ADME NTP Study S0573 Benzethonium chloride

Sex/Species: young adult male F344 rats.
Vehicles: intravenous, 95% ethanol; dermal, 95% ethanol.

CASRN 121-54-0

Radiolabeled with carbon-14 in the benzyl ring; Benzethonium chloride, [benzyl ring(U)-^{14}C]-

Studies Performed:

- Single 0.15 mg/kg dose intravenous study in rats with sacrifice 24 hours postdose.

- Single 0.15 or 1.5 mg/kg dermal administration to rats with covered dose site and sacrifice at 6, 24, 48, 96, and 168 hours postdose.

- 10-day repeat dermal administration study in rats with a single daily dose of 1.5 mg/kg. Dose sites were covered and rats were sacrificed at 6, 24, 48, 96, and 168 hours following the radiolabeled dose. Unlabeled benzethonium chloride was administered on days 1-10 and [^{14}C]benzethonium chloride administered on day 11.

[^{14}C]Benzethonium chloride concentrations in urine and feces over 168 hours after radiolabeled dermal administration were represented in figures in the report that are not shown here. Whole blood levels after a single or repeated dermal administration were generally below the detection limits of 1.63×10^{-3} ug/mL (low dose) and 3.26×10^{-3} ug/mL (high dose). The total percent urinary excretion following a single or repeated dermal application (high dose) was 1.76 ± 0.16% and 1.37 ± 0.19%, respectively. After a single application, equivalents in the feces accounted for 48.2 ± 2.0% and 42.1 ± 3.5% of the applied dose in the low and high dose groups, respectively. After repeated application for 10 days, the total fecal excretion accounted for approximately 26.1 ± 2.6% of the dose.

[^{14}C]Benzethonium chloride concentrations in whole blood after intravenous administration were represented in figures in the report and are not shown here. After 24 hours following the intravenous administration, 3.58 ± 0.65% and 46.40 ± 10.80% of the administered dose were recovered in the urine and feces, respectively.

Toxicokinetics:

Following a single intravenous injection, a plot of the concentration in the blood versus time produced a biexponential curve. Pharmacokinetic modeling of this data showed benzethonium chloride was best fitted to a two compartment model which indicated it is readily distributed into secondary compartments (tissues) from the central compartment

(systemic circulation). The whole blood concentration time profiles were analyzed by fitting the data (based on patterns of residual and visual evaluation of goodness of fit) with the simplest compartmental pharmacokinetic model that described the data satisfactorily. The equation used to fit the [^{14}C]benzethonium chloride equivalent blood concentrations after intravenous injection was $Cp = Ae^{\alpha t} + Be^{-\beta t}$ where coefficients (A, B) and slopes of the semilogarithmic concentration vs. time plot (α, β) were determined by the iterative least-squares regression fitting program of SAS (NLIN).

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TABLE 1. TIME-RELATED TISSUE DISTRIBUTION OF ^{14}C-BENZETHONIUM CHLORIDE EQUIVALENTS (μg/g tissue) AS A PERCENT OF THE APPLIED DOSE AFTER A SINGLE OR REPEATED DERMAL APPLICATION

Treatment Group	Dose Group (mg/Kg)	Tissue Type	Hours After Application				
			6	24	48	96	168
Single	0.15	Liver	BDL	0.11	0.10	BDL	0.06
		Kidney	0.01	0.01	0.02	BDL	BDL
		Skeletal Muscle	BDL	0.02	0.02	BDL	0.04
		Heart	BDL	BDL	BDL	BDL	BDL
		Cardiac Blood	BDL	BDL	BDL	BDL	0.08
		Lungs	BDL	0.01	0.01	BDL	BDL
		Gonads	BDL	BDL	BDL	BDL	BDL
		Perirenal Fat	BDL	0.02	0.01	BDL	0.02
		Non-Appl. Site Skin	0.08 ± 0.02	0.05 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.01
		Appl. Site Skin	47.58 ± 5.27	37.19 ± 3.33	32.79 ± 7.70	14.19 ± 2.74	7.23 ± 1.27
		Residual Carcass	3.28 ± 1.75	2.37 ± 0.45	12.20 ± 1.42	4.27 ± 0.46	2.12 ± 0.21
Single	1.5	Liver	0.07	0.01 ± 0.00	0.02 ± 0.00	0.02	BDL
		Kidney	0.01	BDL	BDL	BDL	BDL
		Skeletal Muscle	0.01	0.01 ± 0.00	0.01	0.01	BDL
		Heart	BDL	BDL	BDL	BDL	BDL
		Cardiac Blood	BDL	BDL	BDL	BDL	BDL
		Lungs	BDL	BDL	BDL	BDL	BDL
		Gonads	BDL	BDL	BDL	BDL	BDL
		Perirenal Fat	BDL	BDL	BDL	BDL	BDL
		Non-Appl. Site Skin	0.42 ± 0.15	0.03 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
		Appl. Site Skin	31.27 ± 7.20	33.51 ± 4.20	20.29 ± 2.79	19.90 ± 4.09	9.81 ± 0.77
		Residual Carcass	1.42 ± 0.45	4.08 ± 1.81	11.15 ± 0.94	3.93 ± 0.50	1.71 ± 0.16

TABLE 1. (Continued)

Treatment Group	Dose Group (mg/Kg)	Tissue Type	Hours After Application				
			6	24	48	96	168
Repeated	1.5	Liver	0.02	0.04 ± 0.00	0.02	0.01	0.01
		Kidney	BDL	0.01 ± 0.00	BDL	BDL	BDL
		Skeletal Muscle	BDL	0.01 ± 0.00	BDL	BDL	BDL
		Heart	BDL	BDL	BDL	BDL	BDL
		Cardiac Blood	BDL	BDL	BDL	BDL	0.07
		Lungs	BDL	BDL	BDL	BDL	BDL
		Gonads	BDL	BDL	BDL	BDL	BDL
		Perirenal Fat	BDL	BDL	BDL	BDL	BDL
		Non-Appl. Site Skin	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	BDL
		Appl. Site Skin	47.43 ± 3.75	31.31 ± 3.21	22.75 ± 4.12	17.53 ± 3.56	6.24 ± 0.56
		Residual Carcass	1.20 ± 0.43	2.13 ± 0.29	13.25 ± 2.82	4.51 ± 0.49	1.69 ± 0.34

Data are expressed as the mean ± SEM of at least 4 animals. In those instances where the sample cell contains fewer than 4 values, only the mean is reported. Values less than 2.5 x background were designated as below detection limits (BDL). Detection limits for the low and high dose group animals were 1.75 and 3.60 ng of ^{14}C-Benzethonium Chloride equivalents/g tissue, respectively.

TABLE 2. ^{14}C-BENZETHONIUM CHLORIDE EQUIVALENTS IN SKIN LAYERS OF THE APPLICATION SITE

Treatment Group	Dose Group (mg/Kg)	Skin Layer Depth (microns)	% Applied Dose	% of the Total Amt. in the Skin
Single	0.15	0-500	22.46	99.60
		500-1000	0.02	0.09
		1000-1500	0.03	0.13
		1500-2000	0.03	0.13
		2000-3000	0.02	0.09
Single	1.5	0-500	29.03	99.55
		500-1000	0.08	0.27
		1000-1500	0.04	0.14
		1500-2000	0.01	0.03
		2000-3000	0.00	0.00
Repeated	1.5	0-500	21.89	99.45
		500-1000	0.10	0.45
		1000-1500	0.01	0.05
		1500-2000	0.01	0.05
		2000-3000	0.00	0.00

After a 24 hour contact period, the application site was washed, removed from the animal, and sliced horizontally into approximately 500 micron layers. The full thickness skin sample was shaved beginning with the deepest dermal layer (2000-3000 microns) and ending with the surface layer of the epidermis (0-500 microns). The data are expressed as the mean (n = 2) except for the single application high dose group (n = 1).

TABLE 3. BIOAVAILABILITY OF BENZETHONIUM CHLORIDE AFTER A SINGLE OR REPEATED DERMAL APPLICATION

Treatment Group	Dose Group (mg/Kg)	Systemic Bioavailability	Total Bioavailability
Single	0.15	52.3 ± 1.8	59.5 ± 2.1
Single	1.5	45.9 ± 3.6	55.6 ± 3.2
Repeated	1.5	29.2 ± 2.3	35.5 ± 1.9

Data are expressed as the mean ± SEM (n = 4) based upon the percent of applied dose recovered in the urine, feces, and tissues exluding the application site skin (systemic bioavailability) or including the application site skin (total bioavailability).

Table 4. Pharmacokinetic Parameters for the [^{14}C]Benzethonium Chloride Equivalents in Whole Blood versus Time Curve Following a Single 1.5 mg/kg Intravenous Administration to Male Rats

Parameter Name	Parameter Value
Cl (mL/min/kg)	14.8 ± 0.9
Vd_{β} (L/kg)	2.3 ± 0.1
Vd_{ss} (L/kg)	5.5 ± 0.3
$t_{1/2}$ (min)	110.2 ± 8.0
K_e (min^{-1})	0.0063

a. AUC (value not given) – The area under the curve was estimated by the linear trapezoidal rule using the SAS program, NLIN.
b. Cl – The total clearance was calculated by dividing the dose (mg/kg) by the AUC (ug/mL•min).
c. $Vd_{ß}$ – The terminal volume of distribution was calculated by dividing the total clearance by the terminal logarithmic slope [ß (min^{-1})].
d. Vd_{ss} – The volume of distribution at steady state was calculated as Dose * $AUMC/AUC^2$ (where AUMC is the time integral of the product of concentration and time).
e. $t_{1/2}$ – The elimination half-life was calculated from the equation, $t_{1/2}$ = 0.693/ ß (min).