

PHARMACOKINETICS AND DISPOSITION OF D&C RED NO. 28 IN MALE FISCHER-344 RATS

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Abstract

D&C Red No. 28 (Red 28) is a U.S. certified color additive that is used in drugs and cosmetics. Currently, it is also being investigated for use as an insecticide for the suppression of fruit flies. Little is known about the pharmacokinetic parameters and extent of absorption of Red 28. Therefore, these studies were performed to determine the pharmacokinetic and disposition parameters of Red 28 in male F-344 rats. Rats were administered either a single intravenous (i.v.) dose (25 mg/kg), a low oral (p.o.) gavage dose (50 mg/kg), or a high p.o. gavage dose (500 mg/kg) of Red 28. Blood, urine and feces were collected at various times and analyzed for Red 28 by HPLC. A solid phase extraction method was developed to clean-up and concentrate the samples prior to HPLC analysis. Following the i.v. dose, the terminal $t_{1/2}$ of Red 28 in blood was 0.6 h, whereas the $t_{1/2}$ following p.o. administration was 2.5-2.8 h. Bioavailability following p.o. administration of the low and high dose was determined to be 0.6% and 2.7%, respectively. Recovery of Red 28 in the urine was less than 1% following the i.v. dose and was not detected after p.o. administration, indicating that urinary excretion was not a significant route of elimination. The major route of elimination following either i.v. or p.o. administration was fecal excretion. Nearly all of the administered Red 28 was excreted in the feces unchanged within the first 24 h, and by 96 h 95-99% of Red 28 was recovered. High p.o. doses of Red 28 (500 mg/kg) resulted in severe diarrhea such that separation of urine and feces was not possible.

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. It is estimated that humans ingest approximately 0.024 lbs. of these colorants per year (1). 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. In Japan it is also used as a food additive in fish cakes and pastries (2). According to the FDA, a safe level of exposure for Red 28 is considered to be 1.25 mg/kg/day (3). Red 28 has a wide variety of other uses that include biological stains, inks, and lacquers for coating and dyeing paper. Because Red 28 has the ability to fluoresce under ultra violet light it is also being investigated for use in fingerprint powders (4).

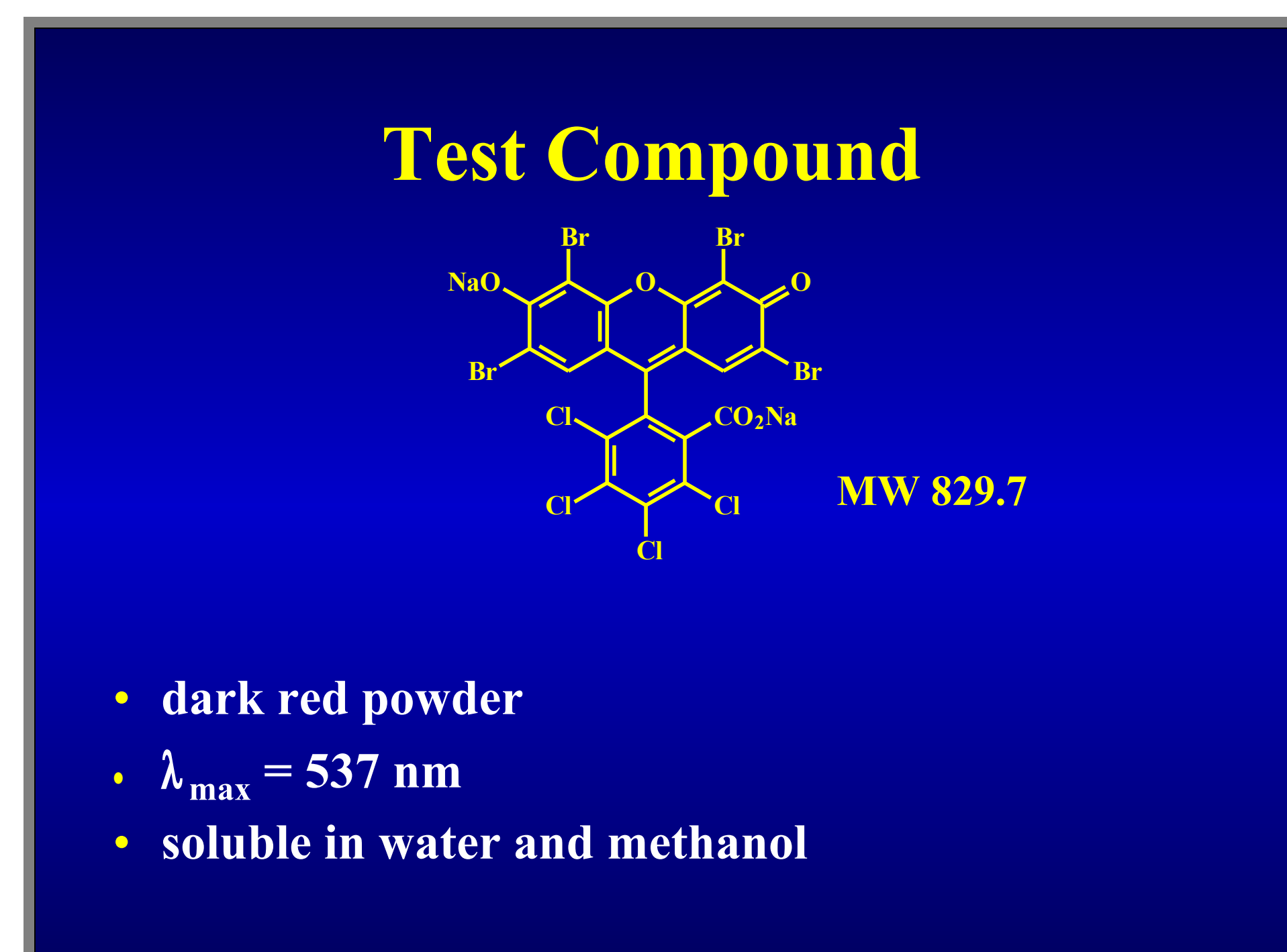
Previous studies have shown Red 28 and similar fluorescein dyes to be mutagenic only in the presence of light in a rec-assay and with *Salmonella typhimurium* (5). However, photoactivation is not required for these dyes to induce genetic mutations in mammalian cells. Red 28 was shown to be mutagenic to human diploid cells at concentrations ranging from 1 to 10 µg/ml (6). Studies performed by Sako et al. demonstrated that Red 28 was toxic to the growth of fetal rat hepatocytes and a dose response relationship was observed (7). Concentrations as low as 10 µM resulted in a reduction in growth. When incorporated into the diet at levels of 3% to 5%, Red 28 was teratogenic to mice, resulting in skeletal anomalies (8). Webb et al. (9) performed previous metabolism and disposition studies in rats with various halogenated fluorescein dyes but no work has been done to determine any pharmacokinetic parameters.

The studies presented here extend our understanding on the absorption and elimination of halogenated fluorescein dyes. No information is available in the literature about the pharmacokinetic properties of Red 28 or similar dyes. Therefore, these studies were designed to determine the pharmacokinetic parameters of Red 28 following i.v. and p.o. administration.

Materials and Methods

Test Compound

D&C Red No. 28 (95 % pure, Warner-Jenkinson Company, Inc.)



Animals

Male F-344 rats (7 weeks, 140-150g) with an indwelling jugular vein cannula (JVC) and male F-344 rats that were surgically unaltered were purchased from Hilltop Lab Animals, Inc. (Scottsdale, PA). Rats were housed in individual Nalgene metabolism cages or wire hanging cages and allowed 5-7 days to acclimate. Food (Teklad 4% Mouse-Rat Diet, Harlan Teklad, Madison, WI) and water were provided *ad libitum*.

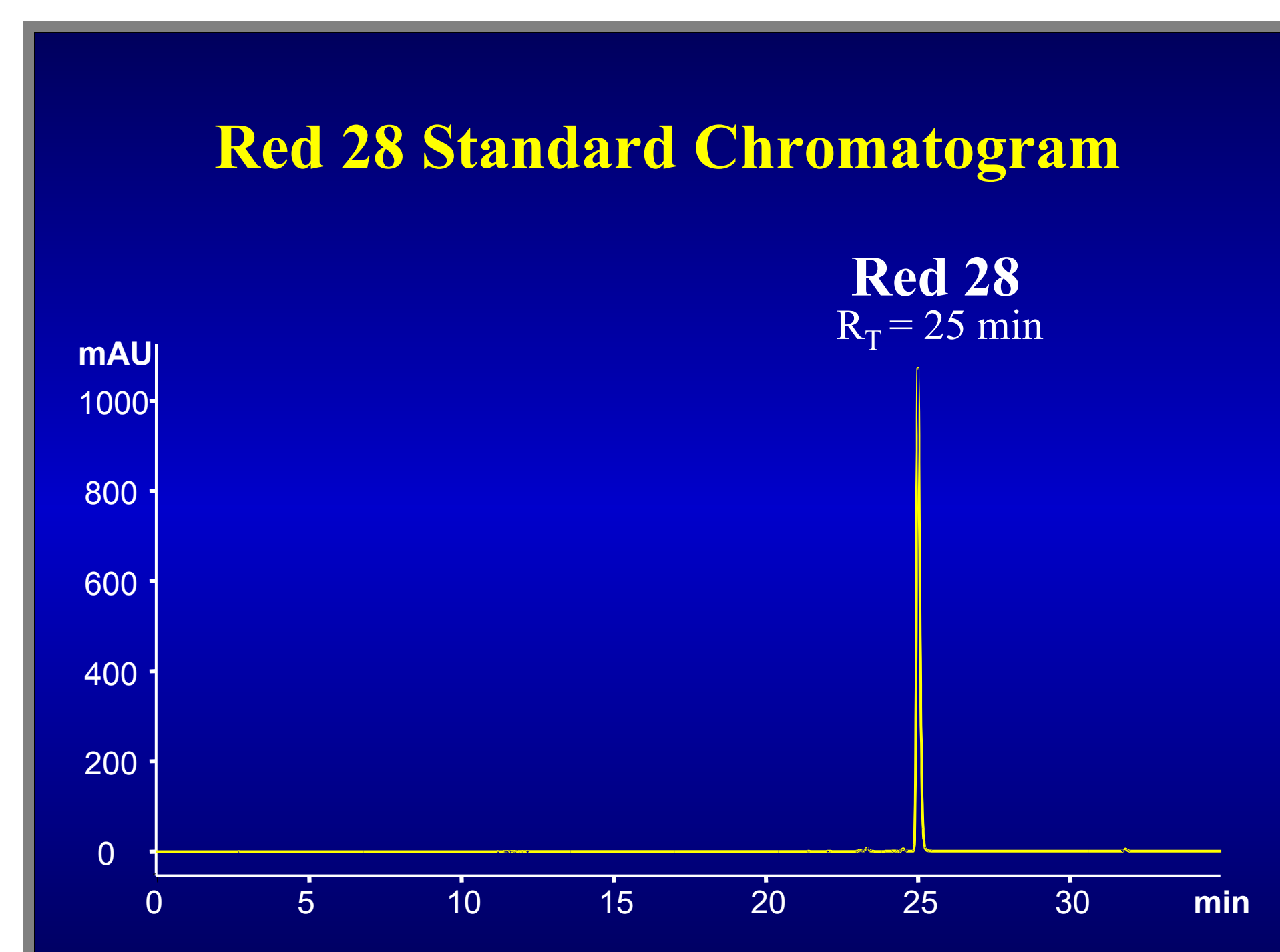
Route of Administration

i.v. (25 mg/kg, 1ml/kg) via JVC or tail vein

p.o. (500 and 50 mg/kg, 2 ml/kg) via oral gavage following a 12-h fasting period, food returned 2 h post dosing

Sample Analysis

Plasma, urine and feces were subjected to SPE using Oasis HLB solid phase extraction cartridges (Waters) and then injected onto the HPLC for quantification of Red 28.



HPLC Analysis

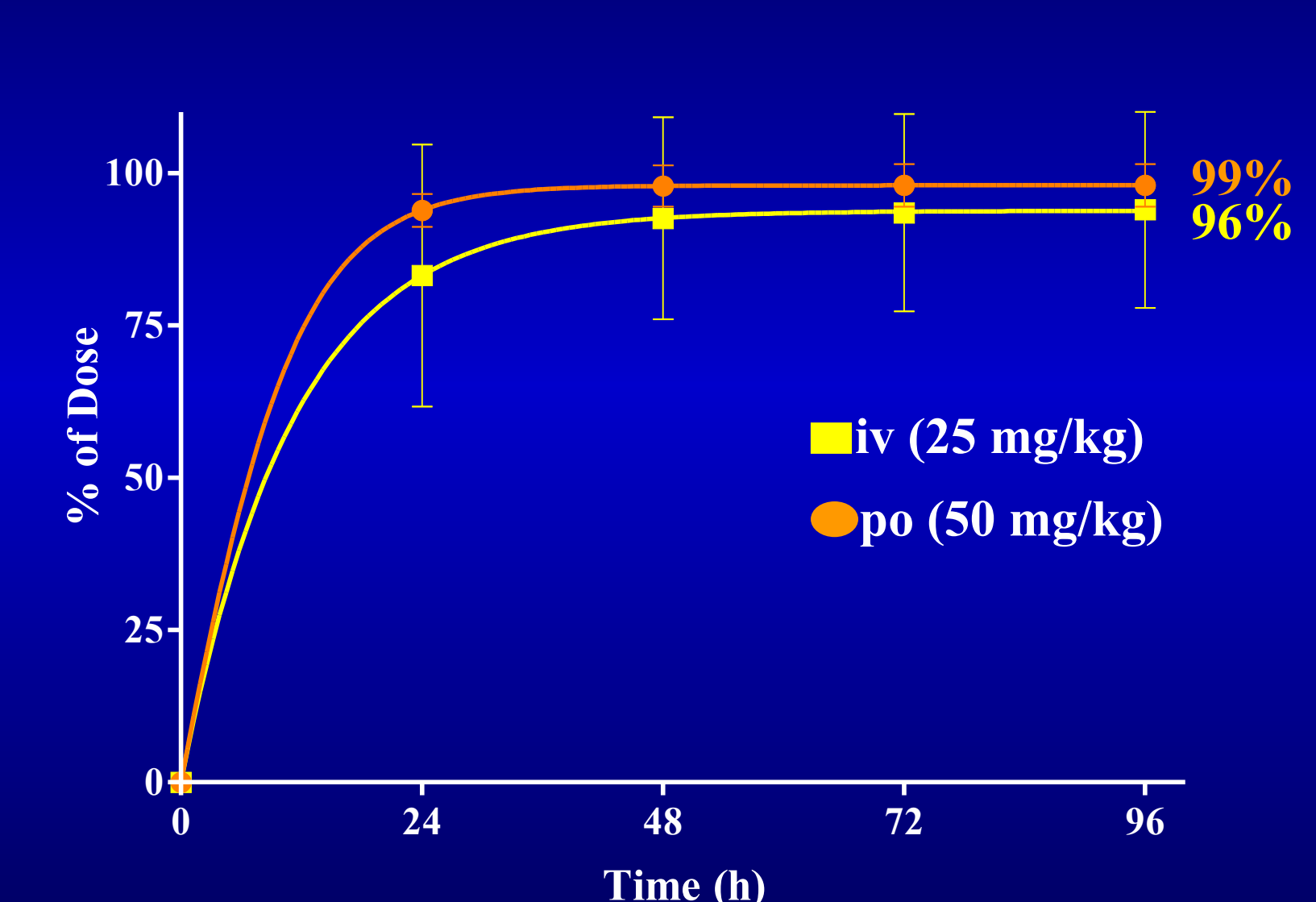
The HPLC (Agilent Technologies) was equipped with an Agilent 1100 quaternary pump, autosampler, and diode array detector monitoring the effluent at 537 nm. Samples (20 µl) were injected onto a Phenomenex Luna 5 µm C18 column (250 x 4.6 mm) and eluted using a gradient mobile phase consisting of methanol and 0.05 M ammonium acetate.

Data Analysis

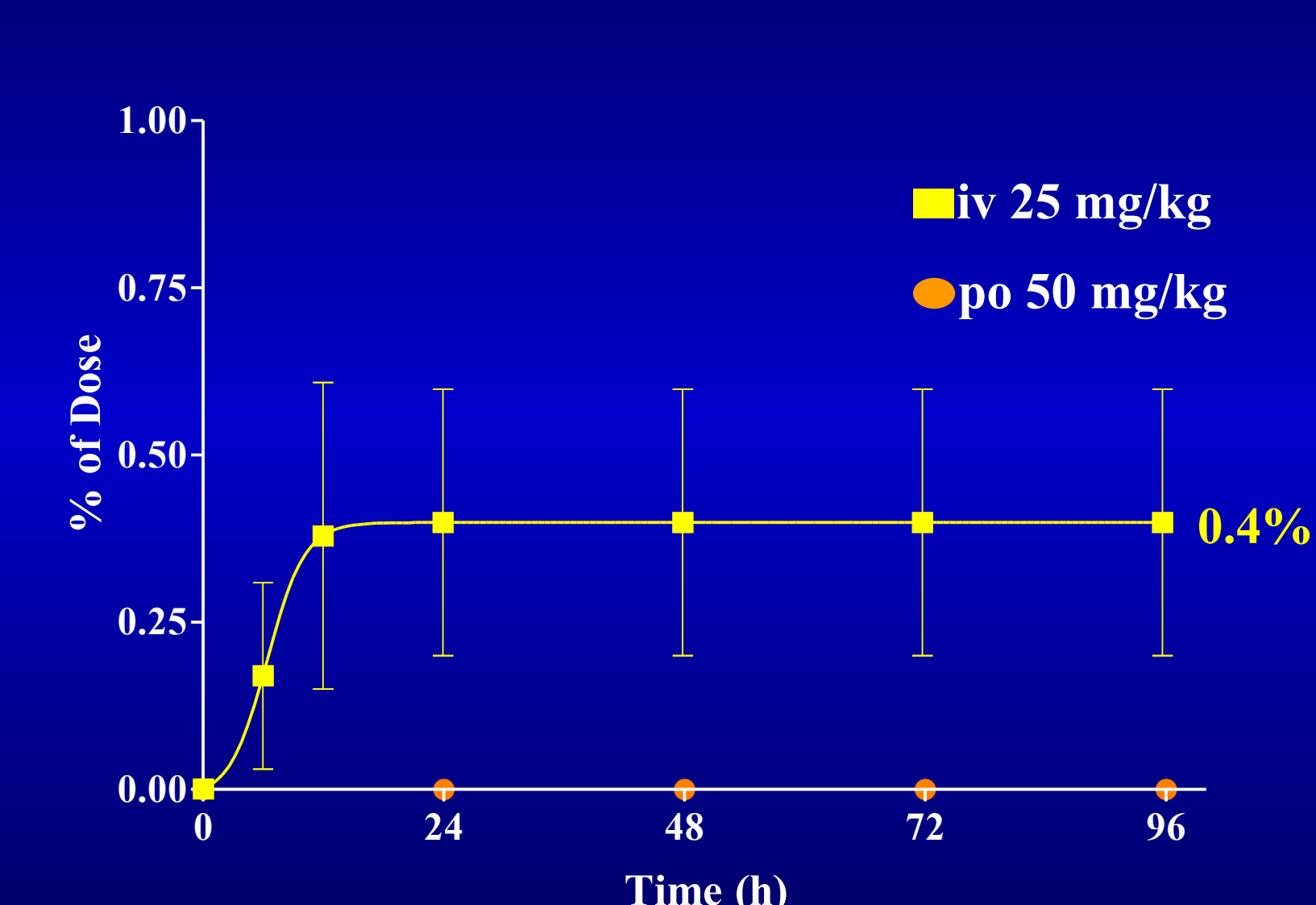
All plasma concentrations were related back to total blood sample. Blood concentration data were analyzed using a computer modeling program (WinNonlin, Scientific Consulting Inc., 1995) to fit the data to a suitable multi-compartment model using non-linear regression analysis and assuming first-order kinetics for all processes.

Results

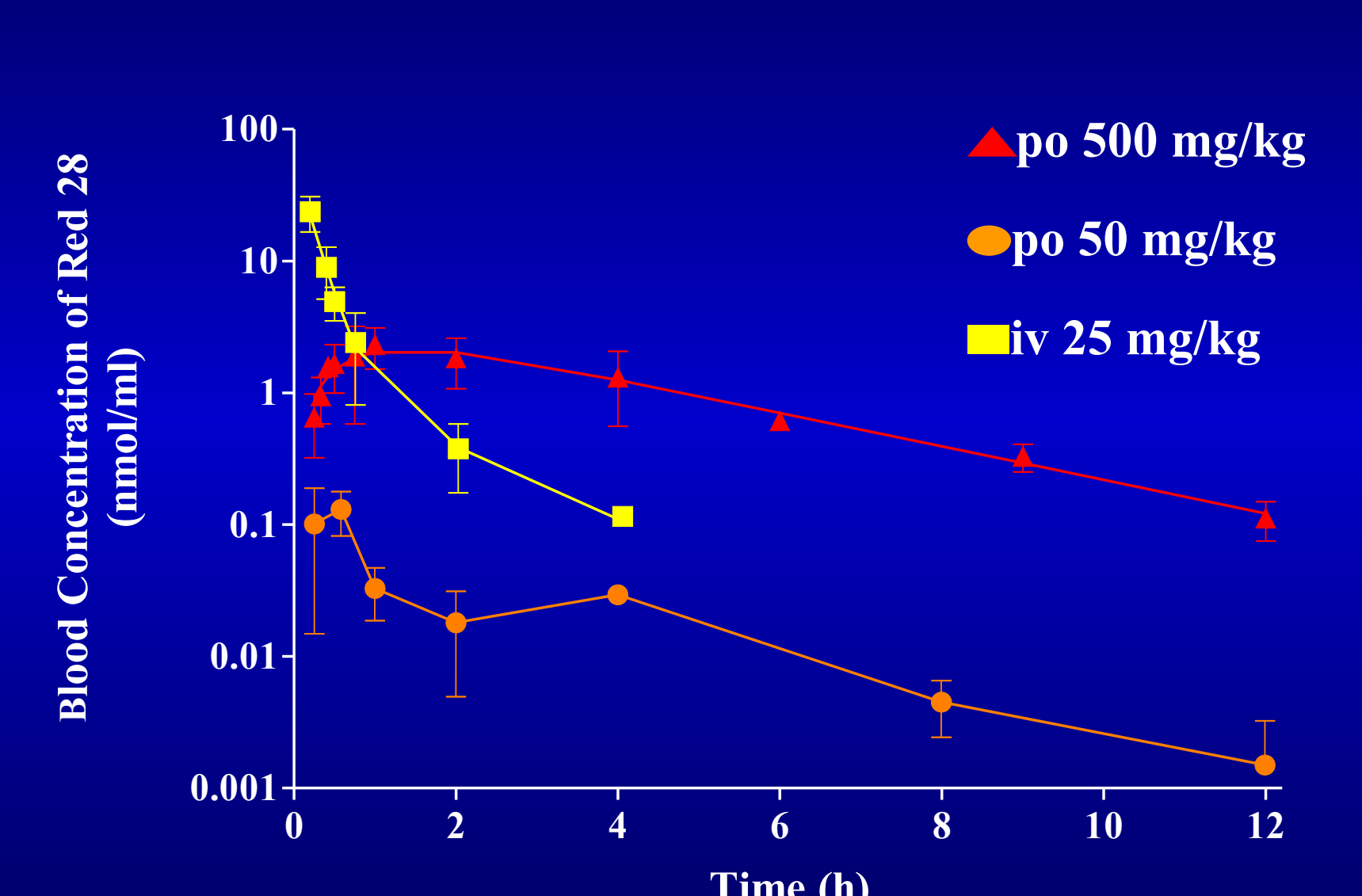
Cumulative Fecal Excretion of Red 28



Cumulative Urinary Excretion of Red 28



Blood Concentration-Time Curves



Pharmacokinetic Parameters

Parameter	i.v.		p.o.	
	25 mg/kg	500 mg/kg	500 mg/kg	50 mg/kg
V_D (mL)	56	na	na	na
C_{max} (nmol/mL)	na	1.81	0.13	
t_{max} (h)	na	1.49	0.58	
AUC (nmol/mL*h)	18.9	10.3	0.24	
$t_{1/2\beta}$ (h)	0.55	2.84	2.53	
CL_R (mL/min)	4.3	na	na	
F (%)	na	2.7	0.64	

na = not applicable

Summary

• Following i.v. administration, 95% of the dose was recovered in the feces and less than 0.5% was recovered in the urine by 96 h. A two-compartment model best described the disappearance of Red 28, suggesting that there was a measurable distribution phase. The average terminal half-life was determined to be 0.55 h.

• Following p.o. administration of Red 28 (500 mg/kg), it was not possible to separate urine and feces due to the severe diarrhea that resulted.

• In order to determine the elimination profile of Red 28 following oral administration, a lower dose (50 mg/kg) was used. Following this dose, 99% was recovered in the feces after 96 h, the majority of which (94%) was excreted within the first 24 h. No dye was recovered in the urine over the 96-h period.

• The terminal half-lives were determined to be 2.8 h and 2.5 h following p.o. administration of a high and a low dose, respectively. Bioavailability was calculated to be 2.7% and 0.64 % for the high and low dose, respectively.

Conclusions

• Dietary exposure may be a better route to assess exposure / elimination because a high oral gavage dose of Red 28 (500 mg/kg) resulted in severe diarrhea that was not observed when the animals were allowed to consume the same level in the diet over a 24-h period. This suggests that the GI distress was due exclusively to the large bolus dose of Red 28.

• Because Red 28 present in the systemic compartment is eliminated exclusively in the feces, urinary excretion is not an adequate indicator of systemic exposure.

References

- Marmion D (1991) *Handbook of U.S. Colorants: Foods, Drugs, Cosmetics and Medical Devices*, 3rd ed., John Wiley and Sons, Inc., New York.
- Ito A, Fujimoto N, Okamoto T, Ando Y, and Watanabe H (1994) *Food Chem. Toxicol.* 32: 517-520.
- U.S. Food and Drug Administration (1982) *Fed. Register*. 47: 42566-42569.
- Sodi G, and Kaur J (2000) Organic fingerprint powders based on fluorescent phloxine B dye. *Defense Science Journal* 50(2): 213-215.
- Yoshikawa K, Kurata H, Iwahara S and Kada T (1978) Photodynamic action of fluorescein dyes in DNA-damage and in vitro inactivation of transforming DNA in bacteria. *Mutation Research* 56: 359-362.
- Kuroda Y (1975) Mutagenesis in cultured human diploid cells. *Mutation Research* 30: 239-248.
- Sako F, Kobayashi N, Watabe H and Taniguchi N (1980) Cytotoxicity of Food Dyes on Cultured Fetal Rat Hepatocytes. *Toxicology and Applied Pharmacology* 54: 285-292.
- Seno M, Fukuda S and Umisa H (1984) A teratogenicity study of phloxine B in ICR mice. *Food Chem. Toxic.* 22: 55-60.
- Webb JM, Fonda M and Brouwer EA (1962) Metabolism and excretion patterns of fluorescein and certain halogenated fluorescein dyes in rats. *J. Pharmacol. Exp. Therap.* 137: 141-147.

Acknowledgements

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