46.02	20.16
TOTAL	222.49	224.72	278.59	229.19	238.75	26.71

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Appendix Table 23: Radioactivity Excreted in the Feces of Rats During the Indicated Intervals After a Single iv Dose of 3 mg ^{14}C-2,4-TDA/kg of Body Weight.

PERCENT OF DOSE

COLLECTION INTERVAL (HR)	90A-4024	90A-4025	90A-4026	90A-4028*	MEAN	SD
0-24 HR	12.386%	16.080%	13.052%	20.246%	15.441%	3.584%
24-48 HR	7.520%	3.164%	7.975%	NS	6.220%	2.656%
TOTAL	19.906%	19.244%	21.027%	- -	20.059%	0.901%

µg eq 2,4-TDA

COLLECTION INTERVAL (HR)	90A-4024	90A-4025	90A-4026	90A-4028*	MEAN	SD
0-24 HR	70.96	94.24	79.27	115.02	89.87	19.34
24-48 HR	43.08	18.54	48.43	NS	3[illegible].58	15.94
TOTAL	114.04	112.78	127.70	- -	118.17	8.27

* No 24-48 hr specimen available.

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Appendix Table 24: Total Percent of Administered 2,4-TDI in Tissues of Male Rats 48 hr and 2 hr After a Single Oral Dose of 60 mg ^{14}C-2,4-TDI/kg of Body Weight.

48 Hr PERCENT OF ADMINISTERED DOSE

TISSUE	89A-4841	89A-4842	89A-4843	MEAN	SD
BLOOD	0.066%	0.037%	0.035%	0.046%	0.017%
CARCASS	0.747%	0.986%	0.588%	0.774%	0.200%
FAT	0.003%	0.001%	0.001%	0.002%	0.001%
KIDNEY	0.023%	0.021%	0.025%	0.023%	0.002%
LIVER	0.113%	0.105%	0.108%	0.109%	0.004%
LUNG	0.003%	0.002%	0.005%	0.003%	0.002%
SKIN	0.175%	0.134%	0.139%	0.149%	0.022%
GI TRACT	0.059%	0.089%	0.164%	0.104%	0.054%
GI CONTENTS	1.593%	3.028%	3.059%	2.560%	0.838%
TOTAL	2.782%	4.403%	4.124%	3.770%	0.867%

2 Hr - PERCENT ADMINISTERED DOSE

TISSUE	89A-4845	89A-4846	89A-4847	89A-4848	MEAN	SD
BLOOD	NA	NA	NA	NA	NA	NA
CARCASS	2.398%	8.118%	2.372%	9.098%	5.497%	3.615%
FAT	0.001%	0.005%	0.002%	0.001%	0.002%	0.002%
KIDNEY	0.060%	0.098%	0.083%	0.068%	0.077%	0.017%
LIVER	0.715%	0.421%	0.443%	0.416%	0.499%	0.145%
LUNG	0.008%	0.017%	0.014%	0.212%	0.063%	0.100%
SKIN	0.755%	1.512%	0.584%	1.637%	1.122%	0.530%
GI TRACT*	7.721%	9.786%	14.744%	8.161%	10.103%	3.085%
GI CONTENT*	73.223%	54.157%	64.017%	71.889%	65.821%	8.346%
TOTAL	84.881%	74.114%	82.259%	91.482%	83.184%	7.185%

NA- Not Analyzed ; *- Samples were run as duplicates and this represents the average of 2 determinations.

METABOLIC FATE OF ^{14}C-TOLUENE-2,4-DIISOCYANATE IN FISCHER 344 RATS

Appendix Table 25: Percent of Recovered Radioactivity in Tissues Immediately Post-Exposure and 48 hr After Male Rats were Exposed to 2 ppm ^{14}C-2,4-TDI Vapors for a 4 hr Period.

48 hr PERCENT OF RECOVERED ^{14}C

TISSUE	89A-7450	89A-7451	89A-7452	89A-7458	MEAN	SD
BLOOD	0.060%	0.136%	0.353%	0.361%	**0.228%**	**0.153%**
CARCASS	8.129%	13.645%	10.463%	7.821%	**10.015%**	**2.692%**
FAT	0.015%	0.008%	0.004%	0.005%	**0.008%**	**0.005%**
KIDNEY	0.298%	0.264%	0.231%	0.200%	**0.248%**	**0.042%**
LIVER	0.372%	0.375%	0.364%	0.375%	**0.372%**	**0.005%**
LUNG	0.220%	0.348%	0.148%	0.406%	**0.281%**	**0.118%**
SKIN	3.365%	7.187%	6.069%	5.754%	**5.594%**	**1.608%**
GI TRACT	1.200%	0.924%	0.415%	0.497%	**0.759%**	**0.359%**
GI CONTENTS	21.531%	27.126%	8.723%	9.154%	**16.634%**	**9.176%**
TOTAL	**35.190%**	**50.013%**	**27.770%**	**24.573%**	**34.137%**	**11.531%**

IMMEDIATELY POST-EXPOSURE
PERCENT OF RECOVERED ^{14}C

TISSUE	89A-7454	89A-7455	89A-74567	89A-7457	MEAN	SD
BLOOD	NA	NA	NA	NA	**NA**	**NA**
CARCASS	71.293%	67.941%	75.246%	71.688%	**71.542%**	**2.987%**
FAT	0.024%	0.016%	0.018%	0.016%	**0.019%**	**0.004%**
KIDNEY	0.597%	0.760%	0.664%	0.754%	**0.694%**	**0.078%**
LIVER	1.698%	1.836%	1.635%	1.529%	**1.675%**	**0.128%**
LUNG	3.804%	3.093%	1.531%	1.572%	**2.500%**	**1.133%**
SKIN	7.981%	10.404%	7.024%	14.022%	**9.858%**	**3.[illegible]%**
GI TRACT	5.938%	3.594%	2.273%	3.190%	**3.749%**	**[illegible]1%**
GI CONTENT	8.628%	11.944%	11.409%	7.059%	**9.760%**	**2.314%**

NA- Not Analyzed

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CERTIFICATE OF AUTHENTICITY

THIS IS TO CERTIFY that the microimages appearing on this microfiche are accurate and complete reproductions of the records of U.S. Environmental Protection Agency documents as delivered in the regular course of business for microfilming.

Data produced 5 (Month) 11 (Day) 92 (Year) Marcia Tubolino Camera Operator

Place Syracuse (City) New York (State)

AMTEK corp