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Absorption and elimination of D&C Red No. 28 in male F-344 rats

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Abstract

D&C Red No. 28 (Red 28) is a US certified color additive used in drugs and cosmetics. Little is known about the extent of systemic absorption and pharmacokinetic behavior of Red 28. Therefore, these studies were performed to determine oral bioavailability and pharmacokinetic parameters of Red 28 in male F-344 rats following single and repeated oral dosing. Rats were administered either a single i.v. dose (50 mg/kg), a low oral gavage dose (50 mg/kg), or a high oral gavage dose (500 mg/kg) of Red 28. Plasma, urine and feces samples were subjected to solid phase extraction (SPE) and analyzed by HPLC for Red 28. Regardless of the dose or route of administration, the terminal $t_{1/2}$ of Red 28 was 2.5 h. The major route of elimination was fecal excretion, with 88% (i.v.) and 98% (50 mg/kg p.o.) of the dose recovered by 96 h. Urinary excretion of Red 28 accounted for 1% of the dose following i.v. administration. No Red 28 was detected in urine after p.o. administration. Biliary excretion was determined experimentally to be the primary route of elimination for systemically available Red 28. Bioavailability following p.o. administration was very low (1–2%) and was not altered significantly by 14 days of dietary pretreatment with Red 28.
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Keywords: D&C Red No. 28; Phloxine B; Pharmacokinetics; Bioavailability

1. Introduction

Colors are an important part of our society. They add enjoyment and appeal to food and are critical for identifying different types and dosages of medication. Color additives are classified as either certified colors or those exempt from certification (e.g., natural products obtained from plant, animal or mineral sources). Certified colors are given a label of FD&C, D&C or external D&C to signify their approved use in foods, drugs and cosmetics, drugs and cosmetics or external drugs and cosmetics, respectively. Each batch of certified colorants manufactured must meet certain specifications as described in the *Code of Federal Regulations* (CFR, 1995).

As its name implies D&C Red No. 28 (Red 28), commonly known as Phloxine B, is a color additive certified for use in both drugs and cosmetics in the United States. It is used in such products as biological stains, inks, and lacquers for coating and dyeing paper (Gurr, 1971). It is also being investigated for use as a fruit fly pesticide (Heitz, 1997). According to the FDA, a safe level of exposure for Red 28 is considered to be 1.25 mg/kg/day (Federal Register, 1982).

Red 28 is a member of the second largest class of color additives known as the xanthene dyes (Fig. 1). Many of these dyes are substituted derivatives of fluorescein (D&C Yellow No. 7), often containing different combinations of halogen atoms. In general this group of dyes displays brilliant fluorescent color ranging from red to greenish-yellow in hue depending on the particular substituents on the ring.

Limited data are available on the systemic exposure to these halogenated fluorescein dyes. Webb et al. (1962) performed previous metabolism and disposition studies

Abbreviations: Red 28, D&C Red No. 28; i.v., intravenous; p.o., oral; SPE, solid phase extraction; F-344, Fischer 344; JVC, jugular vein cannula; EtOH, ethanol; MeOH, methanol; HPLC, high performance liquid chromatography; i.p., intraperitoneal.

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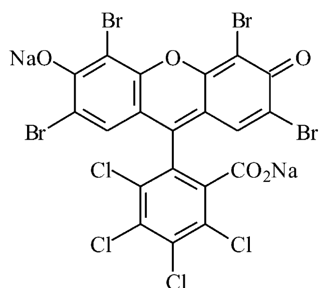

Fig. 1. Chemical Structure of D&C Red No. 28.

in rats with various halogenated fluorescein dyes. After oral dosing with 250 mg/kg of D&C Red No. 27, 100% of the dose was accounted for in the feces and no dye was detected in the urine. No metabolites were detected in the urine or bile for D&C Red No. 27. With increasing halogenation of fluorescein, the amount of dye excreted in the bile increased while the amount of dye excreted in the urine decreased. Monohalogenated fluoresceins were metabolized partially to fluorescein while tri-halogenated and tetra-halogenated derivatives were excreted unchanged in the feces.

This investigation focused on determining the bioavailability of Red 28 (the sodium salt of D&C Red No. 27) following oral administration and to determine if repeated exposure to Red 28 altered its systemic exposure.

2. Materials and methods

2.1. Chemicals

Warner-Jenkinson Company, Inc. provided the Red 28 dye (95% pure). The following chemicals were purchased from the vendors indicated: ammonium hydroxide, ammonium acetate and hydrochloric acid (Spectrum Chemical Co.); *n*-butylamine and Trizma® base (Sigma Chemical Co.); sodium hydroxide (Aldrich Chemical Company, Inc.); ethanol (95%, 190 proof), HPLC grade acetonitrile and HPLC grade methanol (Burdick & Jackson).

2.2. Animals

Male Fischer-344 (F-344) rats (7 weeks) were purchased from Harlan Sprague–Dawley Inc. (Indianapolis, IN). Male F-344 rats fitted with an indwelling jugular vein cannula (JVC) were purchased from Hilltop Lab Animals, Inc. (Scottdale, PA). Upon arrival, rats were housed in individual Nalgene metabolism cages or wire hanging cages and allowed 5–7 days to acclimate. The animal facility has an air circulation of 15 fresh filtered air changes per hour, a room temperature between 68–74 °F, the relative humidity between 40-60%, and a light/dark cycle maintained at 12 h intervals. Food (Teklad 4% Mouse-Rat Diet, Harlan Teklad, Madison, WI) and water were provided ad libitum during this acclimation period. The average weight of rats prior to dosing on week 8 was 145 g $\pm$ 8 g.

2.3. Sample collection

Following dosing, animals were placed in Nalgene metabolism cages to facilitate the collection of urine and feces, which were collected every 24 h up to 96 h post dosing. Cages were rinsed after each collection with approximately 10 ml of 25% ethanol (EtOH) in water. Animals were killed by CO_2 inhalation at the end of the study. All biological samples were stored at −80 °C until analysis.

2.4. Intravenous study

In this study male F-344 rats fitted with an indwelling JVC were used. Animals were administered a single intravenous dose of Red 28 (50 mg/kg; 1 ml/kg) in saline via the JVC. The injection was followed with an equal volume of saline to flush the cannula. Serial blood samples were collected from the animals (100–300 µl) and replaced with an equal volume of saline at various times. Immediately following collection, blood samples were centrifuged (4 min at 12 000 rpm) to separate the plasma, which was later subjected to SPE and analyzed by HPLC for the presence of Red 28 as described below. Plasma was selected for analysis based on the results of in vitro incubations of Red 28 with heparinized blood. They showed that after 2 h of incubation the majority of Red 28 (95% $\pm$ 6%) was present in plasma (data not shown).

2.5. Oral bolus studies

For elimination studies, animals were fasted for 4 or 12 h prior to administration of Red 28. For kinetics studies, animals were fasted for 12 h prior to administration of Red 28. Food was returned to the animals 2 h post dosing.

Male F-344 rats were administered an oral gavage dose of Red 28 (50 mg/kg; 2 ml/kg) in water. For elimination studies, urine, feces and cage rinse (25% EtOH in water) were collected at 24, 48, 72 and 96 h post dosing. To determine plasma kinetics, four animals per time point were killed by CO_2 inhalation at 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h following dosing. Blood (3–4 ml) was immediately collected from the posterior vena cava into a heparinized syringe and processed as above.

In the high dose group, male F-344 JVC rats were administered a single oral gavage dose of Red 28 (500 mg/kg; 2 ml/kg) in water. Serial blood samples (100–300

µl) were collected from the JVC and replaced with an equal volume of saline at various times.

2.6. Dietary feeding study

In this study two different groups of rats were used. Both groups consisted of 28 male F-344 rats. The rats were fed a diet containing blended rat chow (Teklad 4% Mouse-Rat Diet) mixed with Red 28 for 14 days such that their daily intake would be 500 mg/kg. Rats were weighed every other day. Food containers were also weighed every day to determine the amount of consumed diet. The diet was prepared and replaced every three days based on the actual food consumption and weight of the animals over the two-week period. On day 15, after a 12 h fasting period, one group was dosed by oral gavage with the dye (500 mg/kg; 2 ml/kg) in water while the other group was dosed with the vehicle (water, 2 ml/kg). To determine plasma kinetics and background levels of Red 28 from the diet, four rats from each group were killed by CO_2 inhalation at 1, 2, 4, 8, 12, 24 and 48 h post dosing. At these times, blood (4–5 ml) was immediately collected from the posterior vena cava into a heparinized syringe and processed as above.

2.7. Bile studies

The common bile duct was cannulated in rats anesthetized with urethane/saline (1.5 mg/kg, i.p.) as described by Waynforth and Flecknell (1992). Rats were dosed with Red 28 via oral gavage (50 mg/kg; 2 ml/kg) or the tail vein (10 mg/kg; 1 ml/kg). Serial bile samples were collected from animals before dye administration and then every 30 min for 3 h post dosing. After sample analysis, data were combined to summarize the elimination results for each hour.

2.8. Sample preparation

The methods described here were optimized for the detection and recovery of Red 28. Solid phase extraction procedures were developed for Red 28 in each biological matrix. Based on previous experiments (Webb et al., 1962) no metabolites of Red 28 are known to exist. Because Red 28 is a highly water soluble compound, it is reasonable to assume that any water soluble metabolites could be partially extracted using the same procedure as Red 28.

Aliquots (1.5–2.0 ml) from urine and cage rinse samples were mixed with 1 ml of methanol (MeOH)/acetonitrile/*n*-butylamine (1:1:0.05) which was further diluted 1:1 with Tris–HCl buffer (pH 9). Samples were loaded into Oasis 3cc HLB cartridges (Waters) located on a solid phase extraction manifold (Waters). Cartridges were rinsed with 1 ml of water followed by another 1 ml of 20% MeOH in water. The dye was eluted using 2–3 ml of NaOH (0.5 M)/MeOH (1:10). The sample extraction efficiency for urine and cage rinse samples was determined to be 105% ± 4% ($n=6$).

An equal weight of water was added to frozen fecal samples to aid in homogenization and samples were allowed to sit at room temperature for 24 h to assist in thawing and promote absorption of water into formed feces. After thoroughly mixing the samples, aliquots (0.1–0.5 g) from each sample were taken and analyzed for the presence of Red 28. After addition of 1.0 ml of MeOH/acetonitrile/*n*-butylamine (1:1:0.05), samples were vortexed, centrifuged (4 min at 12 000 rpm), and the supernatant removed and saved. The procedure was repeated until the supernatant appeared clear. Combined extracts were diluted 1:1 with Tris–HCl buffer (pH 9) and then treated by solid phase extraction as described above. The extraction efficiency for Red 28 in feces was determined to be 98% ± 4% ($n=12$).

Approximately 0.25 ml of MeOH/acetonitrile/*n*-butylamine was added to each plasma or bile sample, which was then diluted 1:1 with Tris–HCl buffer (pH 9). Samples were loaded into 1cc or 3cc Oasis HLB cartridges (Waters) and rinsed with about 1 ml of water. The dye was eluted with 1 ml of NaOH (0.5 M)/MeOH (1:10) solution. The samples were evaporated and reconstituted in 150–300 µl of MeOH. The extraction efficiency of Red 28 for plasma and bile was determined to be 94% ± 5% ($n=14$).

2.9. HPLC analysis

Samples (20 µl) were injected onto a Phenomenex Luna 5 µm C18 column (250×4.6 mm) and eluted using a mobile phase consisting of MeOH and 0.05 M ammonium acetate. The HPLC (Agilent Technologies) was equipped with an Agilent 1100 quaternary pump, autosampler, and diode array detector. ChemStation data acquisition software (Rev. A.0.9.03[1417]) was used to evaluate the chromatograms. The mobile phase gradient started with 25% MeOH and 75% ammonium acetate and increased to 90% MeOH over a period of 25 min. From 25 to 30 min, it was increased to 100% MeOH, which was held until 35 min, after which the gradient returned to the initial conditions of 25% MeOH for a reequilibration period of 10 min before injection of the next sample. During the HPLC run, all spectra were collected and stored in order to detect the presence of other compounds such as impurities or metabolites. For quantification purposes, the effluent was monitored at 550 nm (the λ_{max} of Red 28). No internal standard was used but aqueous standards of Red 28 ranging from 0.05 to 200 µg/ml were run along with samples in order to generate a calibration curve for quantification. The limit of detection and limit of quantification for Red 28 was 0.007 µg/ml and 0.02 µg/ml, respectively.

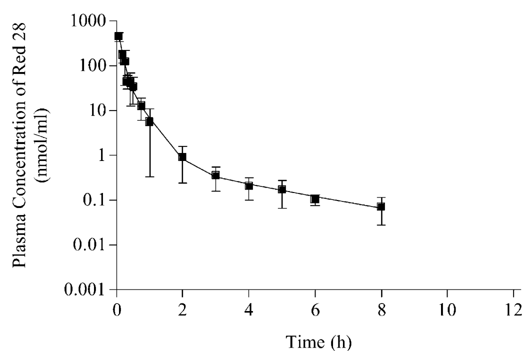

Fig. 2. Semilogrithmic plot showing plasma concentration of Red 28, expressed as nmol/ml, after i.v. administration (50 mg/kg) to male F-344 rats. Data points represent the mean plasma concentration ($n = 5$) along with the standard deviation and the solid line represents the nonlinear regression fit of the data.

2.10. Pharmacokinetic analysis

The oral high dose and i.v. plasma concentration-time curves were analyzed by compartmental analysis. A computer modeling program (WinNonlin, Scientific Consulting Inc., 1995) was utilized to fit the data to a suitable multi-compartment model using non-linear regression analysis and assuming first-order kinetics for all processes. The parameters of the model were used to calculate values for systemic clearance (CL_s), and volume at steady-state (V_{ss}) following i.v. administration. Values for the terminal half-life ($t_{1/2}$), area under the curve from time zero to infinity (AUC), time to maximum plasma concentration of Red 28 (t_{max}), and the maximum plasma concentration of Red 28 (C_{max}) were calculated following both i.v. and oral administration. Average parameter values (±S.D.) were obtained from the arithmetic average with the exception of $t_{1/2}$, which is expressed as the harmonic mean and ‘‘pseudo’’ standard deviation (Lam et al., 1985).

Plasma concentration–time curves from the low oral dose and feeding study were analyzed by non-compartmental methods. The terminal rate constant (k) was calculated from a log-linear regression of the data in the terminal phase (1–12 h). From this value, the terminal half-life ($t_{1/2}$) was determined (0.693/k). The AUC was calculated with the linear trapezoidal rule using the last measured concentration to extrapolate to infinity. C_{max} and t_{max} represent the maximum measured concentration and the corresponding time.

Bioavailability throughout this paper refers to the absolute oral bioavailability (F), which was calculated by dividing AUC following oral administration by AUC following i.v. administration, correcting for dose when appropriate, and assuming that clearance remained constant (Rowland and Tozer, 1995).

3. Results

3.1. Intravenous administration

A multi-compartment model best described the plasma concentration-time curve after i.v. administration of Red 28. The initial concentration of Red 28 in the plasma fell rapidly until 1 h, after which time a slower rate of disappearance was observed. After 3 h, the concentration of Red 28 in the plasma declined at the slowest rate, representing the terminal phase. Red 28 was not detected in the plasma after 8 h (Fig. 2). The terminal half-life was 2.2 h with a terminal rate constant of 0.31 h^{-1}. The remaining pharmacokinetic parameters are listed in Table 1.

Following the single i.v. bolus dose nearly all of the dye was excreted in the feces unchanged (84% after 24 h and 88% by 96 h). Very little of the dye was detected in the urine (1%) after 24 h. Average recovery obtained for Red 28 after i.v. administration was 89%. The elimination profile for Red 28 is shown in Table 2.

3.2. Oral administration

The maximum concentration of Red 28 measured in the plasma was 0.23 nmol/ml (0.01% of the dose in total plasma volume, 31.2 ml/kg, Davies and Morris, 1993) after 35 min. After this time, the plasma concentration decreased (except for a possible small transient increase at 4 h). The terminal half-life was 2.5 h with a terminal rate constant of 0.27 h^{-1}. Oral bioavailability of Red 28, calculated as $AUC_{p.o.}/AUC_{i.v.}$, was determined to be

Table 1
Pharmacokinetic parameters of Red 28 in plasma after i.v. administration (50 mg/kg) to male F-344 rats ($n = 5$)

Parameter	Individual Rat					Mean±SD
k_{term} (h^{-1})	0.438	0.256	0.189	0.313	0.377	0.31±0.10
$t_{1/2}$ (h)	1.58	2.71	3.66	2.21	1.84	2.2±0.7
AUC (nmol·h/ml)	148	190	70.9	80.6	115	121±49
V_{ss} (ml)	11.4	14.0	34.7	21.6	12.9	19±10
Cl (ml/min)	0.95	0.74	1.98	1.74	1.22	1.3±0.5

k_{term}: rate constant associated with the terminal phase. $t_{1/2}$: terminal half-life. AUC: area under the plasma concentration–time curve from time 0 to infinity. V_{ss}: volume of distribution at steady-state. Cl: systemic plasma clearance.

0.35%. The remaining pharmacokinetic parameters are listed in Table 3.

Following administration of the lower oral bolus dose (50 mg/kg) no dye was detected in the urine or cage rinse over the 96 h period. All of the dye recovered (98% of the dose) was excreted as parent compound in the feces with 94% in the first 24 h and another 4% after 48 h. Minimal amounts of dye were excreted in the feces after 48 h. The elimination profile is shown in Table 2.

Following administration of the higher dose (500 mg/kg; 2 ml/kg), a one-compartment model best described the plasma concentration-time profile. The concentration of Red 28 in the plasma reached a maximum of 3.1 nmol/ml (0.02% of the dose in total plasma volume) at 1.5 h and was not detected beyond 12 h post dosing (Fig. 3). The terminal half-life was 2.5 h with a terminal rate constant of 0.28 h^{-1}. Oral bioavailability of Red 28 was determined to be 1.5%. The remaining pharmacokinetic parameters are listed in Table 3.

Table 2
Mean recovery (±S.D.) of Red 28 following i.v. (50 mg/kg) and oral administration (50 mg/kg) to male F-344 rats

	% of dose				
	0–24 h	24–48 h	48–72 h	72–96 h	Total
Feces i.v.	84.2±9.1	3.7±0.9	0.1±0.1	0	88.0±8.5
p.o	94.2±2.9	4.1±2.9	0.2±0.2	0	98.5±3.5
Urine i.v.	1.0±0.3	0.03±0.01	0	0	1.0±0.3
p.o.	0	0	0	0	0

Values are expressed as percent of administered dose excreted within the ranges indicated relative to the time of administration (time 0) via either the jugular vein cannula (i.v.) or oral gavage (p.o.) (n=7 and 8, respectively).

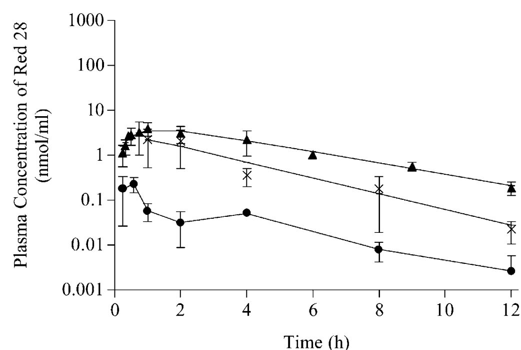

Fig. 3. Semilogrithmic plot showing plasma concentration of Red 28, expressed as nmol/ml, after oral administration of a low (● 50 mg/kg) and high (▲ 500 mg/kg) dose to naïve male F-344 rats. F-344 rats were also fed a diet containing Red 28 diet (500 mg/kg/day) for 14 days and then administered the high dose on day 15 (×). Data points represent the mean plasma concentration along with the standard deviation. For the high dose, serial blood samples (100–300 μl) were obtained from the JVC from each animal (n=6) throughout the study. For the low dose and pretreatment study, four animals were killed at each time point, and 3–5 ml of blood were collected.

Following the large oral bolus dose it was not possible to separate completely the urine and the feces. The large dose produced severe diarrhea beginning 2 h post dosing in the animals that were fasted for 12 h. The diarrhea persisted for the first 24 h, during which the animals were able to produce only a small amount of solid feces (about 2.0 g). In order to promote the formation of solid stool the animals were also fasted for a shorter period of time (4 h instead of 12 h). Red 28 also caused diarrhea in these animals, however, this diarrhea was not evident until 12 h post dosing. There was a large variation in the excretion pattern of these animals. The majority of the dye was excreted in the feces unchanged (48%) but a significant portion was recovered in the urine collector (9%) and the cage rinse (20%) (Data not shown). Because of the fluid nature of the fecal material, it was not possible to determine quantitatively the amount of Red 28 excreted in urine or feces. In fact, total recovery of administered Red 28 was reduced in studies with the large oral bolus dose. Red 28 was adhering to the fur of the rats as well as to the walls and metal grates of the metabolism cages.

3.3. Dietary feeding study

The animals gained weight over the 14-day feeding period on the Red 28 diet. They started the study as 8-week-old rats weighing 168 g±6 g and by 10 weeks they weighed 225 g±10 g. Detectable plasma levels of Red 28 were not present in the animals that received the bolus dose of water following pretreatment with Red 28 in the diet. In the animals that received the bolus dose of Red 28, the average maximum concentration of Red 28 measured in the plasma was 2.2 nmol/ml (0.01% of the bolus dose in total plasma volume). Red 28 was not

Table 3
Mean (±S.D.) pharmacokinetic parameters of Red 28 in plasma after oral administration to male F-344 rats

Parameter	p.o. (50 mg/kg)[a]	p.o. (500 mg/kg) (n=6)	Pretreatment[a]
k_{term} (h^{-1})	0.27	0.28±0.09	0.41
$t_{1/2}$ (h)	2.5	2.5±0.8	1.7
C_{max} (nmol/ml)	0.23	3.1±1.2	2.2
T_{max} (h)	0.58	1.5±0.3	1.0
AUC (nmol·h/ml)	0.43	18±5	7.2
F (%)	0.35	1.5±0.4	0.60

k_{term}: rate constant associated with the terminal phase. $t_{1/2}$: terminal half-life. C_{max}: maximum plasma concentration. T_{max}: time to reach C_{max}. AUC, area under the plasma concentration–time curve from time 0 to infinity. F: absolute oral bioavailability.

[a] Four animals were killed at selected time points and exsanguinated to obtain enough sample to determine the plasma concentration of Red 28. Average concentrations were combined to generate a single plasma concentration–time profile for calculation of each pharmacokinetic parameter.

detected in plasma beyond 12 h post dosing. The terminal half-life was calculated to be 1.7 h with a terminal rate constant of 0.41 h^{-1} (Fig. 3, Table 3). Oral bioavailability of Red 28 was determined to be 0.60%.

Following a 14-day pretreatment regimen with Red 28 in the diet, animals that received the bolus dose of the vehicle (i.e., no Red 28) on day fifteen still excreted dye in the feces 24 h after being placed on the control diet. The amount of Red 28 recovered was 2450±1020 nmol/g feces. The amount of dye recovered in the feces from these animals during the next 24 h declined dramatically (46±48 nmol/g). No dye was detected in the urine or cage rinse of the animals pretreated with Red 28 in the diet. As occurred with naïve animals administered a large oral bolus dose of Red 28, animals that had been pretreated for 14 days developed diarrhea when administered the large bolus dose (500 mg/kg) by oral gavage. Because of this, it was not possible to separate urine and feces to determine the elimination pattern of the dye.

3.4. Bile studies

Following i.v. administration of Red 28 (10 mg/kg; 1 ml/kg) to anesthetized rats ($n=2$) an average of 67% of the dose was recovered in the bile within 3 h (Table 4). Following oral administration of Red 28 (50 mg/kg; 2 ml/kg) only 0.14% of the dose ($n=4$) was recovered in bile within 3 h. The i.v. results are comparable to those reported previously by Webb et al. (1962) (50–75% by 2 h) and Iga et al. (1972) (87% by 4 h).

4. Discussion

These studies were performed to determine the extent of systemic absorption of Red 28 following oral administration. Previous work by Webb et al. (1962) characterized the elimination and metabolism of structurally similar dyes but did not attempt to determine systemic exposure. The studies reported here are the first to determine the bioavailability of Red 28 following oral administration. In these present studies, plasma concentration-time data were obtained in addition to elimination data. These results help characterize the systemic exposure to Red 28.

Table 4
Mean (±S.D.) biliary recovery of Red 28 following i.v. (10 mg/kg) and oral (50 mg/kg) administration to male F-344 rats anesthetized with urethane

	% of dose			
	0–1 h	1–2 h	2–3 h	Total
i.v.	34±4	21±1	12±1	67±6
p.o.	0.03±0.02	0.06±0.01	0.05±0.02	0.14±0.03

Values are expressed as percent of administered dose excreted within the ranges indicated relative to the time of administration (time 0) via either the tail vein (i.v.) or oral gavage (p.o.) ($n=2$ and 4, respectively).

Because urinary elimination of Red 28 is low, it was necessary to use another means to assess systemic exposure from the intestinal tract. The absolute oral bioavailability provides an accurate assessment of systemic exposure. The bioavailability for this dye is very low; about 1% of the dose reaches the systemic circulation following oral administration. In addition, dietary pretreatment with the compound for 14 days does not appear to alter the systemic exposure to Red 28. Because primarily the entire dose is recovered after 96 h as parent compound, metabolism does not appear to be playing any role in the elimination of this dye.

The low oral bioavailability of Red 28 results from poor oral absorption and/or efficient extraction and retention by the liver. Hepatic retention in the liver seems unlikely, as it was not retained in the liver following i.v. administration. Data reported here and by Webb et al. (1962); Iga et al. (1972) show 67–87% of i.v. administered Red 28 to be eliminated in the bile within 2-4 h. Thus, hepatic extraction of Red 28 is efficient and it is readily excreted into the bile. At 3 h after oral administration less than 0.2% of the dose was recovered in the bile. This represents about 60% of the entire amount calculated to reach the systemic circulation following oral administration ($F=0.35\%$ for 50 mg/kg oral bolus dose). Therefore, comparison of the biliary excretion of Red 28 following i.v. and oral administration supports the conclusion that Red 28 is poorly absorbed from the intestinal tract.

Following i.v. administration of Red 28, the majority of the dose is recovered in the feces, with only 1% in the urine even though the entire dose had been delivered to the systemic circulation. Low renal excretion of this compound might be due to plasma protein binding (Lutty, 1978), which prevents glomerular filtration. Because this compound is polar and has a large molecular weight (>500 g/mol), it is a better candidate for biliary secretion (Rowland and Tozer, 1995).

Compounds that are actively secreted into the bile can have biliary clearances that approach hepatic blood flow (Rowland and Tozer, 1995). The clearance measured in these experiments represents plasma, not blood clearance. In order to make a direct comparison, the clearance needs to be adjusted based on the blood-to-plasma concentration ratio (Rowland and Tozer, 1995). Based on previous experiments, Red 28 partitions almost entirely into the plasma (95%), so the blood clearance would be almost double the plasma clearance. The approximate blood clearance for this compound (3–6 ml/min) is lower than the reported rat hepatic blood flow (12–20 ml/min, Derelanko and Hollinger, 1995). Even though high hepatic clearances are not seen in this case, much higher concentrations of Red 28 are measured in the bile compared to the plasma. This sug-

gests the work of an active transport process in order to move Red 28 from the plasma into the bile against a concentration gradient. Organic anion transporting polypeptides (OATPs) in the liver are involved in clearing plasma protein bound compounds from the circulation (Hagenbuch and Meier, 2003). It is possible that Red 28 is among the wide range of substrates (such as bile salts, organic dyes, steroid conjugates and many other xenobiotics) for OATPs. If this is the case, pretreatment with Red 28 in the diet may actually enhance its own elimination through up-regulation of these transporters. This could be a possible explanation for the shorter half-life and lower oral bioavailability of Red 28 seen following the 14-day pretreatment regimen.

The greater bioavailability observed after oral administration of the higher dose (500 mg/kg) of Red 28 is most likely the result of the gastrointestinal distress associated with this large bolus dose. The diarrhea may have caused the animals to become dehydrated, resulting in a lower blood volume and subsequently higher plasma concentrations of the dye. Gastrointestinal distress was also observed by Webb et al. (1962) when D&C Red 27 was administered at the same dose (500 mg/kg). Animals that consumed approximately the same daily amount of Red 28 in the diet did not develop diarrhea even after 14 days of dosing. A lower bolus oral dose of Red 28 (150 mg/kg) administered to female F-344 rats also resulted in diarrhea (data not shown). Gastrointestinal distress was not produced with a bolus dose of 50 mg/kg. The results suggest that the gastrointestinal distress was due exclusively to administration of a large amount of Red 28 as a bolus dose. The diarrhea caused by the high bolus dose of Red 28 did not appear to alter appreciably the systemic exposure or plasma pharmacokinetics of the dye. The half-lives following administration of either the 50 or 500 mg/kg bolus doses of Red 28 were comparable. These results agree with first order kinetics in that half-life is independent of dose. Furthermore, the half-lives following oral and i.v. administration are also similar, suggesting that elimination rather than absorption is the rate-limiting process for excretion of Red 28 following oral administration.

The half-life determined for Red 28 in these studies is significantly different from that previously reported by Iga et al. (1972). Following i.v. administration of Red 28 (100 mg/kg) to male rats, they reported that the half-life was 2-12 min and the plasma concentration data could be described by a single compartment model. Plasma concentration-time data were only measured out to 30 min, perhaps due to the limit of detection of Red 28 with their method. In the present studies, it was determined that the terminal half-life was 2.2 h and the plasma concentration-time data displayed a multi-compartment model. The discrepancy can be explained by the shorter time course measured by Iga et al. (1972). As a result, they did not observe the true terminal phase of the dye. Rather, the half-life they reported is similar to the distribution half-lives observed in the studies reported here.

Other pharmacokinetic parameters were obtained following i.v. administration that have not been previously reported for this compound. The volume of distribution at steady-state for this dye (19 ml) is larger than total plasma volume (about 5 ml for a 150 g rat) (Davies and Morris, 1993), suggesting that some of the dose moves out of the plasma into peripheral compartments (e.g., liver, other tissues). The initial rapid drop in plasma concentration is most likely due to distribution of Red 28 to peripheral compartments, rather than elimination.

Although studies by Webb et al. (1962) have shown a correlation between increased halogenation of fluoresceins and elimination, it is not clear what effect increasing halogenation has on toxicity. It is possible that the high degree of halogenation of Red 28 decreases intestinal absorption and increases fecal elimination. In that regard, it would appear that potential systemic exposure to highly halogenated fluorescein dyes would be much less than lower halogenated fluoresceins dyes. Based on these studies, systemic exposure to orally administered Red 28 in rats is very low and is not altered by repeated oral dosing. The dose of 50 mg/kg, which did not cause any gastrointestinal distress, and which did not result in systemic exposure to the dye, is 40 times greater than the dose estimated by the FDA to be safe for humans.

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