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Uptake and Distribution of ^{14}C during and following Inhalation Exposure to Radioactive Toluene Diisocyanate

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Uptake and Distribution of ^{14}C during and following Inhalation Exposure to Radioactive Toluene Diisocyanate. KENNEDY, A. L., STOCK, M. F., ALARIE, Y., AND BROWN, W. E. (1989). *Toxicol. Appl. Pharmacol.* **100,** 280–292. Inhalation of toluene diisocyanate (TDI) results in toxic responses ranging from pulmonary irritation to immunological sensitization. The use of radioactively labeled isocyanate has made it possible to follow the initial uptake of the compound into the bloodstream independent of the final fate of the isocyanate. This study shows that the rate of uptake into the blood is linear during exposure to concentrations ranging from 0.00005 to 0.146 ppm and that the uptake continues to increase slightly postexposure. It also demonstrates that the radioactivity clears from the bloodstream to a level corresponding to approximately a 100 nM concentration of tolyl group after 72 hr and persists at a nanomolar level even 2 weeks following the exposure. This is similar to the response previously reported by this group for radioactively labeled methyl isocyanate. The initial rate of ^{14}C uptake is also a linear function of the concentration of TDI when expressed either as concentration (ppm) or as concentration multiplied by duration of exposure (ppm · hr). This is discussed in comparison with the toxic responses as a function of both ppm and ppm · hr. Finally, the inclusion of the data on methyl isocyanate indicates that the uptake into arterial blood is a function of exposure concentration, independent of isocyanate structure.

Isocyanates are a group of highly reactive chemicals that are used in a number of industrial and biochemical applications (Brown, 1986). The toxic effects of exposure to isocyanates have been well documented in both animal models and exposed human populations (Karol, 1986) but the mechanism of isocyanate toxicity is not fully understood. The *in vivo* targets of inhaled isocyanates are still unknown and the uptake of isocyanates during inhalation exposure has not been investigated in a systematic fashion. Radioactive isocyanate exposures have been performed using a guinea pig model to address these questions (Ferguson *et al.*, 1988). The uptake and distribution of radioactive toluene diisocyanate (TDI) is the focus of this paper. Biochemical analysis of the material derived from these exposures will further the understanding of the mechanism of isocyanate toxicity.

We have previously reported the uptake and distribution of ^{14}C found following exposure to [^{14}C]methyl isocyanate (MIC; Ferguson *et al.*, 1988). That study demonstrated the initial, rapid, and linear uptake of ^{14}C into the bloodstream of guinea pigs exposed to concentrations of MIC ranging from 0.5 to 15 ppm for periods of 1–6 hr. In addition, ^{14}C was detected in all tissues and body fluids with greater than 90% clearance of radioactivity over a period of 72 hr.

Chemicals of the isocyanate family have varied reactivities and responses both *in vitro* and *in vivo* (Brown *et al.*, 1987). Isocyanates can react with a number of functional groups, many of which are present on biological

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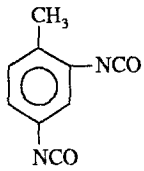
TABLE 1

COMPARISON OF THE CHEMICAL AND BIOCHEMICAL PROPERTIES OF METHYL ISOCYANATE (MIC) AND TOLUENE DIISOCYANATE (TDI)

Property	MIC	TDI
Structure	CH_3NCO	
Molecular weight (g/m)	57.05	174.15
Number of reactive groups	1	2
Density (g/ml)	0.96	1.22
Boiling point (°C)	38	120
Vapor pressure (mm Hg at 20°C)	348	0.025
Potency as sensory irritant (ppm)[a]	1.3	0.2
Potency as pulmonary irritant (ppm)[a]	1.9	>1.0

[a] From Alarie *et al.* (1987).

macromolecules (Chadwick and Cleveland, 1981). Examples include amino, hydroxyl, sulfhydryl, carboxyl, and imidazole groups. Table 1 is a comparative summary of the properties of the two isocyanates of interest to this report. TDI and MIC represent two different chemical structures, each possessing the isocyanate functional group. MIC, an alkyl monofunctional isocyanate, is classified as both a sensory and a pulmonary irritant (Alarie *et al.*, 1987) with only weak immunogenic properties (Karol *et al.*, 1987). TDI is an aromatic bifunctional isocyanate which is primarily a sensory irritant often associated with immunologic sensitization in animal models and some human subjects (Karol, 1985). TDI, unlike MIC, has been shown to have a high degree of specificity in *in vitro* protein modifications (Brown *et al.*, 1982). Diisocyanates, in general, display a stoichiometric inhibition of enzyme activity for both cholinesterases and chymotrypsin (Brown, 1986). The differences in affinities between alkyl and aryl isocyanates observed in *in vitro* reactions with proteins may be significant in the *in vivo* uptake and response as well.

The overall goal of this research is to identify the *in vivo* target molecules labeled as a result of inhalation exposure to radioactive isocyanates and to determine whether a correlation can be made between the modification of these targets and the physiological responses which occur as a result of exposure. The initial phase of this research which is described in this paper involves the analysis of whether differences exist in uptake, distribution, and clearance during and following inhalation exposure as a function of isocyanate concentration. The range of concentrations was chosen to bracket the threshold limit value (TLV) for TDI. Most of the exposures were for 1 hr; however, two longer exposures were also performed to evaluate whether cumulative effects occur. Analysis of clearance and postexposure effects was accomplished by euthanizing individual animals at times up to 3 days postexposure. One experiment extended this analysis to 2 weeks postexposure.

These studies parallel the previously published results of [^{14}C]MIC exposures (Ferguson *et al.*, 1988). By using MIC and TDI as two variants of isocyanate structure, we can also begin to assess the role of chemical structure on uptake and reactivity *in vivo*.

MATERIALS AND METHODS

Chemicals. 2,4-[^{14}C]TDI (12.5 mCi/mmol) was synthesized by New England Nuclear (Boston, MA) with the ^{14}C incorporated in the benzene ring. Gas chromatographic analysis provided by NEN showed the radiochemical to be 99% pure. Derivatization and HPLC analysis of the sample confirmed the reactivity and purity as 2,4-TDI. (See Quantitation of isocyanate concentrations.) Cold 2,4-TDI was received from the same source and was used as a reference compound as well as to establish the instrument settings for the desired exposure concentrations.

Animals. Male Hartley guinea pigs (300–400 g) were purchased from Hazelton Research Products, Inc. (Denver, PA). They were held in an animal room for at least 7 days prior to exposure with food and water provided *ad libitum* and on a 12 hr light/dark cycle. Thirty-two guinea pigs were used in this study. Seven exposure levels were tested with four animals at each concentration. Four additional animals served as controls.

Carotid artery cannulation. Prior to exposure the carotid artery was cannulated following the previously published procedure (Alarie and Stock, 1988; Ferguson *et al.*, 1988) with several modifications described below. In order to facilitate the withdrawal of blood samples, a revised cannulation procedure was used with one of the major alterations being the size of the tubing. A larger intramedic tubing (PE 50) was implanted in the carotid artery. Prior to surgery the cannulae were prepared by cutting two pieces of 0.035 ID manifold pump tubing crosswise and slipping the pieces onto the PE 50 tubing. The pieces were glued with Super Glue at distances of 15 and 95 mm from one end. The pieces now formed two bulb-type anchors on the intramedic tubing. The tubing was attached to a 1 ml syringe of Ringers lactate solution via a 23-gauge needle and then filled with the solution. The opposite end having the 15 mm anchor was inserted into the carotid artery and a suture was placed behind the anchor. The cannula was passed under the skin and over the shoulder without a loop to alleviate the problem of a bend or blockage which occurred when the tubing was looped inside the ventral nape area. The plastic hub used in the original procedure was discarded. By making a larger (25 mm) incision on the dorsal side the excess tubing could be inserted in a pocket made under the incision. The second anchor was sutured inside this incision. The tubing was rolled in a circle and placed in this pocket with only the clay-filled cannula tip sticking out of the incision. The incision was closed with a single suture which could be removed quickly several days later. The animals were then returned to their cages for a couple of days. On the day of the exposure, the suture was snipped and the cannula pulled out of the incision and unrolled. The animal was placed in the exposure chamber and the cannula was passed through one of the chamber ports. The clay-filled tip was cut off and the cannula was attached by a 23-gauge needle to a Ringers lactate-filled 1-ml syringe. The flow of blood in the cannula was checked and, if acceptable, the animal was ready for exposure. If the animals were to be kept for several days after the exposure, the dorsal incision area was anesthetized with 4% topical xylocaine (ASTRA, Pharmaceutical Products Inc.). The cannula was again filled with clay, wound in a circle, returned to the pocket, and sutured. These changes in the procedure kept the animals from picking or scratching the cannula which had previously been mounted on their backs.

Exposure system. A continuous airflow system was used for all seven radioactive TDI exposures. The system was designed according to the protocol of Alarie *et al.* (1987) with only minor alterations as previously presented (Ferguson *et al.*, 1988). For each exposure, four cannulated animals were placed in separate, glass, whole-body plethysmographs which served as exposure chambers. These were attached to a central mixing chamber into which the desired exposure concentration was established. The radioactive TDI was shipped in sealed, glass mini-vials identical to those used for the MIC experiments. To generate the [^{14}C]TDI vapor, house air was dried and delivered over the liquid in the vial once the internal glass septum was broken with a needle. Air flow was controlled by an appropriate flowmeter. Choice of flowmeters and rate settings, ranging from 2.75 ml/min to 5 liters/min, for the radioactive experiments was determined using identical mini-vials containing "cold" TDI and with animals in the exposure chambers. Due to the difference in vapor pressure between the two isocyanates, the icebath used to control vapor generation in the MIC exposures was eliminated for the TDI experiments. A 20-gauge needle also penetrated the top of the vial to deliver the vapor to the chamber. The vapor was drawn into the system by a vacuum pump. Exhaust rates varied between 7 and 30 liters/min depending on the isocyanate concentration desired.

Quantitation of isocyanate concentrations. The quantitation of isocyanate concentrations in the system was monitored throughout all exposures by the periodic sampling of the guinea pig chamber atmospheres using three methods. These air samples were withdrawn from a sampling port directly above the animal. Samples were collected from alternate chambers to determine whether each animal received an equivalent concentration. The first method for the determination of isocyanate concentrations was the Marcali method (Marcali, 1957) as modified by NIOSH (1978). Air samples were bubbled into an impinger containing the Marcali trapping solution for periods of 1–60 min depending on the expected exposure concentration. Samples were processed and then quantified by absorbance at 550 nm relative to a cold 2,4-TDI standard curve. An aliquot of this solution was counted in ACS liquid scintillant (Amersham) for calculation of isocyanate content based on the specific activity of the original compound. The second method involved bubbling air samples at fixed times through Marcali trapping solution and immediate scintillation analysis. Concentration was determined using the specific activity of the original compound. The third method was the derivatization of the isocyanate with *p*-nitrobenzoylpropylamine (PNBPA; Regis Chemical Co.) which was immobilized on glass fiber filters. Air samples were drawn through the filter cassettes at a rate of 2 liters/min for periods of 1–30 min. Derivatized isocyanate was extracted from the filter in acetonitrile and analyzed by reverse-phase HPLC following a previously established method (Schroeder and Moore, 1985) using a C_{18} μbondapak column (0.39 × 15 cm; Waters). An aliquot of the extracted sample was also counted and the isocyanate concentration calculated for comparison based on its specific activity.

Analysis of ^{14}C uptake into the bloodstream. During the exposure 0.5 ml blood samples were collected at approximately 3, 6, 10, 15, and 20 min and every 10 min thereafter for the 1 hr exposures. Samples from the 4-hr exposure were taken following a similar schedule for the first hour and then every 30 min to the end of the exposure. For the 5 hr exposure, samples were taken at 15 min intervals for the first 2 hr and then every 30 min to the

termination. All uptake samples were collected via the carotid cannulae. Alternate animals were sampled when blood flow from the cannula was adequate (see Table 4). The blood samples were immediately mixed in Vacutainer tubes containing 0.149 M buffered sodium citrate (Becton–Dickinson). An aliquot of 200 μl was removed from each sample and transferred to a glass scintillation vial for the determination of ^{14}C content as follows. To each vial, 2.4 ml of NCS tissue solubilizer (Amersham) was added and the suspension was heated at 50°C for 20 min. An aliquot of 0.8 ml of a 20% benzoyl peroxide solution was added followed by an incubation at 50°C for an additional 30 min to decolorize the samples. After cooling to room temperature, organic scintillant (toluene, 2,5-diphenyloxazole, and 1,4,-bis 2-(5-phenyloxazolyl)benzene) was added to bring the final volume to 20 ml. To reduce the level of background radioactivity due to chemiluminescence, the samples were stored in the dark for at least 24 hr before scintillation analysis. Total radioactivity was calculated on a counts per minute per milliliter of blood basis. The remaining portion of whole blood was spun at 478*g* for 15 min. Plasma was separated from cellular components and stored at −20°C. Cellular pellets were kept at 4°C to avoid freeze fracturing of the cell membranes.

Collection of terminal blood, body fluids, and tissues. After each exposure, animals were euthanized (2 ml sodium pentobarbital 50 mg/ml, ip) at times represented in Table 3. Typical times for euthanasia were 0, 6, 24, and 72 hr postexposure. Deviations from this schedule were demanded for exposures 4 and 6 because in both cases, one of the animals was visibly ill during exposure and was euthanized immediately. To assure relevant data for the 0 hr postexposure time point, an additional animal was euthanized and the 6 hr time point forfeited. Exposure 5 extended the schedule to 2 weeks postexposure. Terminal blood samples were collected via cardiac punctures, immediately mixed in a Vacutainer tube containing anticoagulant, and treated identically to the uptake samples previously described. A 2 hr post exposure blood sample was taken from arterial cannulae for exposures 5–7, and a 6 hr sample was taken for exposure 4. Additional cannulae and toebleed samples were collected following exposure 5 at times diagrammed in Fig. 4. Bile was withdrawn from the gall bladder with a syringe and urine samples were similarly collected from the bladder. All fluids were stored at −60°C. To determine the level of ^{14}C in the bile and urine, 100-μl aliquots were counted in Scintiverse Bio-HP (Fisher Scientific, Pittsburgh, PA) and radioactivity was calculated on a counts per minute per milliliter basis following subtraction of background counts.

Trachea, lung, kidney, heart, spleen, and liver were dissected from each of the exposed animals as well as four control animals. For unpaired organs equal sections were taken: one was immediately frozen in liquid nitrogen and stored at −60°C for biochemical studies and the other portion was immersed in 10% buffered formalin for histological analysis. Paired organs were separated and processed similarly.

Tissue solubilization. A representative fragment was cut from each major organ and weighed. The fragment was transferred to a glass scintillation vial. NCS tissue solubilizer was added to a volume six times the total sample weight and the vials were then incubated at 50°C for 24 hr. Samples were cooled and neutralized to pH 7 with glacial acetic acid. Some samples required decolorization with benzoyl peroxide. Organic scintillant was added as described earlier and the samples were incubated in the dark for up to 4 weeks and then counted. Results were corrected for background and blood content using published percentages of blood volume for each organ (Wagner and Manning, 1976) and were then normalized on a counts per minute per gram basis.

RESULTS

Determination of Radiochemical Purity and Specific Activity

To complement the gas chromatographic analysis performed by NEN on the [^{14}C]TDI, a reverse-phase HPLC analysis was conducted on the radiochemical following derivatization with PNBPA. The appropriate peak of 254 nm absorbance was collected (Fig. 1D, peak 3). An aliquot was counted and the total radioactivity contained in the peak was calculated to be 83% of the total injected. Migration distances of cold 2,4-TDI as well as 2,6-TDI, Mondur TD80 (80% 2,4 isomer/20% 2,6 isomer; Mobay), and toluenediamine (TDA) were analyzed under identical conditions. Typical profiles are represented in Figures 1A, B, and C. This analysis showed that the radiolabeled peak comigrates with 2,4-TDI and did not contain other contaminating compounds such as the 2,6 isomer or the hydrolysis product, TDA. A standard curve was generated with cold 2,4-TDI to determine the concentration of TDI in the radioactive sample. The total radioactivity and molar quantity of TDI were used to determine the specific activity of the TDI sample which verified the 12.5 mCi/mmol value obtained by NEN. These analyses not only assayed the specific activity but also confirmed the compound purity. The ability of the radioactive compound to react with the

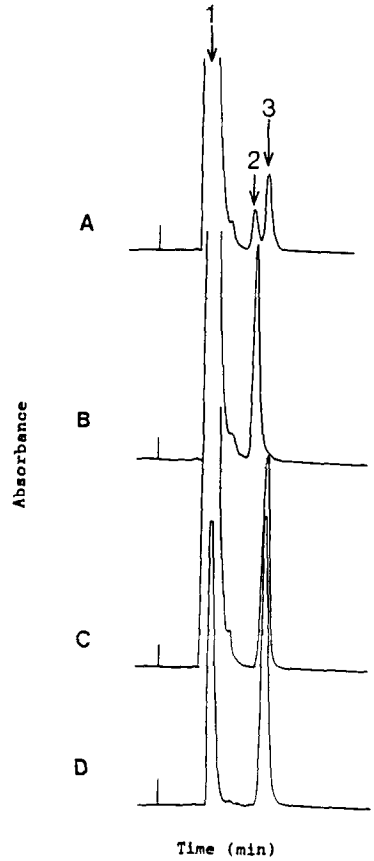


FIG. 1. HPLC analysis of derivatized isocyanates. Typical absorbance profiles at 254 nm of tolyl isocyanates derivatized with PNBPA. (A) TD 80 (80% 2,4-TDI, 20% 2,6-TDI), (B) 2,6-TDI, (C) cold 2,4-TDI. (D) ^{14}C 2,4-TDI. Peak 1 in all profiles is unreacted nitroreagent. Peak 2 and 3 correspond to the 2,6 and 2,4 isomers, respectively.

PNBPA demonstrates that the chemical form used for animal exposure was reactive isocyanate and not, for instance, TDA, the hydrolysis product.

Isocyanate Exposure Concentrations

A series of ^{14}C TDI exposures was conducted over a range of concentrations as given in Table 2. Three methods were used to monitor the exposure atmospheres: the Marcali assay, scintillation analysis of trapping solutions, and HPLC analysis of derivatized isocyanate. The Marcali determination involved the hydrolysis of isocyanate to toluenediamine and conversion to a colorimetric product. The HPLC method detected the derivative of reactive isocyanate with a nitroreagent (PNBPA). Marcali assay solutions were also subjected to liquid scintillation analysis. On the basis of the specific activity of the original compound, an additional measure of isocyanate concentration was thereby achieved. Table 2 summarizes the compiled data available for each experiment. The data are comparable, independent of the assay method. In some cases a value was not available due to the detection limits (exposure 1) or trapping procedures. For the first series of experiments chamber atmospheres were bubbled directly into a solution of PNBPA and the derivatization was not effective. Exposures 5 and 7 were monitored by the PNPBA glass fiber filter method. Average concentrations were assigned based on all values available. Multiple samples (*n*, Table 2) were collected during the exposures and showed only minor fluctuations in chamber atmospheres throughout the exposures (SD, Table 2).

Uptake and Clearance of ^{14}C into the Bloodstream

The uptake of radioactivity in arterial blood during exposure at all concentrations is shown in Fig. 2. At all concentrations the first blood sample showed a detectable level of radioactivity (except at 0.00005 ppm) which increased linearly throughout the exposure. Table 4 gives the uptake equations and *r* values for all data shown in Fig. 2.

Clearance of radioactivity from the bloodstream was analyzed in terminal blood samples as well as postexposure arterial cannula samples or toebleeds. The profile for postexposure clearance at all concentrations is shown in Fig. 3. A specific illustration of the uptake and clearance of the ^{14}C is shown in Fig. 4 for the 0.136 ppm exposure. Following the linear uptake (Fig. 2C and Fig. 4, filled circles) a postexposure increase in blood radioactivity was observed followed by a grad-

TABLE 2

SUMMARY OF [^{14}C] TDI EXPOSURE CONCENTRATIONS

Exposure No.	Isocyanate measurement: Marcali[a] assay Abs 550 nm (ppm) $\bar{x}$[d]	SD[e]	n[f]	Specific[b] activity cpm (ppm) $\bar{x}$	SD	n	PNBPA[c] assay Abs 254 nm (ppm) $\bar{x}$	SD	n	Average concentration (ppm)	Exposure duration (hr)	$C \times t$ (ppm·hr)
1	ND[g]	—	1	0.00005	—	1	NA[h]	—	0	0.00005	1	0.00005
2	0.0044	—	1	0.0038	0.0005	8	NA	—	0	0.004	1	0.004
4	0.0166	0.00185	10	0.0152	0.0009	10	NA	—	0	0.016	5	0.080
3	0.0299	0.0021	2	0.0270	—	1	NA	—	0	0.029	1	0.029
7	0.0895	0.036	15	0.0756	0.034	15	0.0877	0.062	5	0.084	4	0.336
5	0.1343	0.024	8	0.1047	0.024	7	0.1562	0.039	3	0.132	1	0.132
6	0.1667	0.026	6	0.1242	0.020	6	NA	—	0	0.146	1	0.146

[a] Marcali assay (Marcali, 1957).
[b] Quantitation of TDI based on the specific activity of the original compound.
[c] PNBPA derivatization assay (Schroeder and Moore, 1985).
[d] $\bar{x}$ Is the mean ppm value.
[e] SD is the standard deviation.
[f] n Is the number of samples collected during exposure.
[g] ND, value is not detectable.
[h] NA, value is not available due to inadequate trapping.

ual decline over 72 hr (Fig. 3, and Fig. 4 open triangles, filled and open squares). The level of radioactivity found at 72 hr postexposure did not show a significant decline over the subsequent 11 day period but instead, a level of approximately 1000 cpm/ml was maintained even after the second week (Fig. 4). For all of the exposures above 0.029 ppm, a level of nearly 2000 cpm/ml (1938 cpm/ml, SD, 406) was observed at 72 hr, independent of the maximal amount of radioactivity seen in the bloodstream (Table 3). Using the specific activity of the original compound, it is possible to calculate the molar quantity of the tolyl group represented by a cpm value irrespective of the form. For the average value of 1938 cpm/ml, the molar equivalent for the tolyl group is 8.3×10^{-8} M.

As noted earlier, the uptake profiles and clearance of blood radioactivity were similar for all concentrations tested (Table 4). For each exposure concentration, a direct relationship was found between the ppm·hr and the immediate postexposure terminal blood level of radioactivity. This relationship is presented in Fig. 5. The slope of the line is 3.3 $\times 10^4$ (r, 0.99). Using this value, the lowest ppm exposure (0.00005 ppm) blood level immediately postexposure was calculated and found to be 2 cpm which is below the detection limits and explains the absence of radioactivity at this exposure concentration.

Uptake and Distribution of ^{14}C in Body Fluids and Tissues

Aliquots of bile and urine were counted and the results are given in Table 3. The highest level of radioactivity for all exposures was immediately postexposure. The maximum value for the urine was always higher than the bile for equivalent samples on a counts per minute per milliliter basis. A decline in radioactivity was evident by the 6 hr time point and returned toward a baseline level by 72 hr.

The results of the digestion of tissue fragments from several organs including the tra-

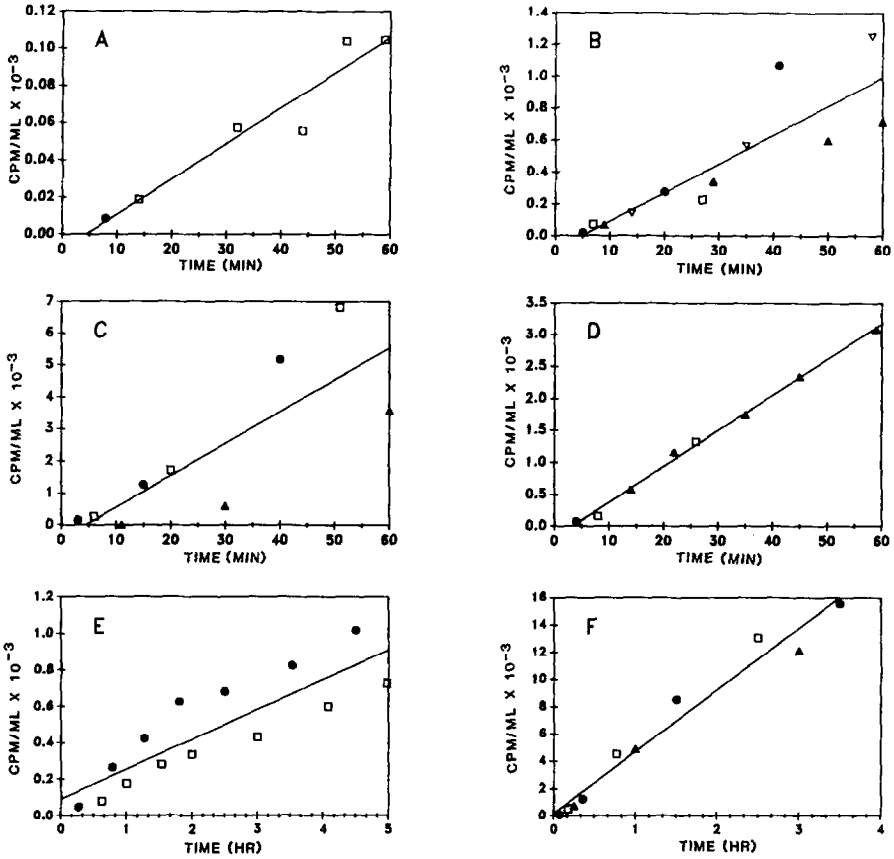


FIG. 2. ^{14}C uptake in arterial blood during exposure of guinea pigs to ^{14}C TDI. Concentrations include, (A), 0.004 ppm; (B), 0.029 ppm; (C), 0.132 ppm; (D), 0.146 ppm for 1 hr. (E, F) Uptake curves for the long-term exposures (exposures 4 and 7, respectively). Samples from individual animals are designated by different symbols. The uptake line represents a linear regression including all points. Regression equations for individual animals and whole graph lines are summarized in Table 4.

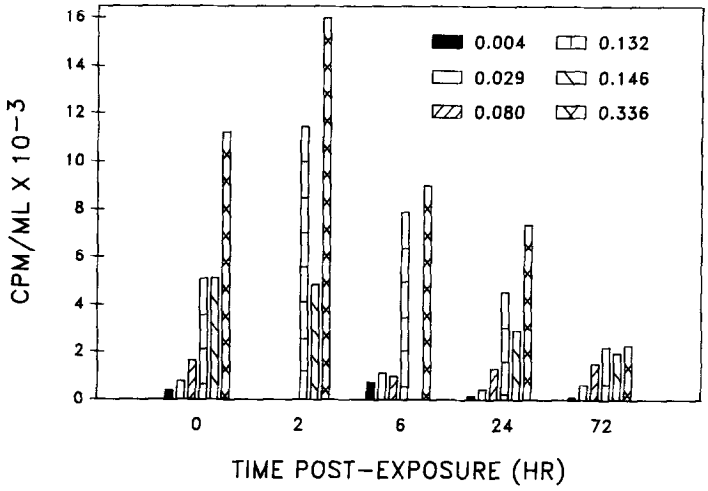


FIG. 3. Clearance of radioactivity from blood following exposure to ^{14}C TDI. 2 hr postexposure samples were obtained from the carotid cannulae for the three highest concentrations. All other timepoints are values derived from terminal blood analysis.

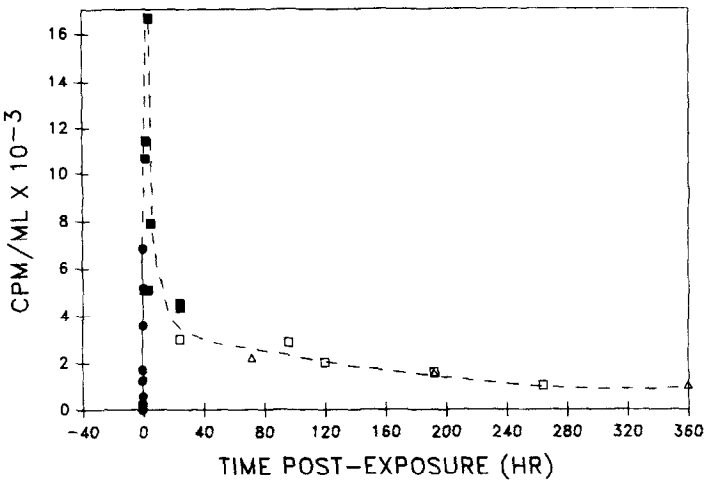


FIG. 4. Uptake and clearance profile of blood radioactivity for the 0.132 ppm, 1 hr exposure. Blood samples were taken from 4 animals and are designated as follows: ●, uptake samples taken from the carotid artery during exposure; △, terminal blood samples collected at the time of euthanasia; ■, post-exposure samples taken from the carotid cannulae; □, post-exposure samples taken from toebleeds.

chea, lung, kidney, liver, heart, and spleen of the 0 hr samples are represented in Fig. 6. As expected for inhalation exposures, the airway tissues show the highest level of radioactivity for all exposures. The 0.00005 ppm exposure did not show detectable levels in any of the tissues measured. After correction for blood content, minimal levels of radioactivity were associated with other tissues, with the kidney demonstrating the most significant level (Fig. 6, inset). Similar to the fluids, the tissues showed a measurable level of radioactivity was maintained in all tissues even after 2 weeks postexposure (Table 5).

DISCUSSION

The fate of reactive vapors *in vivo* and their uptake in blood are phenomena which are not well understood when compared to the wealth of knowledge on the blood uptake of nonreactive, solvent vapors (Fiserova-Bergerova, 1983). We have previously shown that our exposure system and experimental design are effective for working with the highly toxic, reactive vapor, MIC. Isotopic analysis of blood and tissues from such an exposure allowed the direct characterization of uptake and clearance of the radioactive compound (Ferguson *et al.*, 1988). Through comparison of the results from radioactive MIC and TDI exposures, it may be possible to begin to establish relationships between isocyanate structure, uptake, and response.

Exposures to ^{14}C TDI were performed over a range of relatively low concentrations, including levels at and below the TLV for TDI which has been established at 0.005 ppm (Amer. Conf. Gov. Ind. Hyg., 1988). Low concentrations were used to optimize the characterization of the onset of isocyanate response. The Marcali, PNBPA, and radioactivity values yielded comparable exposure concentration results. This confirms that the exposure atmospheres contained reactive TDI at the desired levels, all animals received equivalent concentrations, and chamber atmospheres were maintained throughout the exposure.

Analysis of the uptake and distribution of radioactivity in the TDI-exposed fluids and tissues showed that some form of the labeled compound whether TDI, a conjugate, metabolite, or hydrolysis product, entered and penetrated throughout the entire system, even at the 0.004 ppm level. The urine and bile pro-

TABLE 3

LEVELS OF RADIOACTIVITY IN TERMINAL BODY FLUIDS AS A FUNCTION OF EXPOSURE CONCENTRATION AND TIME OF EUTHANASIA

Exposure no.	^{14}C-TDI exposure (ppm·hr)[a]	Animal no.	Time post-exposure (hr)	Terminal blood (cpm/ml)	Urine (cpm/ml)	Bile (cpm/ml)
1	0.00005	1	0	13	NA[b]	NA
		2	6	0	NA	2
		3	24	0	425	39
		4	72	0	195	16
2	0.004	5	0	195	2,053	91
		6	6	567	886	40
		7	24	115	860	41
		8	72	104	377	27
3	0.029	9	0	741	4,355	465
		10	6	1,088	2,669	65
		11	24	580	1,331	6
		12	72	433	NA	NA
4	0.080 (0.016 × 5 hr)	13	0	1,413	6,365	720
		14[c]	0	811[c]	NA	NA
		15	24	1,283	1,457	265
		16	72	1,389	2,001	176
5	0.132	17	0	5,086	86,835	2027
		18	72	2,202	5,330	376
		19	192	1,555	4,131	365
		20	360	1,055	1,065	27
6	0.146	21[c]	0	2,738[c]	NA	NA
		22	0	4,861	22,224	NA
		23	24	2,851	NA	186
		24	72	1,877	2,624	56
7	0.336 (0.084 × 4 hr)	25	0	11,211	65,850	5766
		26	6	8,986	48,192	3042
		27	24	7,382	31,297	1960
		28[d]	72	2,284	2,555	185

[a] All exposures were for 1 hour except as noted.
[b] NA, not available.
[c] Animal was visibly ill during exposure.
[d] Animal shown to be abnormal upon necropsy.

files demonstrate the rapid penetration of some form of the radioactivity through the system since at all concentrations the highest level of radioactivity in the bile and urine is found immediately following exposure (Table 3). The form of the radioactive compound has not yet been conclusively determined; however, preliminary results from gel filtration chromatography show a change in the distribution of high and low molecular weight radioactive species with time (data not shown). While the total level of radioactivity decreased from the fluids and tissues over a 72 hr period (Tables 3 and 5), exposure 5 illustrated that even after 2 weeks recovery, detectable levels of radioactivity were still present. This is particularly evident in the tissues where direct vapor contact occurred (i.e., trachea and lung) and may be due to the direct, covalent modification of airway macromolecules by reactive isocyanate.

The uptake of ^{14}C into the bloodstream

TABLE 4

LINEAR REGRESSION ANALYSIS FOR ^{14}C UPTAKE IN CAROTID ARTERIAL BLOOD DURING EXPOSURE TO [^{14}C]TDI

Exposure no.	Exposure concentration (ppm·hr)	Animal no.	No. of uptake blood samples	Uptake in blood equation[a]	r value[a]	Composite uptake in blood equation[b]	Composite r value[b]
2	0.004	5	0	—	—	$Y(X) = -8 + 1.9X$	0.96
		6	0	—	—		
		7	5	$Y(X) = -8 + 2X$	0.96		
		8	1	—	—		
3	0.029	9	2	$Y(X)$ 14 + 8X	1.00	$Y(X) = -89 + 18X$	0.87
		10	3	$Y(X) -198 + 30X$	0.98		
		11	4	$Y(X) -39 + 13X$	0.99		
		12	3	$Y(X) -248 + 25X$	0.99		
4	0.080 (0.016 × 5 hr)	13	7	$Y(X)$ 31 + 2.3X	0.99	$Y(X) = 92 + 2.7X$	0.86
		14	0	—			
		15	7	$Y(X)$ 108 + 3.5X	0.97		
		16	0	—	—		
5	0.132	17	0	—	—	$Y(X) = -407 + 98X$	0.82
		18	3	$Y(X) -1146 + 75X$	0.97		
		19	3	$Y(X) -478 + 138X$	0.99		
		20	3	$Y(X) -884 + 149X$	0.99		
6	0.146	21	1	—	—	$Y(X) = -182 + 56X$	0.99
		22	0	—	—		
		23	2	$Y(X) -364 + 65X$	0.99		
		24	5	$Y(X) -135 + 55X$	1.00		
7	0.336 (0.084 × 4 hr)	25	3	$Y(X) -75 + 89X$	0.99	$Y(X) = 179 + 76X$	0.98
		26	3	$Y(X)$ 174 + 67X	0.99		
		27	4	$Y(X)$ 176 + 76X	0.99		
		28	4	—	—		

[a] Linear regression analysis for data from individual animals.
[b] Linear regression analysis for all data at one exposure concentration.

was immediate and increased linearly during exposure over the range of concentrations studied (Fig. 2). The post exposure increase of blood radioactivity (Fig. 4) is presumably due to the processing or desorption of the compound from the sites of entry (i.e., nasal passage, conducting airways, and alveoli) into the bloodstream for clearance. The uptake and clearance blood profiles for labeled MIC were similar (Ferguson *et al.*, 1988).

A linear relationship was observed between the total level of ^{14}C in the blood at the termination of exposure and the concentration multiplied by the duration of exposure (ppm·hr; Fig. 5). This relationship permits the valuable comparison of results for uptake in blood from one study to another. It can only be true that the level for a 1 ppm exposure for 5 hr is the same as a 5 ppm exposure which lasts 1 hr if the fraction retained from each breath is the same and constant as observed over the range of TDI concentrations tested in this study. While it is of interest to note that uptake in arterial blood for TDI followed the $C \times t$ concept, this has previously been shown not to be true for its toxic effects over the same concentration range. For sensory irritation, the response was found to be mainly concentration dependent (Sangha and Alarie, 1979) and this was also true for the sensitization response (Karol, 1983). Through the use of radioactive isocyanates, it has been

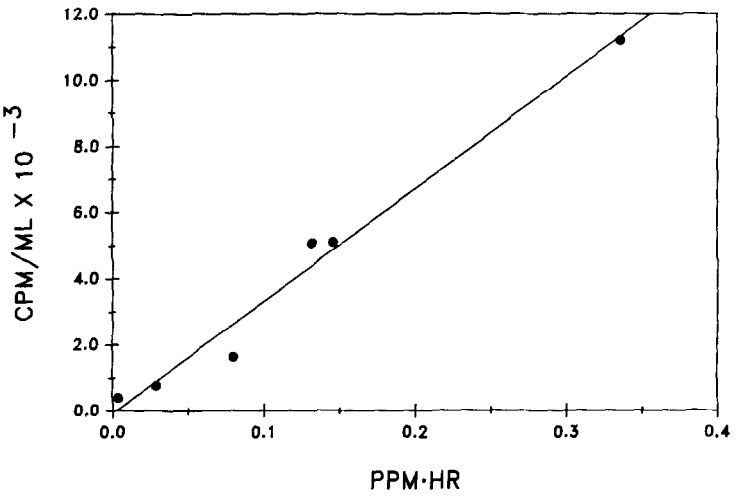


FIG. 5. Relationship between isocyanate concentration (ppm·hr) and level of ^{14}C in terminal blood. Samples taken immediately upon termination of the exposure to ^{14}C TDI (0 hr, Table 3). Data from the lowest exposure category (0.00005 ppm) are not included since the levels of radioactivity are below the detection limits of analysis.

possible to directly detect the compound and thereby observe the $C \times t$ correlation. The difference between this and the results of toxic response studies implies alternative mechanisms.

The linear uptake observed for TDI is the

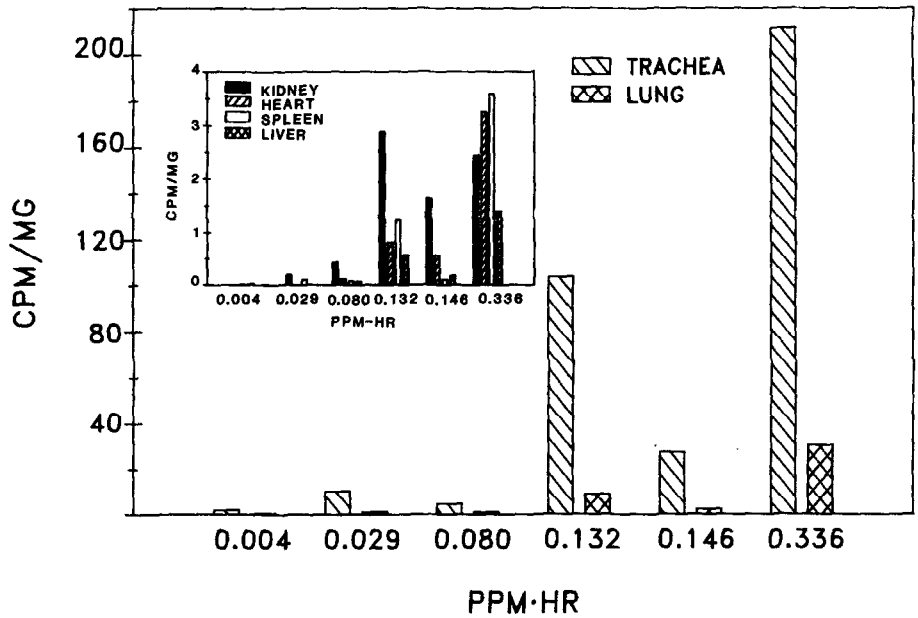


FIG. 6. Tissue distribution of ^{14}C as a function of isocyanate concentration. Level of ^{14}C in trachea and lung immediately following (Table 5, 0 hr) exposure to ^{14}C TDI at indicated concentrations. Inset gives distribution of radioactivity in kidney, heart, spleen, and liver (Table 5). All tissue ^{14}C values were corrected for blood content (see Materials and Methods).

TABLE 5

TISSUE CLEARANCE OF ^{14}C FOLLOWING INHALATION EXPOSURE TO ^{14}C-TDI

Exposure no.	Animal no.	Postexposure time (hr)	CPM/G tissue					
			Trachea	Lung	Kidney	Heart	Spleen	Liver
2	5	0	2,028	570	21	35	—	13
	8	72	349	81	40	—	19	—
3	9	0	10,391	1,232	198	—	102	—
	12	72	1,162	73	335	100	—	—
4	13	0	4,798	1,151	433	109	70	71
	16	72	2,849	1,109	440	242	96	—
5	17	0	103,598	9,058	2880	815	1238	564
	18	72	7,018	1,333	1080	499	70	240
	19	192	19,306	2,660	1078	433	419	151
	20	360	1,875	2,406	226	74	226	134
6	22	0	56,592	8,160	1354	—	697	301
	24	72	7,448	1,346	1301	220	182	176
7	25	0	211,300	30,435	2445	3246	3588	1390
	28	72	9,504	2,454	1377	607	222	384

same as previously reported for the uptake of [^{14}C]MIC (Ferguson, *et al.*, 1988). Since the blood uptake profiles for the two different isocyanates studied are the same, it can be implied that independent of actual mechanism, both isocyanates, in some form, are taken into the bloodstream. Preliminary evidence suggests, however, the form of the radiolabel in the blood following exposure to TDI or MIC is in fact quite different (Kennedy and Brown, 1988). In the case of TDI only one labeled protein was found in the plasma following exposure (Hill *et al.*, 1986), whereas for MIC, a number of labeled proteins were detected as well as radioactivity associated with a low molecular weight substance, presumably methylamine (Kennedy and Brown, 1988). The distinct difference in specificity of *in vivo* targets between the two isocyanates suggests that the chemical structure may play a role in the mechanism of toxicity. However, the uptake in the bloodstream may be independent of the chemical structure and will be tested with other isocyanate compounds.

One of the advantages of using radioactive isocyanates is that it is possible to detect very small quantities of the labeled compound, the tolyl group in this case, and to do so over an extended period of time. After 2 weeks of recovery the animals still had measurable levels of blood radioactivity at a nearly constant amount (8.3×10^{-8} M), regardless of initial dose, which suggests the saturation of a particular target which as a reacted form does not have a rapid turnover rate. Using the specific activity of the labeled MIC, a similar calculation was done for the radioactivity present in the bloodstream after 168 hr. MIC was also present in a nanomolar concentration similar to the value obtained for TDI. The identification of the conjugated molecule(s) is of particular interest in the case of TDI because of its possible role in the stimulation of the documented immune response (Karol, 1980). Characterization of these labeled molecules and other possible *in vivo* targets may further the understanding of the toxic responses to inhaled isocyanates.

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