Metabolism and Distribution of Two ^{14}C-Benzidine-Congener-Based Dyes in Rats as Determined by GC, HPLC, and Radioassays

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Abstract

Absorption, metabolism, and tissue distribution studies were conducted in the rat with ^{14}C-biphenyl ring-labeled Direct Blue 15, a 3,3′-dimethoxybenzidine (DiMxBzd) based azo dye; Direct Red 2, based on 3,3′-dimethylbenzidine (DiMeBzd) and the corresponding benzidine congener amines. Single oral doses of the ^{14}C-labeled dyes (12 mg/kg, 62 μCi/kg) and molar equivalent doses of the respective amines were administered and urine and fecal samples collected at intervals up to 192 hours. Urine specimens were analyzed for ^{14}C content and further characterized by EC/GC for free amines, acetylated metabolites, and conjugates. Feces were assayed for ^{14}C content and for unchanged dosed dyes or amines by HPLC. A comparison of the metabolism of Direct Blue 15 with its base DiMxBzd, indicated that the base was more extensively metabolized and that most of the ^{14}C in various extracts was identified as known metabolites. The metabolism of Direct Red 2 compared with its base, DiMeBzd, indicated that the base was more extensively metabolized, yet only a small percentage of the ^{14}C in extracts was identified as known metabolites. Most of the ^{14}C present in the urine could not be extracted with benzene nor chloroform, indicating high polarity. Distribution studies conducted with both dyes showed that liver, kidney, and lung accumulated and retained higher levels of ^{14}C than other tissues (at 72 hrs). Peak levels of ^{14}C, which occurred 8-12 hours after dosing, were significantly higher with Direct Red 2 than Direct Blue 15. Tissue distribution data (72 hr) for rats dosed with the free amines compared with the dyes showed a generally lower but similar distribution pattern.

Introduction

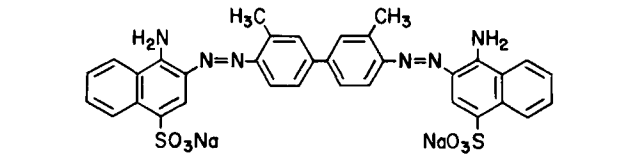
Benzidine- and benzidine-congener-based azo compounds have been widely used in the dye industry for many years. However, benzidine is known to be a human carcinogen (1) and the benzidine-based azo dyes Direct Black 38 (CAS 1937-37-7), Direct Brown 95 (CAS 10300-74-0), and Direct Blue 6 (CAS 2002-46-2) were found to be carcinogenic in rats (2). Furthermore, Direct Black 38 was reported to be converted to benzidine and its metabolites in the hamster (3) and other work has demonstrated that a variety of benzidine-based azo dyes are metabolized to benzidine in the dog (4). The National Institute for Occupational Safety and Health (NIOSH) has therefore evaluated the evidence and issued a special hazard review, which concluded that benzidine-based azo dyes are potential human carcinogens, and that exposure to these dyes should be eliminated (5).

Dyes based on the congeners of benzidine, *o*-tolidine (3,3′-dimethylbenzidine, DiMeBzd) and *o*-dianisidine (3,3′-dimethoxybenzidine, DiMxBzd) possess structures analogous to the benzidine-based dyes and may also be metabolized to the corresponding free amines (4,6,7). Although these congeners are not regulated human carcinogens, they have been shown to be mutagenic (8-10) and to produce tumors in experimental animals (11-18). However, only limited experiments have been conducted to determine whether azo dyes, based on these congeners, are converted to the potentially carcinogenic products in test animals. Lynn *et al.* (4) employed gas chromatography with nitrogen-phosphorus detection (GC/NPD) and gas chromatography/mass spectrometry (GC/MS) to analyze urine from dogs and rats dosed with several DiMeBzd- and DiMxBzd-based azo dyes; the corresponding free amines were detected. Nony *et al.* (19), employing electron-capture/gas chromatography (EC/GC), reported the presence of DiMeBzd and metabolites in the urine of rats and hamsters dosed with Direct Red 2 (DiMeBzd-based); the levels of these metabolites in the rat were about three times more than in the hamster.

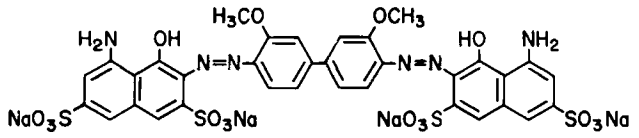
An interagency agreement between NIOSH and the Food and Drug Administration (FDA), with the work to be conducted at the National Center for Toxicological Research (NCTR), was therefore initiated to obtain additional data concerning the absorption, distribution, and metabolism of a DiMeBzd- and DiMxBzd- based dye in rats. Direct Red 2 and Direct Blue 15,

each labeled with ^{14}C, were selected for use in the experiment as representatives of the two classes of dyes; their structures are shown in Figure 1.

In this study, rats were given a single dose (2 mg, 10 μCi) of the dye or the corresponding free amine. The urine was collected at several intervals (up to 192 hr) and assayed for the free amine, its monoacetyl (MoAc) and diacetyl (DiAc) metabolites, and alkaline hydrolyzable conjugates (AHC) by EC/GC, as described by Nony, *et al.* (20). Assays of the MoAc and DiAc metabolites of the congener amines were sought since Morton *et al.* (21) reported that they were present in the metabolism sequence for benzidine. A general scheme for the analytical procedure is presented in Figure 2 (20). Samples of feces collected during the 192-hour study were also analyzed by HPLC to determine the amounts of intact dye or free amines excreted (22). Radiochemical (RC) assays were performed on the same solutions assayed by EC/GC, the discarded fractions, and feces collected at corresponding intervals. Tissues, organs, fluids and excreta from animals sacrificed at various intervals after receiving a single dose of the labeled dye or free amine were assayed radiochemically to determine the extent of absorption and distribution of the ^{14}C.



C.I. Direct Red 2



C.I. Direct Blue 15

Figure 1. Structures of Direct Red 2 and Direct Blue 15.

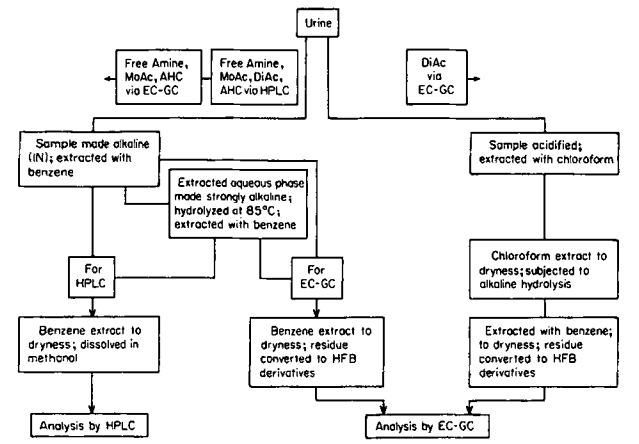


Figure 2. General scheme for extraction, separation, and analysis of benzidine-congener free amines and their monoacetylated (MoAc), diacetylated (DiAc), and conjugated (AHC) products in urine (20).

Experimental

Test Materials

The ^{14}C-Direct Red 2 (CAS 992-59-6) [3,3'-{(3,3'-Dimethyl (1,1'-biphenyl)-4,4'-diyl)*bis*(azo)}*bis*-(4-amino-1-naphthalenesulfonic acid) disodium salt] and ^{14}C-Direct Blue 15, (CAS 2429-74-5) [3,3'-{(3,3'-Dimethoxy(1,1'-biphenyl)-4,4'-diyl)*bis*-(azo)}*bis*-(5-amino-4-hydroxy-2,7-naphthalenedisulfonic acid) tetrasodium salt] each dye was uniformly ring labeled ^{14}C (biphenyl moiety only) with specific activities of 0.176 and 0.180 μCi/μg (*i.e.* 128 and 179 μCi/μmole), respectively, were synthesized by the Dynapol Co. Reduction of the dyes with stannous chloride and subsequent analysis by GC/FID (22) confirmed the presence of the correct benzidine-congener base. Radiometric scans of Direct Red 2 and Direct Blue 15, or their corresponding free amines recovered from reduction of the dyes, developed with several solvent systems on thin layers of silica gel or C_{18} indicated that purities were 99% and 96%, respectively. Aqueous solutions of the ^{14}C-labeled dyes, extracted three times with equal volumes of benzene, yielded 25 and 517 ppm of radiochemical impurities for Direct Red 2 and Direct Blue 15, respectively. Radiometric scans of the extracts developed on thin layers of silica gel indicated that about 150 ppm of the radiochemical impurities from Direct Blue 15 were ^{14}C-DiMxBzd; however, the low level of impurities from Direct Red 2 could not be characterized by the procedure. Based on these assays, and because the ^{14}C-dyes were to be diluted about 33-fold with unlabeled dyes, the ^{14}C-dyes were used as received without further purification. The corresponding ^{14}C-labeled free amines of the Bzd congeners were prepared by reducing an appropriate amount of the ^{14}C-dyes (22).

The unlabeled Direct Blue 15 was obtained from the Atlantic Chemical Corp. via the Dyes Environmental and Toxicology Organization (DETO), and the unlabeled Direct Red 2 was from DETO. Both dyes were purified and analyzed for purity prior to use as described later. The Direct Blue 15 was found to be 65.5% pure and contained 15 ppm of DiMxBzd; the Direct Red 2 was 79.0% pure and contained 0.19 ppm of DiMeBzd.

Aqueous doses of the dyes (1 mL) were prepared to contain 2 mg of dye (based on 100% purity) and 10 μCi of ^{14}C. Therefore, each dose of Direct Blue 15 contained 2.96 mg of the unlabeled dye (65.5% pure) and 0.055 mg of ^{14}C-Direct Blue 15; each dose of Direct Red 2 contained 2.458 mg of the unlabeled dye (79.0% pure) and 0.058 mg of ^{14}C-Direct Red 2.

Doses of the corresponding amine congeners (1 mL) were prepared in aqueous HCl (pH 4) to contain the amount of amine (as the dihydrochloride) available from complete cleavage of the 2-mg dose of dyes and 10 μCi of ^{14}C. Therefore, each dose of DiMxBzd contained 621 μg of unlabeled DiMxBzd·2 HCl and 14 μg of ^{14}C-DiMxBzd; each dose of DiMeBzd contained 765 μg of DiMeBzd·2 HCl (Pfaltz and Bauer, Inc.) and 17 μg of ^{14}C-DiMeBzd.

Animal Experiments

The animals were housed in stainless steel metabolism cages (No. LC-177) from Wahmann Manufacturing Co. An additional 16 mesh stainless steel screen was placed over the animal support screen to retain the smaller fecal pellets and thus separate them from the urine. Urine was collected in a graduated glass tube submerged in solid dry ice and positioned under the drain tube of the cage.

Samples of control rat urine separately spiked with each dye (1 mg/mL) were stored at 5° and 25°C and assayed at various intervals up to 96 hours, to determine whether the compounds were stable under the experimental conditions.

The male Fischer 344 rats, reared at the NCTR, were six weeks old and weighed 150 to 175 g. The rats were kept on a 12-hour light.cycle (6 am to 6 pm) and were allowed free access to water. Control samples of urine and feces were collected during the 36 hours preceding the administration of the single dose (1 mL) of dye or amine by intubation into the stomach. Feed was removed from the cages 12 hours prior to dosing and restored 1 hour after the dose was administered.

Metabolism Tests. Six groups of 4 rats (2 rats/cage) were used in tests with each dye; 5 groups were dosed with the dye and one group, dosed with water, served as a control. Samples of urine and feces from each group (2 cages) were collected for intervals of 0-8, 8-16, 16-24, 24-48, 48-96, 96-144, and 144-192 hours after dosing and stored at −20°C. In tests with the amines, 3 rats which represented one group were used for each amine. The animals were dosed and the urine and feces collected at various intervals and stored in the manner described for dyes.

Distribution Tests. Six groups of 3 rats (1 rat/cage) were dosed with each dye. At intervals of 2, 4, 8, 12, 24, and 72 hours after the dose was administered, three rats were removed, and anesthetized with carbon dioxide; a blood sample was taken from each animal. Each animal was then killed with carbon dioxide and the various tissues, organs, fluids, carcass, and the urine and feces from the cage were separately collected and stored at −20°C unitl analysis. In tests with the amines, 3 rats in separate cages were used for each amine. The animals were removed from the cages 72 hours after the dose was administered, anesthetized, and the various samples were separately collected and stored as described for the dyes.

Preparation of Samples for Analysis

Portions of the urine were taken from the composited samples for extraction and preparation for analysis by EC/GC and for direct analysis of ^{14}C content. The feces from each sampling interval were weighed and ground to a uniform consistency with a mortar and pestle. Weighed portions of the mixture were extracted and prepared for analysis by HPLC, EC/GC or combusted to determine ^{14}C content.

Stomach, small intestine, and large intestine (including the cecum) were partially opened at each end with a razor blade to permit complete extrusion of the contents; the stomach and cecum were also washed with about 3 mL of water after the contents were extruded. The contents and any washings for each tissue were combined and separately analyzed for each animal. The bulk of the urine present in the urinary bladder was removed with a syringe and needle and added to the sample of urine. The organs, tissues, and blood were weighed, then macerated with a razor blade until a homogenous sample was obtained. (Exception: the entire urinary bladder and spleen were separately combusted to determine ^{14}C content.) The carcass was weighed and macerated with 100 mL of water in a Waring blender operated at high speed for 10 minutes. Appropriate amounts (0.5 g or less) of the prepared samples were then combusted to determine ^{14}C content.

Analytical Chemical Methodology

EC/GC Assays. Samples of urine and selected samples of feces from the metabolism tests with dyes and amines were assayed as described by Nony *et al.* (20). Separate portions of the same sample were assayed for the benzidine-congener free amines and their MoAc and AHC metabolites or for the DiAc metabolite as shown in Figure 2. The following is a brief description of the procedure. The benzidine-congener free amine and its MoAc and DiAc metabolites were extracted from urine under alkaline conditions with benzene; the aqueous phase was subjected to alkaline hydrolysis and again extracted with benzene to remove AHC metabolites as the benzidine-congener amine. The two benzene extracts were then subjected to derivatization with heptafluorobutyric anhydride with trimethylamine as a catalyst and analysis by EC/GC. Any DiAc metabolites present in the initial benzene extract were not derivatized or detected. A second sample, made stongly acid with HCl, was extracted with chloroform to remove the DiAc metabolite and thus separate it from any free benzidine-congener or MoAc metabolite. The extracted DiAc metabolite was then hydrolyzed to the free amine, derivatized, and analyzed as described. Assays of several samples of feces from the metabolism studies with dyes were also performed by the EC/GC procedure for urine to determine the levels of benzidine-congener amines and MoAc, DiAc and AHC metabolites excreted via the feces. Minimum detectable levels for the two benzidine-congener free amines and their MoAc and DiAc metabolites in rat urine were at least 12 ppb. Parallel RC assays were performed on each extract as well as discarded fractions and plugs of sodium sulfate used to dry the extracts.

HPLC Assays. The amounts of intact dyes excreted in fecal samples from the metabolism tests were determined as described previously (22). Briefly, the fecal samples from the Direct Blue 15 tests were extracted with dimethylformamide-50% water, cleaned up on a guard column of octadecylsilane (ODS) and analyzed on an ODS column using a mobile phase of 45% acetonitrile: 55% phosphate buffered tetrabutylammonium hydrogen sulfate (0.01 *M*, pH 7.3). Direct Red 2 was assayed in the same manner, except the mobile phase contained 50% acetonitrile. The detector was set at 600 and 500 nm for Direct Blue 15 and Direct Red 2, respectively. The minimum detectable level (based on twice background) for both dyes in feces was about 0.2 ppm.

Fecal samples (0.5 g) from the metabolism tests with amines were analyzed as described previously (20) according to the scheme illustrated in Figure 2. The minimum detectable level (based on twice background) for the amine, MoAc and DiAc metabolites of each dye in feces, was about 0.2 ppm.

RC Assays. The amount of ^{14}C in samples from the metabolism and distribution tests was determined with a Searle Analytic Model 92 liquid scintillation analyzer. Samples of raw urine (100 μL) were analyzed in 20-mL vials containing 15 mL of Aquasol™ (New England Nuclear). Parallel RC assays were also performed on all extracts analyzed by the EC/GC procedure as well as the fractions discarded. The volumes of these solutions added to the Aquasol™ cocktail for analysis ranged from 0.1 to 2.0 mL depending upon the volume and nature of the liquid, and the amount of ^{14}C present. Chloroform extracts which were not compatible with the liquid scintillation procedure were evaporated to dryness and the residue dissolved in benzene prior to analysis; the acidic and basic solutions (1 mL) were adjusted to pH 7 before addition to the cocktail. The liquid scintillation procedure was monitored for chemical and color quench by using the channels ratio technique; however, quench corrections were made by using the internal standard procedure. The efficiency of the RC procedure was about 93%. Recovery

factors used to correct the EC/GC data were also applied to corresponding RC assays.

All fecal samples (0.5 g) were oxidized to carbon dioxide by a Packard Model C306 sample oxidizer. Samples were placed in the furnace burner where oxidation products were quantitatively transferred by the fully automated instrument to a column that was flushed with 7 mL of Carbo-Sorb™ (Packard Instrument Co.), a base-containing absorber fluid that traps the carbon dioxide, and delivered into a scintillation vial along with 10 mL of Permafluor™ (Packard). The vials containing the oxidized samples were then subjected to RC assays by liquid scintillation, which yielded an efficiency of 78% which includes the 99% recovery from sample oxidation.

Samples of tissues, organs, blood, and carcass were assayed exactly as described for feces except that the amounts combusted varied from 0.1 g for fatty samples such as adipose and brain to 0.5 g for muscle, liver, blood and other non-fatty samples.

Purification and Analysis of the Unlabeled Dyes

The unlabeled Direct Blue 15 and Direct Red 2 were purified and analyzed for purity as described previously (22) and for amine impurities as described by Nony *et al.* (20,23). For purification, the dye was mixed with about twice its weight with cold water (3 °C), filtered on a Buchner funnel and washed with three additional portions of cold water to remove water soluble impurities. The residue on the filter was dissolved in water, and extracted four times with benzene to remove impurities of aromatic amines. The aqueous phase was freeze-dried to recover the purified product. Assays for purity were conducted as follows: an aqueous solution of the dye was reduced with 0.2 *M* stannous chloride in 12 *N* HCl at 40 °C for about 3 hours (to complete decolorization), made strongly alkaline and the benzidine-congener free amine was extracted with chloroform and analyzed by GC/FID. The purity of the dye was calculated on the basis of the amount of benzidine-congener amine obtained from a known amount of dye as compared to the amount theoretically obtainable by using the following factors: DiMxBzd × 4.06 = Direct Blue 15; DiMeBzd × 3.41 = Direct Red 2. Amine impurities were extracted from an aqueous solution of the dye with benzene, converted to heptafluorobutyric anhydride (HFB) derivative and assayed by EC/GC.

Results and Discussion

The rats, after being dosed with the dyes or amines, showed no outward signs of shock or discomfort and all animals survived to the end of the experiment. The weight gained by treated rats was similar to that of controls and normal intake of water and feed was observed with all animals. The coloration of urine and feces samples was normal except that fecal pellets from dosed rats were colored red or blue during intervals of peak excretion of the dyes.

In experiments concerning the stability of the dyes in rat urine, both compounds were found to be stable at 5 °C for 96 hours; however, significant amounts of the corresponding free amines were detected after 48 hours at 25 °C. This degradation was probably due to microbial action. Since all samples of urine were collected under dry ice and stored at −20 °C until analysis, any amounts of free amines in the urine, in excess of the small amount dosed as impurities, must result from breakdown in the animals.

Absorption and Excretion

Previous studies conducted at the NCTR (23) and elsewhere (4) on the metabolism of benzidine and benzidine-congener-based azo dyes did not determine the degree of absorption of the dyes. Table I shows excretion profiles for Direct Blue 15 when given as a single oral dose (2 mg, 10 μCi) to each 150-175 g rat (ca. 12 mg/kg and 62 μCi/kg). Most of the dose (*i.e.*, 74.4% as ^{14}C) was excreted in the feces with about 18.8% found in the urine. Peak fecal and urinary excretion of ^{14}C occurred during the 8-16 hour interval, although detectable amounts of radioactivity were still being excreted 144-192 hours after dosing.

HPLC assays of the feces showed that about 12% of the dose was excreted as the unchanged dye. The period of peak excretion was 8-16 hours, with no intact dye detected after 48 hours. The percentage of dose in the feces as ^{14}C (74.4%) compared with the percentage of intact dye (12%) indicated that 84% of the fecal radioactivity was unidentified metabolic products, possibly from bile or some form of azo reduction. The high levels of radioactivity found in the urine (18.8%) indicated extensive metabolism.

Comparable excretion profiles from Direct Red 2 dosed in a manner identical to Direct Blue 15 are presented in Table II.

Table I. Excretion Profiles of ^{14}C-Direct Blue 15 as Determined by RC or HPLC*

Sampling Interval (hr)	Urine, Feces and Dose Excreted**				
	Urine Excreted (mL)	% Dose in Urine	Feces Excreted (g)	% Dose in Feces	% Dose as Intact Dye in Feces (HPLC)
Pretreatment (36)	33. ± 5.	<0.002 ± 0.001	3.36 ± 1.46	0.0002 ± 0.0000	<0.004 ± 0.002
0 - 8	4.0 ± 0.7	2.22 ± 0.26	2.73 ± 0.78	2.10 ± 1.30	0.871 ± 0.589
8 - 16	8.8 ± 2.0	6.59 ± 0.78	4.70 ± 0.46	43.9 ± 6.5	9.91 ± 2.11
16 - 24	8.4 ± 1.3	4.34 ± 0.39	4.23 ± 0.64	15.0 ± 2.4	1.02 ± 0.15
24 - 48	31. ± 5.	4.40 ± 0.64	9.58 ± 1.45	7.70 ± 4.34	0.235 ± 0.104
48 - 96	76. ± 10.	1.08 ± 0.15	30.8 ± 4.0	5.21 ± 2.14	Nil
94 - 144	80. ± 5.	0.103 ± 0.007	31.2 ± 4.2	0.348 ± 0.066	Nil
144 - 192	83. ± 8.	0.055 ± 0.008	34.6 ± 1.5	0.141 ± 0.028	ND
Total	–	18.79	–	74.40	12.04

*Results for post-treatment samples are corrected for background of pretreatment samples.

**Mean and standard deviation of 5 groups of 4 rats each. Nil denotes results that do not exceed background; ND indicates that the assay was not determined.

Most of the dose (*i.e.*, 73.5% as ^{14}C) was excreted in the feces with about 20.6% found in the urine. Peak fecal and urinary excretion intervals were the same as those of Direct Blue 15. HPLC assays of the feces showed 10.1% of the dose excreted as unchanged dye. The excretion profiles of the dye in feces were identical to those of Direct Blue 15, with more than 86% of the ^{14}C excreted in the feces as unidentified metabolites. A comparison of the data in Tables I and II illustrate that excretion profiles of the two dyes are essentially the same.

Attempts were made to determine the levels of free amines and their acetylated metabolites via EC/GC in feces from rats dosed with both dyes. Samples of the 8-16 hour interval from rats dosed with Direct Blue 15 or Direct Red 2 were found to contain about 2.5 and 13 ppm of the free amines, respectively. Further assays were abandoned because of low recoveries and the presence of excessive amounts of interfering substances in the extracts caused a rapid deterioration of the GC system.

Since the two dyes were reduced to their respective benzidine congener bases, excretion profiles with molar equivalent doses of DiMxBzd and DiMeBzd were determined in rats (Table III) for comparison with data presented for the dyes in Tables I and II. Data in Table III indicate that about 52-59% of the doses were excreted in the feces as ^{14}C and about 35-40% in the urine from rats dosed with the two benzidine-congener bases. Although the intervals for peak urinary and fecal excretion of ^{14}C were identical to those for the dyes, fecal excretion of only 1-6% of the dose was observed for the free amines including acetylated metabolites; whereas, 10-12% of the dosed dyes were excreted as intact compounds. Unidentified metabolites comprised about 97-89% of the ^{14}C excreted via the feces from rats dosed with DiMxBzd and DiMeBzd. These data indicate that biliary metabolites, unextractable by the described procedures, were probably excreted via the feces (24). The free benzidine-congener amines showed significantly more metabolism than the dyes (19-21%) since higher levels of the amines were excreted in the urine (35-40%). The amines also showed significantly more urinary excretion than the dyes since less fecal excretion of ^{14}C occurred with the amines (52-59% of the dose) than with the dyes (73-74%).

Metabolism

Studies recently completed at the NCTR with nine benzidine-congener dyes consistently showed metabolic breakdown to free

Table II. Excretion Profiles of ^{14}C-Direct Red 2 as Determined by RC or HPLC*

Sampling Interval (hr)	Urine, Feces and Dose Excreted**				
	Urine Excreted (mL)	% Dose in Urine	Feces Excreted (g)	% Dose in Feces	% Dose as Intact Dye in Feces (HPLC)
Pretreatment (36)	44. ± 4.	<0.001 ± 0.002	4.15 ± 1.70	0.0002 ± 0.0000	<0.005 ± 0.002
0 - 8	4.4 ± 1.6	0.98 ± 0.46	4.00 ± 0.78	1.82 ± 2.04	0.519 ± 0.594
8 - 16	10.3 ± 1.2	8.29 ± 1.06	5.53 ± 0.51	34.7 ± 8.7	8.26 ± 2.76
16 - 24	12.6 ± 0.6	6.38 ± 1.51	5.45 ± 0.77	17.6 ± 3.5	1.10 ± 0.40
24 - 48	42. ± 7.	3.52 ± 0.84	13.5 ± 2.2	12.4 ± 4.1	0.244 ± 0.132
48 - 96	104. ± 30.	1.32 ± 0.29	34.7 ± 2.7	6.06 ± 1.70	Nil
96 - 144	119. ± 41.	0.110 ± 0.029	33.1 ± 4.0	0.650 ± 0.165	Nil
144 - 192	107. ± 35.	0.050 ± 0.018	32.5 ± 3.4	0.284 ± 0.080	ND
Total	–	20.65	–	73.51	10.12

*Results for post-treatment samples are corrected for background of pretreatment samples.
**Mean and standard deviation of 5 groups of 4 rats each. Nil denotes results that do not exceed background; ND indicates that the assay was not determined.

Table III. Excretion Profiles of ^{14}C-Dimethoxybenzidine or ^{14}C-Dimethylbenzidine by RC or HPLC*

Sampling Interval (hr)	Urine, Feces and Dose Excreted**									
	Dimethoxybenzidine					Dimethylbenzidine				
	Urine Excreted (mL)	% Dose in Urine	Feces Excreted (g)	% Dose in Feces	% Dose in Feces as DiAc Metabolites†	Urine Excreted (mL)	% Dose in Urine	Feces Excreted (g)	% Dose in Feces	% Dose in Feces as DiAc Metabolites†
Pretreatment (36)	37.	<0.03	8.92	<0.003	<0.012	58.	<0.005	1.58	<0.001	<0.002
0 - 8	12.	17.5	5.13	9.69	0.69	8.	11.1	4.62	8.51	2.60
8 - 16	28.	10.7	5.76	23.6	0.70	21.	17.3	5.57	26.9	3.37
16 - 24	17.	2.92	5.42	9.89	0.07	19.	4.43	2.81	8.38	0.25
24 - 48	55.	3.14	11.9	5.67	Nil	70.	4.62	10.9	9.60	Nil
48 - 96	102.	0.84	23.1	2.40	Nil	123.	1.89	21.6	4.04	Nil
96 - 144	80.	0.12	24.5	0.52	ND	89.	0.20	15.8	0.77	ND
144 - 192	109.	0.09	24.9	0.28	ND	132.	0.12	18.0	0.44	ND
Total	–	35.31	–	52.05	1.46	–	39.66	–	58.64	6.22

*Results for post-treatment samples are corrected for background of pre-treatment samples.
**Samples are composites from 3 rats. Nil denotes results that do not exceed background; ND indicates that the assay was not determined.
†Determined by HPLC: the main constituent was the amine and acetylated metabolite.

amines and their acetylated products in rats (25). The experiments described in this paper were performed concurrently with ^{14}C-labeled dyes to gain additional information on the metabolism of these classes of compounds.

Urinary excretion profiles of DiMxBzd, MoAcDiMxBzd, and AHC from rats given a single oral dose (2 mg μCi) of ^{14}C-Direct Blue 15 are shown in Table IV. Excretion profiles of the DiAcDiMxBzd metabolites obtained from assays of separate aliquots of the urine samples are presented in Table V. Results reported in Tables IV and V are from parallel GC and RC assays of fractions outlined in Figure 2. Metabolites assayed via GC were: AHC (0.48% of the dose) MoAcDiMxBzd (0.27%), free DiMxBzd (0.22%) and DiAcDiMxBzd (0.22%) (Table IV). Peak excretion occurred during the 8-16 hour interval, with no metabolites detected after 25 hours by GC. The free amine fraction assayed via RC acounted for 0.68% of the dose; however, 90% of the DiAcDiMxBzd (Table V) was also present in the free amine fraction and detected by RC but not by GC. Therefore, after corrections for this discrepancy between the two methods, essentially all of the ^{14}C in the free amine fraction was ascribed to known metabolites as determined by GC analysis. Also, all of the ^{14}C in the AHC fraction was attributed to DiMxBzd by GC analysis. However, it should be noted that 94% of the total ^{14}C found in raw urine was not extracted by benzene at alkaline pH (Table IV). In Table V, all of the ^{14}C in the raw urine remained unextractable by chloroform. The nature of these unextractable water soluble metabolites is not known.

Urinary excretion profiles of DiMeBzd, MoAcDiMeBzd, AHC, and DiAcDiMeBzd from rats given a single oral dose (2 mg μCi) of ^{14}C-Direct Red 2 determined as described for Direct Blue 15 are presented in Tables V and VI. The metabolites assayed via GC were: DiAcDiMeBzd (0.21% of the dose), DiMeBzd (0.15%), AHC (0.12%) and MoAcDiMeBzd (0.08%) (Table VI). The interval of peak excretion was 8-16 hours, and no metabolites were detected by GC after 48 hours. The free amine fraction assayed by RC represented 1.8% of the dose and, after correction for the DiAcDiMeBzd present, only 13.2%

Table IV. Excretion Profiles of DiMxBzd, MoAcDiMxBzd and AHC (as DiMxBzd) of ^{14}C-Direct Blue 15 by (GC) and (RC) Assays*

Sampling Interval (hr)	μg-Equivalents of Dye Indicated**					
	Free Amine Fraction†			AHC Fraction†		Unextractable Metabolites†
	DiMxBzd (GC)	MoAcDiMxBzd (GC)	Total (RC)	DiMxBzd (GC)	Total (RC)	(RC)
Pretreatment (36)	<0.71 ± 0.07	<0.12 ± 0.06	<0.01 ± 0.01	<0.22 ± 0.04	<0.02 ± 0.03	<0.01 ± 0.01
0 - 8	2.59 ± 0.78	7.19 ± 3.54	12.3 ± 2.3	12.8 ± 2.1	11.7 ± 2.1	153. ± 18.
8 - 16	13.8 ± 5.6	10.9 ± 2.9	32.7 ± 9.0	21.3 ± 5.6	20.6 ± 4.4	473. ± 56.
16 - 24	1.39 ± 0.51	3.52 ± 1.19	7.43 ± 1.11	3.99 ± 0.36	4.42 ± 1.07	335. ± 30.
24 - 48	Nil	Nil	1.56 ± 0.27	Nil	1.35 ± 0.39	349. ± 51.
48 - 96	Nil	Nil	0.459 ± 0.125	Nil	0.329 ± 0.087	85. ± 12.
96 - 144	Nil	Nil	0.103 ± 0.056	Nil	0.144 ± 0.091	7.60 ± 0.52
144 - 192	Nil	Nil	0.064 ± 0.038	Nil	0.108 ± 0.043	4.36 ± 0.64
% ^{14}C in Raw Urine	1.26	1.53	3.88	2.70	2.74	93.63
% Dose	0.22	0.27	0.68	0.48	0.48	17.59

*Results for post-treatment samples are corrected for background of pretreatment samples.
**Mean and standard deviation from 5 groups of 4 rats each. Nil denotes results that do not exceed background. ND denotes assays not determined.
†Extracted with benzene under strongly alkaline conditions.

Table V. Excretion Profiles of DiAcDiMxBzd and DiAcDiMeBzd in Urine by GC and RC Assays*

Sampling Interval (hr)	μg-Equivalents of Dye Indicated**					
	Direct Blue 15†			Direct Red 2†		
	DiAcDiMxBzd (GC)	Total (RC)	Unextractable Metabolites (RC)	DiAcDiMeBzd (GC)	Total (RC)	Unextractable Metabolites (RC)
Pretreatment (36)	<0.07 ± 0.10	<0.02 ± 0.10	<0.01 ± 0.01	<0.17 ± 0.03	<0.02 ± 0.01	<0.01 ± 0.01
0 - 8	4.09 ± 0.87	4.23 ± 0.67	168. ± 20.	0.848 ± 0.254	2.68 ± 1.17	68. ± 31.
8 - 16	11.3 ± 2.8	11.4 ± 2.5	503. ± 59.	9.49 ± 1.65	55.1 ± 13.6	476. ± 61.
16 - 24	2.34 ± 1.14	2.81 ± 0.64	343. ± 31.	6.53n ± 1.27	32.9 ± 17.5	381. ± 90.
24 - 48	Nil	0.689 ± 0.211	351. ± 51.	Nil	7.99 ± 3.23	265. ± 62.
48 - 96	Nil	0.232 ± 0.072	86. ± 12.	Nil	1.94 ± 0.85	90. ± 20.
96 - 144	Nil	0.176 ± 0.361	8.22 ± 0.56	Nil	0.217 ± 0.111	7.27 ± 1.93
144 - 192	Nil	0.087 ± 0.047	4.42 ± 0.65	ND	ND	3.96 ± 1.41
% ^{14}C in Raw Urine	1.18	1.31	97.4	1.02	6.10	78.2
% Dose	0.22	0.24	18.3	0.21	1.26	16.1

*Results for post-treatment samples are corrected for background of pretreatment samples.
**Mean and standard deviation from 5 groups of 4 rats. Nil denotes results that do not exceed background. ND denotes assays not determined.
†Extracted with chloroform under strongly acid conditions.

of the ^{14}C compounds in the fraction responded to GC analysis. The AHC fraction (RC) contained 1.7% of the dose; however, GC assays accounted for only 7.4% of the ^{14}C metabolites. Excretion profiles for DiAcDiMeBzd in Table V indicate that 16.7% of the ^{14}C compounds in that fraction were detected by GC. Therefore, GC assays of the specified metabolites of Direct Red 2 account for only a small percentage of the ^{14}C present in the various fractions. Excessive amounts of unidentified ^{14}C compounds in the fractions from Direct Red 2 was in sharp contrast to the total accountability found with Direct Blue 15. However, most of the ^{14}C in raw urine from both dyes remained unextracted with benzene or chloroform.

Results from metabolism of the two benzidine-congener amines in rats were compared with those from their respective dyes by administering molar equivalent doses of the free amines. Urinary excretion profiles of DiMxBzd, MoAcDiMxBzd, AHC, and DiAcDiMxBzd from rats dosed with DiMxBzd are shown in Tables VII and VIII. The metabolites determined by GC were: AHC (1.56% of dose), DiMxBzd (1.18%), DiAcDiMxBzd (0.93%), and MoAcDiMxBzd (0.35%). The peak excretion intervals and the duration of excretion for each metabolite detected by GC showed some variation, but generally peaked earlier and was eliminated sooner than the same metabolites from rats dosed with Direct Blue 15. GC analysis accounted for all of the ^{14}C metabolites in the free amine fraction from rats dosed with Direct Blue 15, while 78% of the AHC (RC) could be attributed to known metabolites by GC. However, in contrast to Direct Blue 15, only 35% of the ^{14}C products in the DiAcDiMxBzd fraction responded to GC analysis. Most of the ^{14}C in raw urine was not extracted with benzene or chloroform as observed with dosed dyes.

A comparison of the metabolism of Direct Blue 15 with its base (DiMxBzd) shows: A) the base was more extensively metabolized and excreted; AHC was the major product, B) the dye was metabolized more slowly producing DiAcDiMxBzd as its major product, C) GC assays accounted for all of the ^{14}C products in the free amines and AHC fractions from both the base and the dye, and D) while GC accounted for all of the

Table VI. Excretion Profiles of DiMeBzd, MoAcDiMeBzd and AHC (as DiMeBzd) of ^{14}C-Direct Red 2 by GC and RC Assays*

Sampling Interval (hr)	μg-Equivalents of Dye Indicated**					
	Free Amine Fraction			AHC Fraction		Unextractable Metabolites†
	DiMeBzd (GC)	MoAcDiMeBzd (GC)	Total (RC)	DiMeBzd (GC)	Total (RC)	(RC)
Pretreatment (36)	<3.13 ± 0.5	<0.87 ± 0.27	<0.01 ± 0.01	<0.41 ± 0.13	<0.02 ± 0.03	<0.10 ± 0.10
0 - 8	1.33 ± 0.62	0.71 ± 0.32	6.45 ± 3.03	1.31 ± 0.49	5.98 ± 2.69	66. ± 32.
8 - 16	5.16 ± 1.01	4.01 ± 0.73	77.0 ± 11.1	6.34 ± 0.22	5.88 ± 12.9	509. ± 65.
16 - 24	2.09 ± 0.47	2.00 ± 0.33	46.3 ± 18.6	2.31 ± 1.00	47.2 ± 15.9	399. ± 95.
24 - 48	3.13 ± 0.56	Nil	13.8 ± 3.3	Nil	15.2 ± 4.4	251. ± 60.
48 - 96	Nil	Nil	3.33 ± 0.83	Nil	5.75 ± 2.32	96. ± 21.
96 - 144	Nil	Nil	0.317 ± 0.106	Nil	0.602 ± 0.294	7.9 ± 2.1
144 - 192	ND	ND	ND	ND	ND	–
% ^{14}C in Raw Urine	0.71	0.41	9.48	0.60	8.08	80.4
% Dose	0.15	0.08	1.84	0.12	1.67	16.6

*Results for post-treatment samples are corrected for background of pretreatment samples.
**Mean and standard deviation from five groups of 4 rats each. Nil denotes results that do not exceed background. ND denotes assays not determined.
†Extracted with benzene under strongly alkaline conditions.

Table VII. Excretion Profiles of DiMxBzd, MoAcDiMxBzd and AHC (as DiMxBzd) of ^{14}C-Direct Blue 15 by (GC) or (RC) Assays*

Sampling Interval (hr)	μg-Equivalents of Compound Indicated**					
	Free Amine Fraction†			AHC Fraction †		Unextractable Metabolites†
	DiMxBzd (GC)	MoAcDiMxBzd (GC)	Total (RC)	DiMxBzd (GC)	Total (RC)	(RC)
Pretreatment (36)	<0.09	<0.12	<0.01	<0.13	<0.02	<0.01
0 - 8	16.8	4.71	28.0	19.2	22.5	209.
8 - 16	0.644	0.406	2.75	2.32	3.45	151.
16 - 24	Nil	Nil	0.374	0.544	0.922	42.6
24 - 48	Nil	Nil	0.495	0.990	2.59	42.3
48 - 96	Nil	Nil	Nil	Nil	Nil	12.4
96 - 144	Nil	Nil	Nil	Nil	Nil	1.75
144 - 192	Nil	Nil	Nil	Nil	Nil	1.30
% ^{14}C in Raw Urine	3.34	0.98	6.06	4.42	5.65	88.22
% Dose	1.18	0.35	2.14	1.56	2.00	31.19

*Results for post-treatment samples are corrected for background of pretreatment samples.
**Samples are composites from 3 rats.
†Extracted with benzene under strongly alkaline conditions.

^{14}C products in the DiAcDiMxBzd fraction from the dye, only 35% of the radioactivity from the base could be attributed to DiAcDiMxBzd in that fraction.

Urinary excretion profiles of DiMeBzd, DiAcDiMeBzd, AHC, and MoAcDiMeBzd from rats dosed with DiMeBzd are shown in Tables VIII and IX. The metabolites determined by GC were: DiAcDiMeBzd (2.1% of the dose), AHC (0.4%), DiMeBzd (0.4%) and MoAcDiMeBzd (0.2%) with peak excretion intervals similar to DiMxBzd. Only 13.9% of the ^{14}C products in the free amine fraction, 11.0% in the AHC fraction and 34.4% in the DiAcDiMeBzd fraction were attributed to known metabolites by GC. These results were similar to those observed with the parent dye. Again, the bulk of the ^{14}C in raw urine was not extracted with the two solvents employed.

A comparison of the metabolism of Direct Red 2 with its base (DiMeBzd) shows: A) the base was more extensively metabolized and excreted; DiAcDiMeBzd was the major metabolite for both the dye and base, and B) only a small percentage of the ^{14}C in the extracted fractions from both base and dye responded to GC assays for specific metabolites.

The metabolism patterns for the dyes correlated well with their corresopnding bases. As expected, the bases were metabolized more extensively than the dyes.

Preliminary results recently reported by Ilias *et al.* (26) on the metabolic fate of ^{14}C-benzidine in the rat showed that the major portions of the radioactivity appeared in the feces, with about 20% found in the urine. In bile exteriorized animals, up to 70% of the dose was eliminated via the bile. A variety of N-acetylated and N-glucuronide metabolites were reported. The results are consistent with those reported for DiMxBzd and DiMeBzd in the present study.

Tissue Distribution

Results from tissue distribution tests with the two dyes deter-

Table VIII. Excretion Profiles of DiAcDiMxBzd of ^{14}C-DiMxBzd and DiAcMeBzd of ^{14}C-DiMeBzd by GC and RC Assays*

	μg-Equivalents of Compound Indicated**					
	Dimethoxybenzidine†			Dimethylbenzidine†		
	DiAcDiMxBzd Fraction			DiAcDiMeBzd Fraction		
Sampling Interval (hr)	DiAcDiMxBzd (GC)	Total (RC)	Unextractable Metabolites (RC)	DiAcDiMeBzd (GC)	Total (RC)	Unextractable Metabolites (RC)
Pretreatment (36)	<0.06	<0.02	<0.01	<0.15	<0.02	<0.01
0 - 8	2.59	7.80	250.	6.30	32.3	116.
8 - 16	10.9	30.2	129.	10.6	53.4	157.
16 - 24	0.164	1.07	41.7	0.385	8.90	49.8
24 - 48	Nil	0.478	43.3	2.26	10.8	57.5
48 - 96	Nil	Nil	12.4	Nil	1.48	25.3
96 - 144	Nil	Nil	1.75	Nil	Nil	3.56
144 - 192	Nil	Nil	1.30	Nil	Nil	2.11
% ^{14}C in Raw Urine	2.62	7.58	91.88	5.28	15.31	58.92
% Dose	0.93	2.68	32.48	2.09	6.08	23.29

*Results for post-treatment samples are corrected for background of pretreatment samples.
**Samples are composites from 3 rats.
†Extracted with chloroform under strongly acid conditions.

Table IX. Excretion Profiles of DiMeBzd, MoAcDiMeBzd and AHC (as DiMeBzd) of ^{14}C-Dimethylbenzidine by GC or RC Assays*

	μg-Equivalents of Compound Indicated**					
	Free Amine Fraction†			AHC Fraction†		
Sampling Interval (hr)	DiMeBzd (GC)	MoAcDiMeBzd (GC)	Total (RC)	DiMeBzd (GC)	Total (RC)	Unextractable Metabolites† (RC)
Pretreatment (36)	<0.99	<0.95	<0.01	<0.12	<0.02	<0.01
0 - 8	4.14	1.52	41.5	3.94	21.1	124.
8 - 16	0.792	1.44	43.2	3.07	34.5	113.
16 - 24	0.961	Nil	6.16	0.414	6.06	219.
24 - 48	0.192	Nil	8.76	0.430	7.13	62.5
48 - 96	Nil	Nil	1.84	Nil	2.92	26.9
96 - 144	Nil	Nil	Nil	Nil	Nil	3.56
144 - 192	Nil	Nil	Nil	Nil	Nil	2.11
% ^{14}C in Raw Urine	0.87	0.42	14.54	1.13	10.27	78.95
% Dose	0.35	0.17	5.77	0.45	4.08	31.35

*Results for post-treatment samples are corrected for background of pretreatment samples.
**Samples are composites from 3 rats.
†Extracted with benzene under strongly alkaline conditions.

mined at six intervals (up to 72 hr) by assays of twenty different tissues, fluids, organs, and contents are presented in Tables X and XI. Additionally, similar test results with the corresponding amine bases determined at 72 hours are shown in Table VII.

Tissue distribution data for rats dosed with Direct Blue 15 are shown in Table X and, as expected, the oral dose was easily traced from the stomach contents through the GI tract. At 2 hours, in addition to expected accumulation in the GI tract, significant ^{14}C was found in urinary bladder, liver, kidney, carcass, and lung. At 8 hours, all tissues showed peak ^{14}C content except brain, heart, lung, and small intestine which all peaked at 4 hours. Excluding the GI tract, the highest content of ^{14}C was in liver, kidney, lung, and carcass. At 72 hours, liver kidney, lung, and carcass contained highest levels of ^{14}C. These results are similar to those cited for benzidine distribution in tissues (24).

Tissue distribution data for rats dosed with Direct Red 2 are shown in Table XI. High concentrations of ^{14}C were observed in the GI tract as with Direct Blue 15. At 2 hours, in addition to the GI tract, significant accumulations were found in liver, lung, kidney, and carcass. At 12 hours, most tissues showed peak ^{14}C accumulation with liver, lung, and kidney containing the highest levels. At 72 hours, highest accumulations were in liver, urinary bladder, kidney, lung, and carcass. The peak levels of ^{14}C in rats dosed with Direct Red 2 were consistently higher than Direct Blue 15.

Tissue distribution data determined at 72 hours from rats dosed with DiMxBzd and DiMeBzd are shown in Table XII. The

Table X. Distribution of ^{14}C-Direct Blue 15 in Tissues at Several Intervals*

	μg-Equivalents of ^{14}C-DiMxBzd in Tissue at Sampling Interval Indicated					
Tissue	**2 hr**	**4 hr**	**8 hr**	**12 hr**	**24 hr**	**72 hr**
Adipose	0.043 ± 0.025	0.130 ± 0.072	0.164 ± 0.139	0.103 ± 0.046	0.058 ± 0.030	0.021 ± 0.001
Blood	0.126 ± 0.077	0.200 ± 0.081	0.291 ± 0.153	0.149 ± 0.022	0.121 ± 0.060	0.041 ± 0.010
Brain	0.024 ± 0.005	0.075 ± 0.038	0.046 ± 0.017	0.030 ± 0.011	0.019 ± 0.007	0.003 ± 0.004
Carcass	0.333 ± 0.247	0.515 ± 0.309	1.03 ± 0.84	0.421 ± 0.496	0.206 ± 0.046	0.039 ± 0.012
Feces	0.016 ± 0.023	0.870 ± 1.42	90.4 ± 79.3	565. ± 391.	452. ± 182.	144. ± 29.
Heart	0.115 ± 0.072	0.241 ± 0.105	0.219 ± 0.100	0.159 ± 0.033	0.120 ± 0.041	0.060 ± 0.016
Kidney	0.452 ± 0.297	0.777 ± 0.235	1.14 ± 0.53	0.919 ± 0.210	0.860 ± 0.352	0.395 ± 0.173
Large Intestine	5.10 ± 4.06	14.9 ± 11.3	15.0 ± 3.3	12.2 ± 2.1	9.66 ± 6.23	0.244 ± 0.199
and Contents	57.3 ± 44.9	838. ± 501.	668. ± 249.	271. ± 92.	378. ± 326.	0.713 ± 0.905
Liver	0.867 ± 0.574	3.52 ± 2.05	4.06 ± 1.67	2.99 ± 0.33	3.14 ± 1.74	1.14 ± 0.37
Lung	0.232 ± 0.102	0.864 ± 0.543	0.672 ± 0.377	0.698 ± 0.239	0.366 ± 0.196	0.043 ± 0.004
Muscle	0.040 ± 0.018	0.330 ± 0.163	0.437 ± 0.291	0.279 ± 0.096	0.047 ± 0.014	0.016 ± 0.003
Spleen	0.156 ± 0.130	0.295 ± 0.093	0.362 ± 0.299	0.227 ± 0.028	0.124 ± 0.019	0.016 ± 0.024
Small Intestine	2.13 ± 0.54	7.14 ± 3.58	5.51 ± 2.42	3.76 ± 0.50	3.78 ± 2.48	0.106 ± 0.044
and Contents	826. ± 546.	398. ± 196.	146. ± 96.	70.6 ± 9.5	44.3 ± 22.9	0.714 ± 0.486
Urinary Bladder	1.59 ± 0.31	4.04 ± 2.41	9.62 ± 7.88	3.99 ± 0.71	8.10 ± 6.70	0.404 ± 0.471
Stomach	22.3 ± 6.4	5.37 ± 4.26	0.588 ± 0.089	0.298 ± 0.047	0.193 ± 0.031	0.053 ± 0.027
and Contents	149. ± 42.	54.7 ± 74.6	1.34 ± 1.87	0.264 ± 0.267	0.790 ± 0.750	0.028 ± 0.021
Testes	0.033 ± 0.022	0.116 ± 0.062	0.136 ± 0.071	0.031 ± 0.041	0.050 ± 0.018	0.022 ± 0.006
Urine	2.8 ± 0.15	9.0 ± 4.75	26.7 ± 16.4	24.6 ± 7.0	26.3 ± 10.3	17.0 ± 12.0

*Mean and standard deviation from 3 rats.

Table XI. Distribution of ^{14}C-Direct Red 2 in Tissues at Several Intervals*

	μg-Equivalents of ^{14}C-DiMeBzd in Tissue at Sampling Interval Indicated					
Tissue	**2 hr**	**4 hr**	**8 hr**	**12 hr**	**24 hr**	**72 hr**
Adipose	0.157 ± 0.016	0.112 ± 0.010	0.661 ± 0.106	1.43 ± 0.54	0.122 ± 0.036	0.048 ± 0.025
Blood	0.121 ± 0.063	0.284 ± 0.090	2.01 ± 0.36	4.50 ± 1.44	0.437 ± 0.154	0.108 ± 0.053
Brain	0.045 ± 0.023	0.066 ± 0.033	0.688 ± 0.147	1.40 ± 0.40	0.067 ± 0.033	<0.002 ± 0.001
Carcass	0.136 ± 0.013	0.444 ± 0.415	1.34 ± 0.37	3.02 ± 0.90	0.388 ± 0.027	0.104 ± 0.051
Feces	0.006 ± 0.008	0.048 ± 0.046	0.581 ± 0.540	82.2 ± 140.	408. ± 15.	169. ± 35.
Heart	0.143 ± 0.057	0.264 ± 0.092	3.96 ± 2.07	5.00 ± 0.96	0.386 ± 0.178	0.066 ± 0.032
Kidney	0.231 ± 0.087	0.563 ± 0.128	2.91 ± 0.59	5.39 ± 1.29	1.08 ± 0.24	0.459 ± 0.160
Large Intestine	13.6 ± 4.8	12.3 ± 6.2	32.2 ± 9.0	24.2 ± 6.2	8.30 ± 1.80	0.462 ± 0.394
and Contents	265. ± 149.	338. ± 55.	562. ± 25.	447. ± 117.	81.1 ± 16.5	6.20 ± 3.89
Liver	0.963 ± 0.429	1.85 ± 0.42	8.60 ± 1.33	15.9 ± 5.8	8.50 ± 2.53	3.64 ± 1.04
Lung	1.43 ± 0.35	2.38 ± 0.90	6.39 ± 1.32	11.7 ± 1.5	8.35 ± 1.54	2.08 ± 0.79
Muscle	0.130 ± 0.074	0.328 ± 0.175	1.76 ± 0.54	3.58 ± 1.03	0.234 ± 0.072	0.030 ± 0.011
Spleen	0.153 ± 0.056	0.342 ± 0.121	1.68 ± 0.36	3.63 ± 1.26	0.403 ± 0.111	0.135 ± 0.041
Small Intestine	7.63 ± 4.62	17.1 ± 3.6	6.89 ± 2.37	8.43 ± 2.65	1.78 ± 0.67	0.208 ± 0.104
and Contents	657. ± 496.	559. ± 332.	79.7 ± 29.1	77.4 ± 39.5	32.2 ± 6.0	2.26 ± 2.15
Urinary Bladder	2.14 ± 2.09	3.37 ± 2.93	15.5 ± 0.5	18.1 ± 9.7	12.4 ± 11.6	0.560 ± 0.364
Stomach	13.6 ± 15.8	45.8 ± 40.9	2.90 ± 1.05	4.29 ± 1.52	1.05 ± 0.72	0.121 ± 0.072
and Contents	454. ± 448.	781. ± 594.	5.7 ± 4.9	2.17 ± 0.86	1.66 ± 1.23	0.310 ± 0.481
Testes	0.049 ± 0.037	0.185 ± 0.033	1.11 ± 0.17	2.44 ± 0.55	0.323 ± 0.135	0.049 ± 0.010
Urine	**	6.8 ± 10.8	33.0 ± 3.2	42.6 ± 20.2	73.0 ± 29.0	34.4 ± 12.9

*Mean and standard deviation from 3 rats.

**None excreted.

Table XII. Distribution of ^{14}C-DiMxBzd and ^{14}C-DiMeBzd in Tissue Samples at 72 Hours*

Sample	DiMxBzd μg Equiv/g	DiMeBzd μg Equiv/g
Adipose	<0.014 ± 0.000	<0.014 ± 0.000
Blood	0.027 ± 0.002	0.039 ± 0.011
Brain	0.006 ± 0.001	0.004 ± 0.002
Carcass	0.015 ± 0.003	0.026 ± 0.011
Feces	22.6 ± 1.6	26.8 ± 2.5
Heart	0.036 ± 0.011	0.021 ± 0.006
Kidney	0.127 ± 0.029	0.130 ± 0.032
Large Intestine	0.034 ± 0.006	0.058 ± 0.019
and Contents	0.302 ± 0.134	0.551 ± 0.219
Liver	0.568 ± 0.065	0.907 ± 0.252
Lung	0.049 ± 0.006	0.459 ± 0.063
Muscle	0.008 ± 0.002	0.009 ± 0.003
Spleen	0.030 ± 0.006	0.036 ± 0.010
Small Intestine	0.035 ± 0.003	0.101 ± 0.054
and Contents	0.395 ± 0.169	1.13 ± 0.70
Urinary Bladder	0.147 ± 0.029	0.048 ± 0.016
Stomach	0.061 ± 0.056	0.145 ± 0.099
and Contents	0.104 ± 0.080	0.768 ± 0.665
Testes	0.023 ± 0.006	0.019 ± 0.008
Urine	4.85 ± 0.66	6.61 ± 0.82

*Mean and standard deviation from 3 rats.

highest accumulation of ^{14}C from rats dosed with DiMeBzd was in the liver, urinary bladder, and kidney. The highest accumulated levels of ^{14}C in rats dosed with DiMeBzd occurred in the liver, lung, stomach, and kidney. The levels of ^{14}C found in animals dosed with DiMxBzd did not differ appreciably from those dosed with DiMeBzd. The levels of ^{14}C at 72 hours in tissues from rats dosed with amines were generally lower than comparable tissues from rats dosed with dyes; however, the general distribution of ^{14}C in rats dosed with both amines and dyes was similar. It should be noted that both dyes significantly accumulate in the liver, a target organ for rats dosed with benzidine (24) and the site of tumors in rats dosed with benzidine-based azo dyes (2).

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