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FOR THE COMMANDER

CORNELIUS R. FAY III
Lieutenant Colonel, MS
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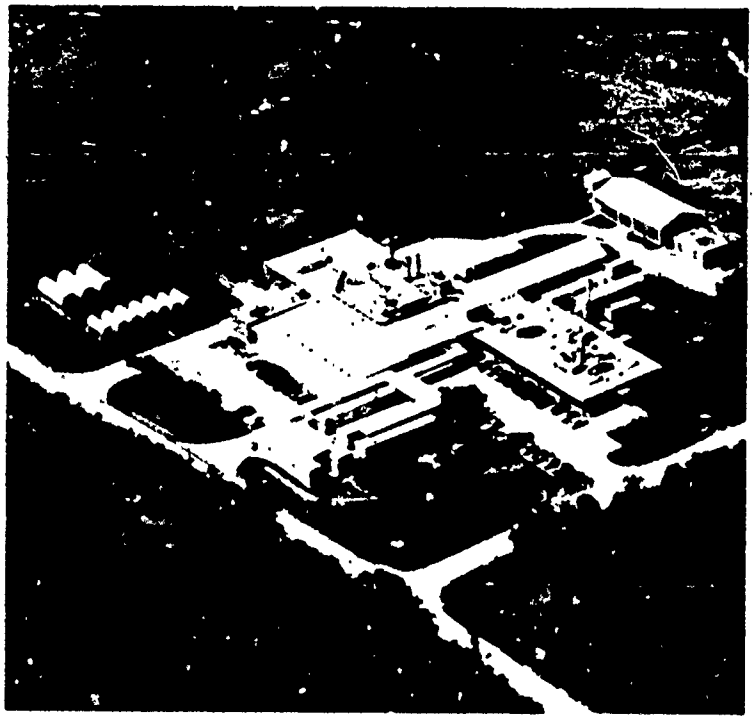
REPORT

(1)

FINAL REPORT



Task 90-15: Crossover Comparison of the Pharmacokinetics of Atropine and Pralidoxime Chloride in Three Multichambered Autoinjector Systems and the Mark I

To

U.S. Army Medical Research

and Development Command

Institute of Chemical Defense

March, 1991

FINAL REPORT

Contract DAMD17-89-C-9050
A Medical Research and Evaluation Facility (MREF) and Studies Supporting the Medical Chemical Defense Program

on

TASK 90-15: CROSSOVER COMPARISON OF THE PHARMACOKINETICS OF ATROPINE AND PRALIDOXIME CHLORIDE IN THREE MULTICHAMBERED AUTOINJECTOR SYSTEMS AND THE MARK I

to

U.S. ARMY MEDICAL RESEARCH
AND DEVELOPMENT COMMAND

March, 1991

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19 ABSTRACT (Continue on reverse if necessary and identify by block number)

Eight sheep were injected in a crossover study with four different autoinjectors with at least a 1-week wash out between autoinjectors. The pharmacokinetics of atropine and pralidoxime chloride was used to compare the different autoinjectors against the standard Mark I. Results were normalized for dose since there was significant difference in the doses contained between the different autoinjectors. When two of the autoinjectors (MCA-A and MCA-B) were used, the atropine and 2-PAM were absorbed more rapidly than with the standard or the third autoinjector (MCA). The maximum 2-PAM concentrations were also higher with these two. The MCA-A resulted in higher maximum atropine concentrations.

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In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health (NIH), Publication No. 86-23, Revised 1985).

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FINAL REPORT

on

TASK 90-15:
CROSSOVER COMPARISON OF THE PHARMACOKINETICS OF ATROPINE AND PRALIDOXIME CHLORIDE IN THREE MULTICHAMBERED AUTOINJECTOR SYSTEMS AND THE MARK I

to

U.S. ARMY MEDICAL RESEARCH
AND DEVELOPMENT COMMAND

March, 1991

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QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to management and the study director as follows:

Phase	Date
Catheter insertion and removal, IM injection of four animals (1 of 4 type injectors per animal) blood collectors, centrifugation, and serum storage.	07/25/90
Set-up and analysis of plasma samples for 2-PAM analysis by spectrometric auto analyzer	07/31/90
Audit-chemistry notebook and data.	08/27/90
Stock preparation, reagent addition	09/06/90
Precipitation of antibodies	09/07/90
Audit/atropine injector chemistry data	10/24/90
Audit/atropine RIA data	11/07/90
Audit/Draft Final Report	01/03/91

Report to Study Director and Management: 7/25, 8/3, 8/27, 9/7, 10/24, 11/27/90, and 1/3/91.

To the best of my knowledge, the methods described were the methods followed and the data presented accurately represent data generated during the study.

James D Carter 1/7/90

Quality Assurance Unit Date
Health and Environment Group

GOOD LABORATORY PRACTICES COMPLIANCE STATEMENT

To the best of my knowledge, all aspects of this study were conducted in compliance with the U.S. Food and Drug Administration's Good Laboratory Practices regulations (21 CFR Par. 58). This report was reviewed by Battelle's Quality Assurance Unit to verify that the information contained herein accurately depicts the data collected in the study.

Carl T. Olson 1-4-91

Carl T. Olson, D.V.M., Ph.D. Date
Study Director

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TASK 90-15: CROSSOVER COMPARISON OF THE PHARMACOKINETICS OF ATROPINE AND PRALIDOXIME CHLORIDE IN THREE MULTICHAMBERED AUTOINJECTOR SYSTEMS AND THE MARK I

1.0 INTRODUCTION

The U.S. Army Medical Materiel Development Activity (USAMMDA) is currently evaluating candidate multichambered antidote autoinjector systems in order to select one to replace the Mark I (MKI) as a field treatment for nerve agent intoxication. USAMMDA requires information on the comparative pharmacokinetics of the two active components, atropine and pralidoxime chloride (2-PAM), following injection with the multichambered systems to select the one autoinjector optimal for further development. The objective of this task was to determine, in compliance with the Food and Drug Administration's Good Laboratory Practices (GLP), the pharmacokinetics of atropine and 2-PAM in sheep when delivered using either of three candidate autoinjector systems or the MKI. The experiment was designed as a crossover study, with each of eight sheep receiving atropine and 2-PAM delivered by each of the four autoinjector systems with at least a one-week washout period between injections.

2.0 EXPERIMENTAL DESIGN

2.1 Test Animals

Sheep were used for this study because of similarities with man in body weight and because sheep have been used in similar pharmacokinetic studies with intramuscularly (IM) administered drugs.[1,2,3] Approximately 1-year-old wethers of Rambouillet-Columbia breeding were obtained from Thomas D. Morris, Inc., Reisterstown, MD. All sheep had serology performed prior to shipment to Battelle's Medical Research and Evaluation Facility (MREF) and were negative for antibody titers for the Q fever causative organism, Coxiella burnetii. Upon arrival at the MREF, sheep were examined by a veterinarian, and blood and fecal samples were obtained for clinical pathological and gastrointestinal parasite evaluations. Sheep were held in quarantine for a minimum of seven days prior to use in the study. All animals

were tagged in the ear to retain positive identification, and were maintained in an outdoor fenced area with available shelter until brought into the laboratory for experimentation. Sheep were fed Purina Rumilab® with limited quantities of locally purchased hay. Water was supplied from Battelle's West Jefferson water system ad libitum. The water is analyzed quarterly for potability, and annually for contaminants. No contaminants which would interfere with the results of the study are known to be present in the water or feed.

Sheep were shorn, brought into the laboratory, and maintained on straw bedding in animal rooms kept at 65 15 F with a relative humidity of 50 20 percent. Fluorescent lighting with a light/dark cycle of 12 hr each per day was used. Sheep were acclimated to placement in a sling suspended from a stand for a minimum of 20 min per day for two days prior to experimentation, and they routinely adapted rapidly to this method of restraint. At the start of the study, the sheep weighed between 65 and 81 kg and appeared to be in good physical condition.

2.2 Materials and Methods

Three candidate multichambered autoinjectors containing atropine and 2-PAM and the fielded MKI autoinjector system were provided by USAMMDA. The MKI autoinjector system (Lot RU8243/RU7213), consisting of two separate injectors - an atropen which is designed to deliver 2 mg of atropine sulfate equivalents in an approximately 0.7 mL volume and a 2-PAM injector designed to deliver 600 mg 2-PAM in an approximately 2 mL volume, was used as a standard. One of the multichambered autoinjectors was a dispersion model (MCA; Lot RD1071) designed to deliver both 2 mg atropine sulfate equivalents and 600 mg 2-PAM in a single injection of approximately 2.7 mL. The other two candidate multichambered autoinjectors (MCA-A, Lot FDM90C09R and MCA-B, Lot FDM90C08P) were also designed to deliver amounts of atropine and 2-PAM similar to that of the MKI in single injections of approximately 2.7 mL. Contents from samples of each autoinjector type were analyzed to confirm identity and quantitate the amount of atropine (MREF SOP-89-55) and 2-PAM (MREF SOP-88-39) delivered by each system. All autoinjectors were weighed

prior to use, and again after injections were made, to confirm delivery of the autoinjector contents.

On each day of study, four sheep were restrained in nylon web slings suspended from metal stands. Sheep were given atropine/2-PAM IM in the anterior lateral area of the right thigh, in the area of the vastus lateralis head of the quadriceps femoris muscle, dosing one sheep with each autoinjector system each day. Ten-mL blood samples were taken from a jugular vein through an indwelling catheter (French 8 Catheter Sheath Introducer System, Cordis Corp., Miami, FL) or with a disposable 10-mL syringe and 18-ga 1.5-inch needle (Becton Dickinson, Rutherford, NJ) if the catheter was not patent. The 17-cm rigid plastic vessel dilator, rather than the sheath assembly itself, was used as the catheter because the flexible sheath assembly would collapse and become crimped whenever an animal turned his head to the side. The vessel dilator was loosely fixed in place with a stay suture placed in the skin, and a three-way stopcock was attached to the catheter. Blood samples were taken prior to injection of atropine/2-PAM and at 1, 2, 3, 4, 5, 6, 8, 12, 15, 20, 40, 60, 80, 120, 180, and 240 min after injection. Seven-tenths mL of heparinized physiologic saline (30 units/mL) was used as a block in the three-way stopcock and indwelling catheter to prevent clotting of blood during the longer intervals between blood collections. The heparin block was removed by withdrawing a 1-mL volume before drawing the 10-mL blood sample for analysis. Five mL of the 10-mL blood sample drawn using a 10-mL disposable syringe was immediately placed in a prelabeled, heparinized glass vacutainer® (Becton Dickinson). The other 5 mL was placed in a prelabeled 13-mL polypropylene tube with cap. This tube was placed on its side and the blood allowed to clot at room temperature for at least 1 hr. Sheep were removed from slings after the 120-min blood samples were drawn and allowed access to feed and water. Catheters were left in place until after the 4-hr blood samples were drawn.

The heparinized blood samples were transferred to labeled polypropylene tubes and centrifuged at approximately 1,500 X G for 15 min. Then the plasma was removed with pipettes, put into labeled polypropylene tubes, and frozen at approximately -70 C until assayed for 2-PAM. Analyses for 2-PAM concentration were conducted at the MREF using an ultraviolet spectrophotometric technique with a Technicon (Tarrytown, NY) autoanalyzer

(MREF SOP-88-50). After the blood samples in the non-heparinized tubes had clotted, the blood clots were gently separated from the sides of the tubes with applicator sticks. The tubes were then centrifuged at approximately 800 X G for 15 min and the serum was pipetted into labeled polypropylene tubes and frozen at approximately -70 C. Serum samples were hand-carried to the laboratory of Dr. Larry Miller at Battelle's Columbus site for determination of atropine concentrations using radioimmunoassay (RIA) techniques (SOP Number: TOX VI-014-00).

2.3 Pharmacokinetic Analyses

The study used a Latin squares design which was balanced for sequence of injection, day of testing effects, and residual effects. The sequence in which sheep received injections is given in Table 1. Once blood concentrations of atropine and 2-PAM were determined, concentrations as a function of time, maximum concentrations, times to maximum concentrations, areas under the blood concentration-time curves from 0 to 240 min, absorption and elimination rate constants, and apparent volumes of distribution were estimated using the pharmacokinetic model which best represented the data.

TABLE 1. TREATMENT SCHEDULE

	Sheep Number			
	87	127	93	104
Day 1	MKI	MCA	MCA-A	MCA-B
Day 3	MCA	MCA-A	MCA-B	MKI
Day 5	MCA-B	MKI	MCA	MCA-A
Day 7	MCA-A	MCA-B	MKI	MCA
	117	129	116	123
Day 2	MKI	MCA	MCA-A	MCA-B
Day 4	MCA-B	MKI	MCA	MCA-A
Day 6	MCA	MCA A	MCA-B	MKI
Day 8	MCA-A	MCA B	MKI	MCA

Sixty-four separate pharmacokinetic analyses were performed - serum atropine following use of each of the four autoinjector systems in eight sheep, and plasma 2-PAM for each autoinjector used in eight sheep. Although blood sampling times were established, it was not always possible to draw samples exactly at desired times, usually because of blood flow in the catheters. Times at which blood samples were actually obtained were recorded for each animal at each sampling time and pharmacokinetic parameters were estimated using the actual times of blood collection. Atropine concentrations less than 1 ng/mL, the limit of reliable quantitation, were considered as zero for the pharmacokinetic analyses. The quantifiable limit for 2-PAM was 0.3 μg/mL and values below this concentration were also considered as zero for pharmacokinetic analyses.

Statistical analyses of the pharmacokinetic data were accomplished to determine if any significant differences existed among the 2-PAM and atropine pharmacokinetic parameters estimated for the four different autoinjectors. Empirical data for C_{max}, t_{max}, and AUC_{0-240}, i.e., the actual highest blood concentration measured, the actual sampling time of this highest concentration, and the area under the measured blood concentrations over time curve to 240 min derived by the trapezoidal method, as well as pharmacokinetic parameters predicted by models were statistically evaluated. The correlations between empiric and model estimates were determined to assess the "goodness of fit" of the models.

Pharmacokinetic parameters were analyzed to determine if there were any effects due to autoinjector or week of testing, and to assess the variability in the pharmacokinetic parameters among the animals. Experiments in which the same animals are tested on multiple occasions using different treatment regimens on different testing days are called crossover designs. By using a crossover design, comparisons between the pharmacokinetic parameters across autoinjectors can be made on an individual animal basis. Controlling for the animal-to-animal variability by using each animal as its own control provides more precise comparisons across the autoinjectors. Special considerations may arise because the effects of a treatment administered in one test period may carry over to the next test period (residual effect). Therefore, an animal's blood levels may be affected directly by the

most recent treatment and also by a residual effect from the previous treatment. A relatively long washout and recovery period between dosing was used to prevent residual effects.

An analysis of variance appropriate for crossover designs was carried out for each empirical and model-based estimated pharmacokinetic parameter to assess the statistical significance of the effects of interest. The effects included in the analysis of variance are given in the following equation for a generic pharmacokinetic parameter Y:

$$Y = \mu + \beta + \gamma + \tau + \rho + \epsilon$$

where μ = average value of the pharmacokinetic parameter,

β = effect of animal,

γ = effect of week of testing,

τ = direct effect of the autoinjector used that week,

ρ = residual effect of the dose injected in the preceding week of testing, and

ϵ = uncontrolled variation within an animal.

3.0 RESULTS

3.1 Chemistry

Results of chemical analyses for atropine and 2-PAM content from three injectors of each autoinjector system are presented in Table 2. 2-PAM content exceeded 600 mg in all systems, but atropine content averaged 1.73 mg in the MKI, 1.95 mg in the MCA, 2.09 mg in the MCA-A, and 2.12 mg in the MCA-B.

TABLE 2. CHEMICAL ANALYSES FOR ATROPINE AND 2-PAM CONTENT OF AUTOINJECTOR SYSTEMS

Atropine Analyses:

Expected 2.0 mg atropine sulfate equivalents in each syringe.

Syringe Type	Lot No. (Date of Manufacture)	Measured Volume (mL)	Measured Concentration (mg/mL)	Atropine Sulfate Equivalents (mg)
MKI (A138)	RU7213	0.685	2.43	1.66
MKI (A142)	(9/85)	0.660	2.33	1.54
MKI (A167)		0.670	2.97	1.99
MCA (08)	RD1071	2.85	0.67	1.91
MCA (20)	(5/90)	2.80	0.72	2.02
MCA (31)		2.80	0.69	1.93
MCA-A (06A)	FDM90C09R	2.70	0.79	2.13
MCA-A (18A)	(3/9/90)	2.80	0.73	2.04
MCA-A (38A)		2.70	0.78	2.11
MCA-B (12B)	FDM90C08P	2.65	0.79	2.09
MCA-B (23B)	(3/9/90)	2.78	0.79	2.20
MCA-B (37B)		2.65	0.78	2.07

2-PAM Analyses:

Expected 600 mg 2-PAM in each syringe.

Syringe Type	Lot No. (Date of Manufacture)	Measured Volume (mL)	Measured Concentration (mg/mL)	2-PAM (mg)
MKI (P134)	RU8243	1.98	325.5	644
MKI (P155)	(9/85)	1.93	326.0	629
MKI (P190)		2.00	325.4	651
MCA (08)	RD1071	2.85	249.2	710
MCA (20)	(5/90)	2.80	237.9	666
MCA (31)		2.80	241.1	675
MCA-A (06A)	FDM90C09R	2.70	235.9	637
MCA-A (18A)	(3/9/90)	2.80	224.0	627
MCA-A (38A)		2.70	235.9	637
MCA-B (12B)	FDM90C08P	2.65	234.6	622
MCA-B (23B)	(3/9/90)	2.78	236.5	657
MCA-B (37B)		2.65	235.1	623

3.2 Pharmacokinetics

Mean concentration values for all sheep at all time points in a pharmacokinetic evaluation for each autoinjector system were graphed using a personal computer to determine the type of model best fit by the data and to determine initial estimates, or "seed" values, for parameters of that model. Mean data best fit a two-compartment model for both atropine and 2-PAM, although in some animals, especially with atropine, a one-compartment model could have been used.

Parameters estimated were A, B, α, β, and k_a in the following equation:

$$C(t) = A(e^{-\alpha t}-e^{-k_a t}) + B(e^{-\beta t}-e^{-k_a t}), \quad (1)$$

where C(t) is the serum concentration at time t after dosing, A is the y-intercept of the points in the distribution or the fast composite rate phase regressed to time zero, α is the slope of this distribution or the fast composite rate phase line, B is the y-intercept of the points in the elimination or slow composite rate phase regressed to time zero, and β is the slope of this elimination or slow rate composite phase line; k_a is the first order rate constant for appearance of a drug in the systemic circulation.

Equation (1) is derived from the following more commonly used equation for a 2-compartment model.

$$C(t) = Ae^{-\alpha t} + Be^{-\beta t} - Ke^{-kt}, \quad (2)$$

where k is equal to k_a in equation (1). By definition, K = A + B (at t = 0, the amount of drug in the body, C(0), equals 0). Therefore,

$$C(t) = Ae^{-\alpha t} + Be^{-\beta t} - Ke^{-kt} =$$

$$Ae^{-\alpha t} + Be^{-\beta t} + (-A-B)e^{-k_a t} =$$

$$Ae^{-\alpha t} - Ae^{-k_a t} + Be^{-\beta t} - Be^{-k_a t} =$$

$$A(e^{-\alpha t}-e^{-k_a t}) + B(e^{-\beta t}-e^{-k_a t})$$

After obtaining the initial parameter estimates for each sheep, the data were transmitted to a VAX mainframe computer for more precise estimation of the same parameters using the Statistical Analysis System (SAS; Cary, NC) NONLIN regression procedure, and for calculation of the following:

C_{max} = peak or maximum concentration (ng of atropine/mL or μg of 2-PAM/mL)

$= A(e^{-\alpha t_{max}} - e^{-k_a t_{max}}) + B(e^{-\beta t_{max}} - e^{-k_a t_{max}})$,

t_{max} = time after dosing when C(t) was maximum (min)

$= [\ln(k_a/k_{el})]/(k_a - k_{el})$

AUC_{0-240} = area under the drug concentration versus time curve from t = zero to t = 240 min (ng of atropine*min/mL or μg of 2-PAM*min/mL)

$= \frac{1}{2} C(t_0) + \sum_{i=1}^{239} C(t_i) + \frac{1}{2} C(t_{240})$ by the trapezoidal rule

k_{el} = the first-order rate constant for drug elimination by all routes (min^{-1})

$$= \frac{\alpha\beta[A(k_a-\alpha)+B(k_a-\beta)}{A\beta k_a + B\alpha k_a - (A+B)\alpha\beta}$$

$V_{d\beta}$ = overall apparent volume of distribution of a drug that obeys two-compartment model kinetics as calculated by the area method (L)

$= V_1(k_{el}/\beta)$ where $V_1 = Dose/(A+B)$

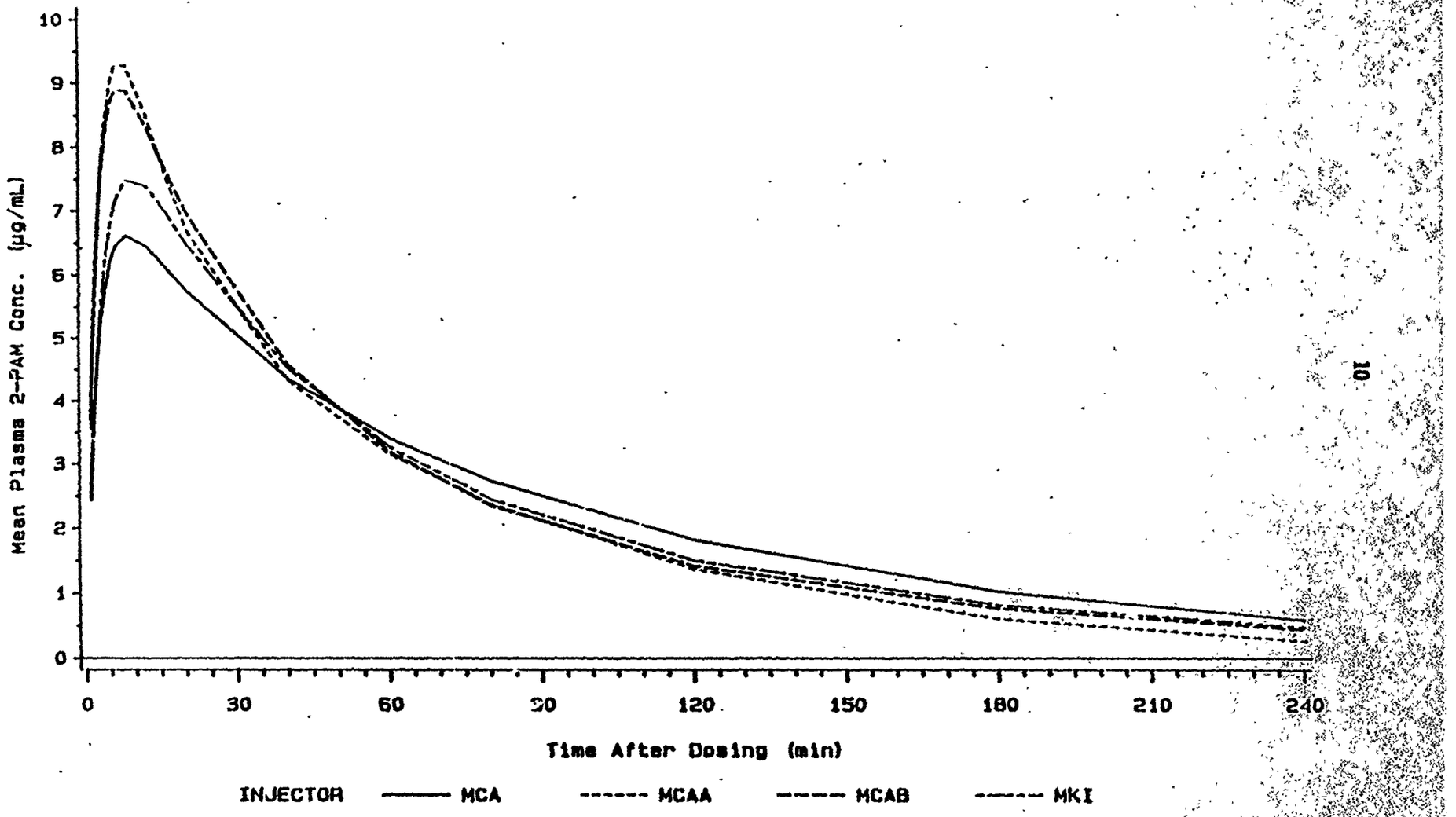
A sample of the computer program used is included in Appendix D.

3.3 Statistical Analyses

3.3.1 2-PAM Pharmacokinetics

Measured 2-PAM plasma concentrations for each animal at each time point for all four autoinjector systems and pharmacokinetic parameters are presented in Appendix C. Figure 1 is a graph of mean plasma 2-PAM

FIGURE 1. MEAN PLASMA 2-PAM CONCENTRATIONS FOLLOWING INJECTION OF EIGHT SHEEP USING FOUR DIFFERENT AUTOINJECTORS



concentrations over time following injection of eight sheep using each of the four autoinjectors. Empirically derived values of the 2-PAM pharmacokinetic parameters AUC_{0-240}, C_{max}, and t_{max} are presented in Table 3. 2-PAM pharmacokinetic parameters calculated from the two-compartment model are shown in Tables 4 and 5. Model-based estimates of AUC_{0-240}, C_{max}, and t_{max} are plotted against the empirically determined values in Figures 2, 3, and 4. The plots demonstrate that strong linear relationships exist between model-based and empirically determined values. Correlations were determined to be statistically different (at the 5 percent significance level) from zero for all three parameters. Correlations calculated between the two sets of estimates are:

Parameter	n	Correlation	P-value
AUC_{0-240}	32	0.996	0.0002
C_{max}	32	0.997	0.0001
t_{max}	32	0.957	0.0003

A hypothesis test was conducted for each pharmacokinetic parameter to assess the statistical significance of any residual effects; results are shown in Table 6. Residual effects were determined to be statistically insignificant for all but one of the parameters analyzed, empirically estimated C_{max} ($P = 0.04$). Considering the number of parameters analyzed, the marginal significance of one out of ten parameters is compatible with what may result from random chance. Therefore, residual effects were dropped from the model, and a second analysis of variance was carried out to assess the effects of autoinjector, animal-to-animal variability, and week of testing.

Table 7 summarizes the results of the statistical analyses and hypothesis testing for autoinjector, animal-to-animal, and week of testing variability. The average values of the pharmacokinetic parameters estimated for each of the four autoinjectors are shown in the second through fifth columns of the table. Because the experiments were balanced across autoinjector systems, the standard errors of the averages are identical for each of the autoinjectors. The standard error of the average pharmacokinetic

TABLE 3. 2-PAM PHARMACOKINETIC PARAMETERS AUC_{0-240}, C_{max}, and t_{max} DERIVED[a] FROM EMPIRICAL DATA

Animal	Test Week	Auto Injector	AUC_{0-240} (μg*min/mL)	C_{max} (μg/mL)	t_{max} (min)
87	1	MKI	535.6	6.69	6.0
	2	MCA	500.8	7.59	6.0
	3	MCA-B	460.3	8.61	5.0
	4	MCA-A	413.4	8.07	5.0
93	1	MCA-A	649.5	9.14	5.0
	2	MCA-B	645.8	10.36	8.0
	3	MCA	505.6	4.18	40.0
	4	MKI	574.0	8.09	12.0
104	1	MCA-B	478.2	5.10	5.0
	2	MKI	486.7	4.72	20.0
	3	MCA-A	455.6	5.92	5.0
	4	MCA	526.7	6.25	16.0
116	1	MCA-A	501.2	8.96	6.0
	2	MCA	571.9	9.71	12.0
	3	MCA-B	462.0	7.77	8.0
	4	MKI	526.0	7.89	16.0
117	1	MKI	550.8	7.20	8.0
	2	MCA-B	645.1	10.40	8.0
	3	MCA	528.9	6.70	12.0
	4	MCA-A	568.2	9.40	5.0
123	1	MCA-B	838.3	14.97	6.0
	2	MCA-A	729.4	13.09	6.0
	3	MKI	706.5	14.22	6.0
	4	MCA	763.9	15.03	5.0
127	1	MCA	584.9	3.57	61.5
	2	MCA-A	501.2	10.61	6.0
	3	MKI	444.8	6.58	16.0
	4	MCA-B	472.4	11.06	4.0
129	1	MCA	595.4	4.66	20.0
	2	MKI	641.3	7.62	6.0
	3	MCA-A	665.1	10.64	8.0
	4	MCA-B	558.9	6.00	20.0

[a] AUC_{0-240} was calculated from the observed 2-PAM concentration-time curve using the trapezoid method; C_{max} is the maximum observed concentration, and t_{max} is the time point corresponding to the maximum observed concentration.

TABLE 4. 2-PAM PHARMACOKINETIC PARAMETERS A, B, α, β AND k_a FROM TWO-COMPARTMENT MODEL

Animal	Test Week	Auto Injector	A	B	α	β	k_a (min^{-1})
87	1	MKI	1.20	5.55	0.0115	0.0114	0.842
	2	MCA	26.43	6.98	0.1459	0.0141	0.202
	3	MCA-B	17.40	7.55	0.2105	0.0171	0.345
	4	MCA-A	31.93	6.66	0.2093	0.0170	0.283
93	1	MCA-A	7.87	3.23	0.0228	0.0095	0.460
	2	MCA-B	7.87	3.95	0.0220	0.0132	0.577
	3	MCA	25.63	1.53	0.0239	0.0040	0.034
	4	MKI	0.74	10.08	0.0035	0.0205	0.246
104	1	MCA-B	-0.06	5.70	0.0023	0.0103	0.528
	2	MKI	4.73	13.98	0.0087	0.0619	0.085
	3	MCA-A	16.06	5.96	0.2349	0.0124	0.292
	4	MCA	0.64	7.10	0.0159	0.0134	0.277
116	1	MCA-A	32.05	7.31	0.1755	0.0158	0.247
	2	MCA	21.10	6.29	0.0869	0.0129	0.182
	3	MCA-B	7.06	6.81	0.0908	0.0156	0.263
	4	MKI	9.15	4.60	0.0423	0.0113	0.178
117	1	MKI	5.07	3.38	0.0258	0.0081	0.532
	2	MCA-B	11.61	0.83	0.0236	0.0016	0.483
	3	MCA	13.36	5.96	0.1022	0.0110	0.172
	4	MCA-A	34.51	6.67	0.1526	0.0125	0.223
123	1	MCA-B	16.13	6.16	0.0527	0.0094	0.328
	2	MCA-A	13.05	6.40	0.0614	0.0107	0.364
	3	MKI	98.28	7.30	0.1287	0.0121	0.166
	4	MCA	31.10	10.69	0.1334	0.0157	0.254
127	1	MCA	8.37	6.68	0.0152	0.0074	0.021
	2	MCA-A	8.15	7.13	0.0892	0.0164	0.468
	3	MKI	8.20	0.58	0.0231	0.0008	0.284
	4	MCA-B	37.94	8.17	0.1827	0.0196	0.268
129	1	MCA	-2.31	8.16	0.0174	0.0095	0.132
	2	MKI	5.24	7.82	0.2266	0.0114	0.394
	3	MCA-A	56.44	8.17	0.1326	0.0130	0.172
	4	MCA-B	-4.94	8.72	0.0800	0.0136	0.461

TABLE 5. 2-PAM PHARMACOKINETIC PARAMETERS CALCULATED FROM A, B, α, β, AND k_a BASED ON TWO-COMPARTMENT MODEL

Animal	Test Week	Auto Injector	k_{el} (min^{-1})	AUC_{0-240} ($\mu g*min/mL$)	C_{max} ($\mu g/mL$)	t_{max} (min)	V_1 (L)	V_{d3} (L)
87	1	MKI	0.011	545.0	6.27	5.2	89.0	89.1
	2	MCA	0.027	495.3	7.62	11.5	18.0	34.6
	3	MCA-B	0.031	444.2	8.31	7.7	24.0	43.4
	4	MCA-A	0.036	401.3	7.70	8.4	15.5	32.7
93	1	MCA-A	0.016	623.0	9.28	7.5	54.1	91.5
	2	MCA-B	0.018	621.8	10.18	6.2	50.8	69.0
	3	MCA	0.014	498.0	4.04	45.2	22.1	74.4
	4	MKI	0.015	563.4	8.02	12.1	55.5	11.0
104	1	MCA-B	0.011	485.8	5.11	7.5	106.4	110.2
	2	MKI	0.015	482.4	4.54	25.0	32.1	7.6
	3	MCA-A	0.019	449.0	5.60	10.1	27.2	41.0
	4	MCA	0.014	518.8	6.30	11.4	77.5	78.5
116	1	MCA-A	0.033	474.7	8.58	9.4	15.2	32.0
	2	MCA	0.029	556.6	9.76	12.0	21.9	49.4
	3	MCA-B	0.024	451.9	7.68	10.0	43.2	66.2
	4	MKI	0.021	519.5	7.85	13.7	43.7	79.9
117	1	MKI	0.014	538.6	7.22	7.1	71.0	119.6
	2	MCA-B	0.012	628.6	10.18	7.8	48.2	364.2
	3	MCA	0.020	521.2	6.53	14.3	31.0	55.3
	4	MCA-A	0.030	548.4	8.89	10.4	14.6	34.9
123	1	MCA-B	0.022	822.3	14.56	8.8	26.9	62.5
	2	MCA-A	0.022	713.1	12.78	8.2	30.9	65.0
	3	MKI	0.039	695.4	13.07	11.4	5.7	18.5
	4	MCA	0.033	733.8	14.34	9.2	14.4	30.3
127	1	MCA	0.009	582.4	3.47	69.8	39.9	48.7
	2	MCA-A	0.027	485.2	10.26	6.4	39.3	65.4
	3	MKI	0.008	449.8	6.44	13.0	68.3	653.2
	4	MCA-B	0.044	447.4	10.36	8.1	13.0	28.9
129	1	MCA	0.008	596.5	4.72	22.5	102.7	88.2
	2	MKI	0.014	632.8	7.39	8.7	45.9	58.5
	3	MCA-A	0.030	650.5	10.21	12.3	9.3	21.5
	4	MCA-B	0.008	545.2	5.25	9.0	58.7	89.5

FIGURE 2. PLOT OF MODEL-BASED AUC AGAINST EMPIRICAL AUC FOR 2-PAM

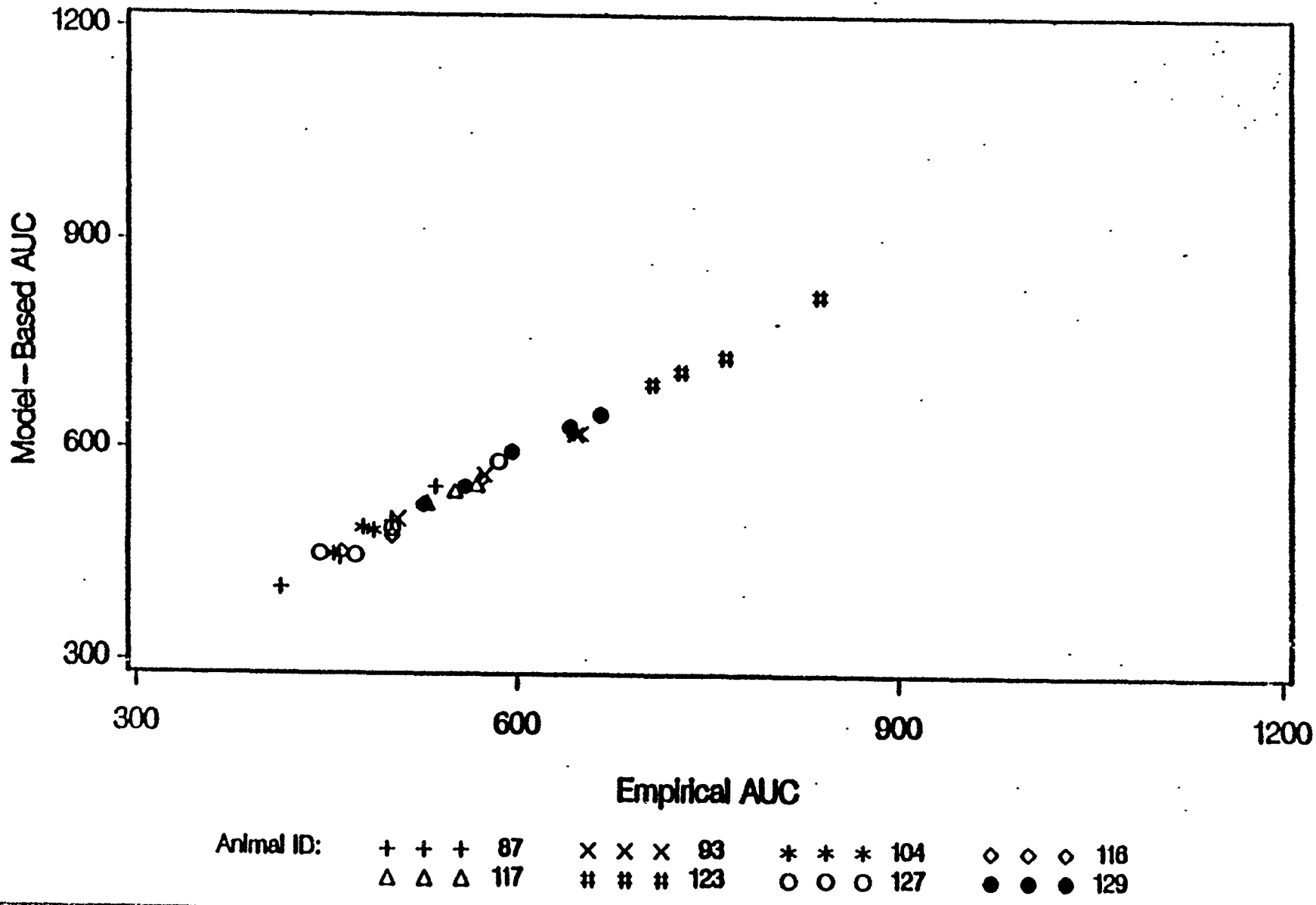
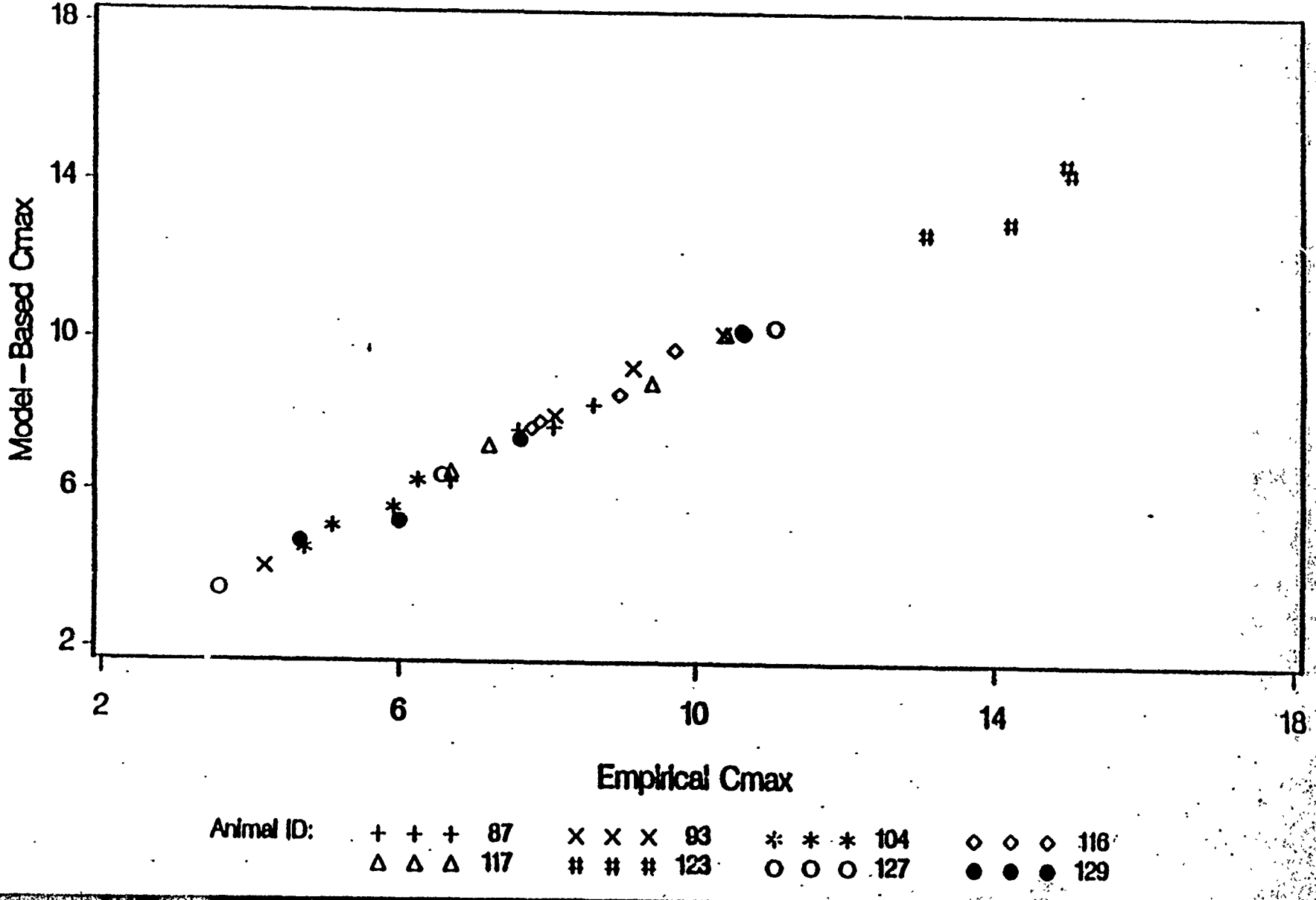


FIGURE 3. PLOT OF MODEL-BASED Cmax AGAINST EMPIRICAL Cmax FOR 2-PAM



effects is significant and yet the multiple comparison procedure fails to identify significant differences between any two autoinjector means. Multiple comparison tests identified significant differences between average values of empirically estimated t_{max}, and model-based k_a for specific pairs of autoinjectors. For empirically estimated t_{max}, the MCA-A group mean was significantly less than the MCA group mean. For k_a, the MCA-B group mean was significantly greater than the MCA group mean.

The variations in the pharmacokinetic parameters over the four weeks of testing were determined to be statistically insignificant for all but two of the parameters, empirically estimated and model-based AUC_{0-240}. There was no observable trend in AUC_{0-240}, however, over the weeks of testing, i.e., average weekly 2-PAM AUC_{0-240} neither consistently increased nor decreased. The between animal variance component was determined to be statistically significant for both the empirically estimated and model-based parameters AUC_{0-240} and C_{max}. These animal effects were strongly influenced by the effect of animal 123, which achieved higher 2-PAM blood concentrations than other animals for all four autoinjectors.

Analyses of variance and multiple test comparison results for the 2-PAM pharmacokinetics parameters may be summarized as follows:

(1) Autoinjector effects were statistically significant for empirically estimated C_{max} and t_{max}, and model-based t_{max} and k_a. Autoinjector effects were marginally significant for model-based C_{max}.

(2) Effects of test week were not significant for eight of ten analyzed parameters. Effects of animal-to-animal variation were significant for four parameters, empirically estimated and model-based AUC_{0-240} and C_{max}, largely due to the effect of one animal which had consistently higher maximum concentrations than other animals.

(3) The MCA-B autoinjector group mean was the highest for k_a, one of the two highest for both empirical and model-based C_{max}, and the shortest for model-based t_{max}. The MCA group mean k_a was less than half of that estimated for the other groups, and the MCA mean (empirical and model-based) t_{max} was twice as long as that estimated for the other groups.

TABLE 6. ASSESSMENT OF CARRY-OVER EFFECTS FOR 2-PAM PHARMACOKINETIC PARAMETERS

Parameter	F-Value	P-Value
	Empirically[a] Derived Parameters	
AUC_{0-240}	1.19	0.35
C_{max}	3.56	0.04
t_{max}	1.47	0.26
	Model[b] Based Parameters	
k_a	1.08	0.39
k_{el}	0.62	0.61
AUC_{0-240}	0.88	0.47
C_{max}	3.1	0.06
t_{max}	1.37	0.29
V_1	1.87	0.18
$V_{d\beta}$	1.08	0.39

(a) Derived from observed 2-PAM concentration-time curve.
(b) Two-compartment model.

TABLE 7. SUMMARY OF STATISTICAL ANALYSIS OF AUTOINJECTOR, ANIMAL TO ANIMAL, AND WEEK OF TESTING VARIABILITY FOR 2-PAM PHARMACOKINETIC PARAMETERS

Pharmacokinetic Parameter (units)	Effect of Autoinjector: Model Predicted Average MCA	MCA-A	MCA-B	MKI	SE[a] of Average	F-Value	P-Value[b]	Animal Variability[c]: σ^2_A	σ^2_A/σ^2_e	P-Value	Week of Dosing: F-Value	P-Value
Empirical Parameters												
AUC_{0-240} (μg•min/mL)	572.26	588.43	578.12	558.21	17.15	0.17	0.919	8,218.8	3.489	0.000	3.24	0.045
C_{max} (μg/mL)	7.21	9.48	9.28	7.83	0.81	3.29	0.045	5.886	1.979	0.000	1.73	0.198
t_{max} (min)	21.56	5.75	8.00	11.25	3.79	3.41	0.040	1.903	0.817	0.422	0.43	0.732
Model-Based Parameters												
k_a (min^{-1})	0.159	0.314	0.407	0.341	0.055	3.00	0.032	0.000	0.000	0.725	1.91	0.164
k_{el} (min^{-1})	0.019	0.027	0.021	0.017	0.003	1.90	0.105	0.000	0.235	0.122	1.00	0.109
AUC_{0-240} (μg•min/mL)	502.02	543.15	555.90	553.35	10.00	0.23	0.072	7,544.4	3.317	0.000	3.43	0.039
C_{max} (μg/mL)	7.10	9.10	0.05	7.00	0.00	2.03	0.007	5.100	1.700	0.000	1.05	0.213
t_{max} (min)	24.40	9.00	0.10	12.01	4.10	3.25	0.040	0.000	0.000	0.592	0.00	0.575
V_1 (L)	40.93	25.70	50.92	51.30	10.37	1.92	0.103	123.21	0.143	0.207	2.13	0.132
$V_{d\beta}$ (L)	57.42	40.00	104.24	133.43	45.75	0.77	0.525	0 000	0.000	0.040	0.39	0.759

(a) Standard error of the estimated average value of the pharmacokinetic parameter for each autoinjector. Because the experimental design was balanced across delivery system, the standard errors are the same for each delivery system.

(b) Observed significance level for the effect of autoinjector.

(c) σ^2_A = Estimate of the animal-to-animal variance component.

σ^2_A/σ^2_e = Ratio of the variance components estimated for animals to the variance component estimated for uncontrolled error.

P-value = Observed significance level for the animal-to-animal variance component.

parameter for each autoinjector is displayed in the sixth column of the table. For each pharmacokinetic parameter, a statistical hypothesis test was performed to determine if the effect of autoinjector was statistically significant. The value of the F tests and their observed significance levels are given in the next two columns of the table.

The component of variation due to the effects of different animals was estimated for each pharmacokinetic parameter. The estimates of the between animal variance components (σ_A^2) are displayed in column nine of Table 7. Negative estimates of the variances were reported as zero. To assess the magnitude of the animal to animal variability, the between animal variance components were statistically compared to the variance component estimated for the variability within animals (σ_B^2). Ratios of the two variance components, and statistical significance levels for the between animal variance component are contained in the tenth and eleventh columns of the table. For each pharmacokinetic parameter, a statistical hypothesis test was performed to determine if the effect of week of testing was statistically significant. The value of the F tests and their observed significance levels are displayed in the last two columns of the table.

Autoinjector mean values were statistically different (at the 5 percent significance level) for the empirically estimated parameters C_{max} and t_{max} and for the model-based parameters t_{max}, and k_a. Autoinjector effects were marginally significant for the model-based estimated C_{max} ($P = 0.067$). The analysis of variance F-Test for autoinjector effects compares the parameter variability between autoinjector group means to the variability of that parameter within each autoinjector group to determine if differences between autoinjector group means are statistically significant. While the F-test may determine that the four autoinjector group means are significantly different from one another, it will not identify the manner in which group means are different. Therefore, multiple comparisons were performed (at the 5 percent significance level) to determine which pairs of autoinjector group means were statistically different using Tukey's Studentized Range Test.[4] Because this procedure appropriately adjusts significance levels to compensate for the simultaneous hypothesis testing for all six combinations of two autoinjector group means, situations arise where the F-Test for autoinjector

effects is significant and yet the multiple comparison procedure fails to identify significant differences between any two autoinjector means. Multiple comparison tests identified significant differences between average values of empirically estimated t_{max}, and model-based k_a for specific pairs of autoinjectors. For empirically estimated t_{max}, the MCA-A group mean was significantly less than the MCA group mean. For k_a, the MCA-B group mean was significantly greater than the MCA group mean.

The variations in the pharmacokinetic parameters over the four weeks of testing were determined to be statistically insignificant for all but two of the parameters, empirically estimated and model-based AUC_{0-240}. There was no observable trend in AUC_{0-240}, however, over the weeks of testing, i.e., average weekly 2-PAM AUC_{0-240} neither consistently increased nor decreased. The between animal variance component was determined to be statistically significant for both the empirically estimated and model-based parameters AUC_{0-240} and C_{max}. These animal effects were strongly influenced by the effect of animal 123, which achieved higher 2-PAM blood concentrations than other animals for all four autoinjectors.

Analyses of variance and multiple test comparison results for the 2-PAM pharmacokinetics parameters may be summarized as follows:

(1) Autoinjector effects were statistically significant for empirically estimated C_{max} and t_{max}, and model-based t_{max} and k_a. Autoinjector effects were marginally significant for model-based C_{max}.

(2) Effects of test week were not significant for eight of ten analyzed parameters. Effects of animal-to-animal variation were significant for four parameters, empirically estimated and model-based AUC_{0-240} and C_{max}, largely due to the effect of one animal which had consistently higher maximum concentrations than other animals.

(3) The MCA-B autoinjector group mean was the highest for k_a, one of the two highest for both empirical and model-based C_{max}, and the shortest for model-based t_{max}. The MCA group mean k_a was less than half of that estimated for the other groups, and the MCA mean (empirical and model-based) t_{max} was twice as long as that estimated for the other groups.

3.3.2 Atropine Pharmacokinetics

Serum atropine concentrations measured for each animal at each time point for the four autoinjector systems and pharmacokinetic parameters are presented in Appendix C. Figure 5 is a graph of mean serum atropine concentrations over time following injection of eight sheep using each of the four autoinjectors. Empirically estimated values of the atropine pharmacokinetic parameters AUC_{0-240}, C_{max}, and t_{max} are presented in Table 8. Atropine pharmacokinetic parameters calculated from the two-compartment model are shown in Tables 9 and 10. Absorption rate constants (k_a) were not determined for three animals (93, 104, 123) in the MCA-A group due to the extremely rapid absorption of atropine in these three animals. For these three animals, the model-based estimated k_a values were so large that they were essentially unquantifiable. Therefore, their estimated values are not reported in Table 9. This problem, however, did not appear to adversely affect the ability of the pharmacokinetic model to estimate the remaining parameters for these animals.

Model-based estimates of AUC_{0-240}, C_{max}, and t_{max} are plotted against estimated values from empirical data in Figures 6, 7, and 8. The plots demonstrate that there exists a strong linear relationship between the model-based and empirically estimated values of AUC_{0-240}, and C_{max} (except for two outlying values). Correlations were computed between the empirically estimated and model-based values of AUC_{0-240}, C_{max}, and t_{max}. The correlation between the model-based and empirically estimated values of C_{max} was also calculated with the two outliers omitted. Correlations were determined to be statistically different (at the 5 percent significance level) from zero for all three parameters. Calculated correlations between the two sets of estimates are given below.

Parameter	n	Correlation	P-value
AUC_{0-240}	32	0.804	0.0001
C_{max}	32	0.950	0.0001
C_{max}	30	0.983	0.0001
t_{max}	32	0.601	0.0003

FIGURE 5. MEAN SERUM ATROPINE CONCENTRATIONS FOLLOWING INJECTION OF EIGHT SHEEP USING FOUR DIFFERENT AUTOINJECTORS

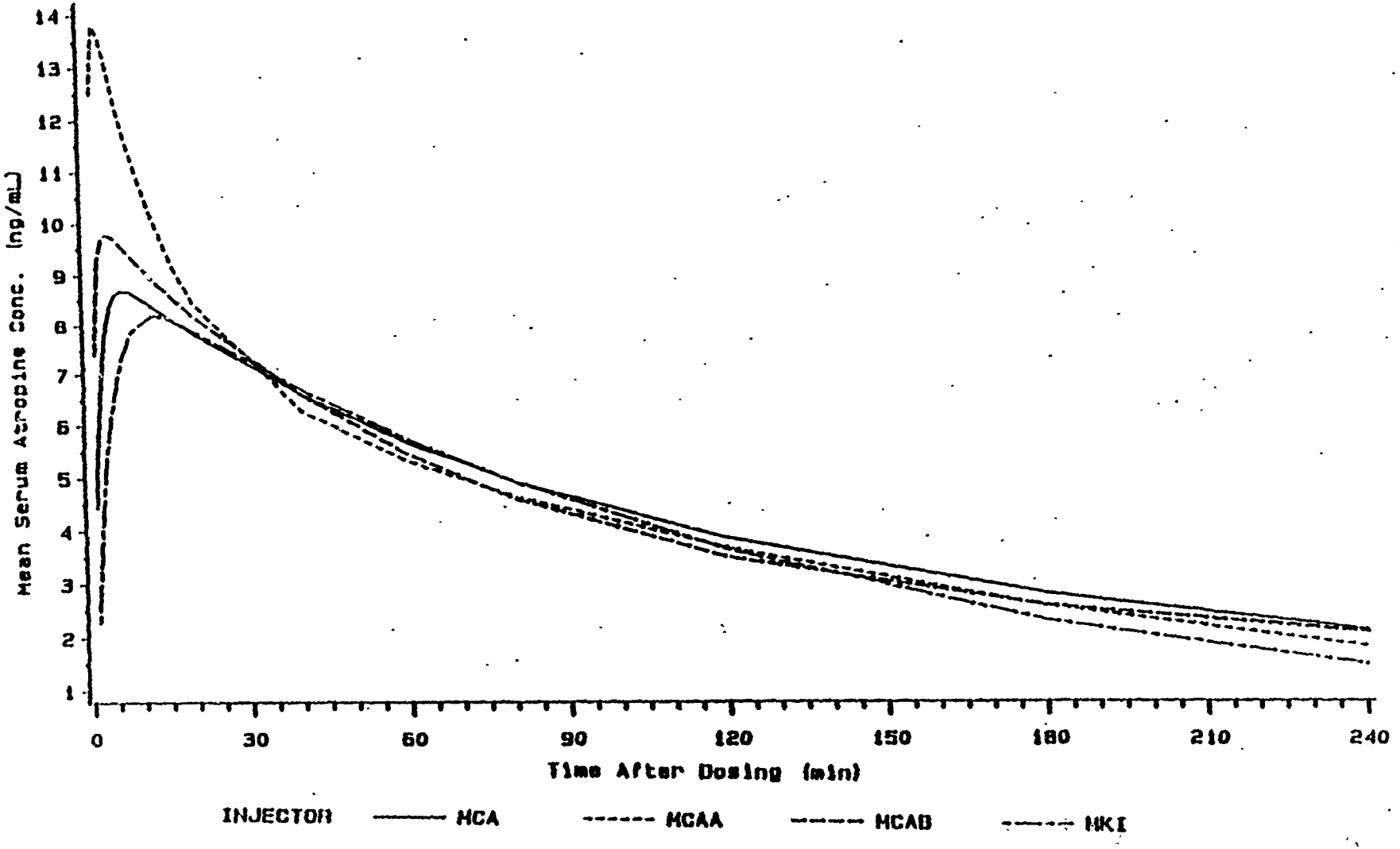


TABLE 8. ATROPINE PHARMACOKINETIC PARAMETERS AUC_{0-240}, C_{max}, and t_{max} DERIVED[a] FROM EMPIRICAL DATA

Animal	Test Week	Auto Injector	AUC_{0-240} (ng*min/mL)	C_{max} (ng/mL)	t_{max} (min)
87	1	MKI	837.8	7.80	20.0
	2	MCA	909.9	8.78	4.0
	3	MCA-B	937.3	11.05	2.0
	4	MCA-A	885.3	11.86	3.0
93	1	MCA-A	1,026.2	13.46	1.0
	2	MCA-B	938.5	10.01	2.0
	3	MCA	954.2	6.21	20.0
	4	MKI	746.5	9.38	4.0
104	1	MCA-B	1,042.3	9.02	16.0
	2	MKI	889.8	10.59	6.0
	3	MCA-A	1,088.0	15.55	5.0
	4	MCA	883.9	10.39	6.0
116	1	MCA-A	1,189.3	14.63	4.0
	2	MCA	1,050.4	12.42	6.0
	3	MCA-B	1,018.8	10.82	5.0
	4	MKI	969.4	9.31	16.0
117	1	MKI	942.4	8.16	16.0
	2	MCA-B	1,154.6	9.47	6.0
	3	MCA	944.9	13.41	12.0
	4	MCA-A	899.6	13.95	4.0
123	1	MCA-B	1,315.6	13.73	4.0
	2	MCA-A	1,248.0	18.05	1.0
	3	MKI	1,199.7	9.24	20.0
	4	MCA	1,235.9	16.98	3.0
127	1	MCA	1,404.0	9.00	40.0
	2	MCA-A	983.3	18.45	2.0
	3	MKI	1,199.6	9.06	40.0
	4	MCA-B	979.1	12.63	4.0
129	1	MCA	1,058.3	7.16	8.0
	2	MKI	1,012.0	8.45	20.0
	3	MCA-A	1,184.4	12.38	3.0
	4	MCA-B	876.2	9.33	4.0

(a) AUC_{0-240} was calculated from the observed atropine concentration-time curve using the trapezoid method; C_{max} is the maximum observed concentration, and t_{max} is the time point corresponding to the maximum observed concentration.

TABLE 9. ATROPINE PHARMACOKINETIC PARAMETERS A, B, α, β AND k_a FROM TWO-COMPARTMENT MODEL

Animal	Test Week	Auto Injector	A	B	α	β	k_a (min^{-1})
87	1	MKI	0.72	7.85	0.0327	0.0081	0.359
	2	MCA	7.14	5.89	0.0603	0.0048	0.278
	3	MCA-B	11.28	7.52	0.3277	0.0066	1.001
	4	MCA-A	7.22	6.47	0.1231	0.0059	1.306
93	1	MCA-A	6.24	6.88	0.0527	0.0057	(*)
	2	MCA-B	7.65	1.55	0.0105	0.0031	5.039
	3	MCA	1.02	5.97	0.0240	0.0039	0.279
	4	MKI	4.09	6.08	0.0210	0.0099	0.514
104	1	MCA-B	1.88	5.70	0.0103	0.0039	1.171
	2	MKI	8.64	8.89	0.1384	0.0083	0.235
	3	MCA-A	7.01	7.86	0.0677	0.0061	(*)
	4	MCA	9.09	7.69	0.1979	0.0076	0.638
116	1	MCA-A	7.50	10.40	0.1825	0.0075	0.931
	2	MCA	8.55	5.70	0.0387	0.0043	0.648
	3	MCA-B	6.73	4.62	0.0273	0.0032	1.116
	4	MKI	2.03	8.05	0.0217	0.0074	0.298
117	1	MKI	0.46	8.49	0.0188	0.0076	0.357
	2	MCA-B	5.08	4.58	0.0112	0.0035	1.022
	3	MCA	15.33	6.14	0.0685	0.0051	0.166
	4	MCA-A	9.79	5.50	0.0600	0.0055	1.153
123	1	MCA-B	9.38	4.52	0.0187	0.0026	2.032
	2	MCA-A	10.00	8.31	0.0600	0.0058	(*)
	3	MKI	-0.24	10.54	0.2228	0.0068	0.235
	4	MCA	10.79	8.87	0.1032	0.0058	0.925
127	1	MCA	-8.13	14.01	0.0410	0.0071	0.216
	2	MCA-A	16.31	9.39	0.2002	0.0095	1.359
	3	MKI	-9.24	11.94	0.0677	0.0075	0.852
	4	MCA-B	14.74	9.39	0.1889	0.0089	0.462
129	1	MCA	0.86	7.43	0.0339	0.0048	0.253
	2	MKI	0.96	9.11	0.0186	0.0075	0.157
	3	MCA-A	6.89	5.97	0.0421	0.0031	2.064
	4	MCA-B	5.01	4.81	0.0293	0.0044	0.601

* It was not possible to adequately estimate k_a from data collected due to rapid absorption of atropine.

TABLE 10. ATROPINE PHARMACOKINETIC PARAMETERS CALCULATED FROM A, B, α, β, AND k_a BASED ON TWO-COMPARTMENT MODEL

Animal	Test Week	Auto Injector	k_{el} (min^{-1})	$AUC_{0-24\theta}$ ($\mu g*min/mL$)	C_{max} ($\mu g/mL$)	t_{max} (min)	V_1 (L)	V_{d_β} (L)
87	1	MKI	0.009	828.5	7.52	10.6	201.8	214.5
	2	MCA	0.009	907.9	8.46	12.8	149.7	273.1
	3	MCA-B	0.013	921.5	9.75	4.4	112.8	222.7
	4	MCA-A	0.011	879.5	10.81	3.7	152.7	293.6
93	1	MCA-A	0.010	1,016.5	13.12	0.0	159.4	276.9
	2	MCA-B	0.007	929.3	9.08	1.3	230.4	561.2
	3	MCA	0.004	953.4	6.24	15.1	279.0	315.1
	4	MKI	0.013	730.9	8.92	7.4	170.2	215.4
104	1	MCA-B	0.005	1,051.7	7.36	4.8	279.6	330.5
	2	MKI	0.011	912.2	8.54	13.5	98.6	136.0
	3	MCA-A	0.011	1,090.7	14.82	0.1	140.6	246.2
	4	MCA	0.013	866.8	9.69	6.2	116.2	205.6
116	1	MCA-A	0.012	1,175.0	12.97	4.8	116.8	180.6
	2	MCA	0.009	1,050.0	11.96	6.7	136.8	285.5
	3	MCA-B	0.007	1,011.3	10.42	4.6	186.8	388.7
	4	MKI	0.008	962.0	8.64	12.3	171.6	196.7
117	1	MKI	0.008	935.4	8.01	10.9	193.2	199.1
	2	MCA-B	0.005	1,158.7	9.25	5.1	219.4	342.7
	3	MCA	0.012	940.7	9.09	17.3	90.8	204.9
	4	MCA-A	0.013	885.1	12.94	4.0	136.7	319.4
123	1	MCA-B	0.006	1,296.3	13.34	2.9	152.5	362.8
	2	MCA-A	0.011	1,240.7	18.26	0.1	114.1	225.3
	3	MKI	0.007	1,198.6	9.21	15.5	168.0	167.8
	4	MCA	0.011	1,227.3	14.96	4.8	99.2	195.1
127	1	MCA	0.004	1,394.6	8.41	18.8	331.9	186.0
	2	MCA-A	0.022	945.6	17.53	3.1	81.3	189.1
	3	MKI	0.002	1,193.8	5.56	7.0	642.1	195.9
	4	MCA-B	0.017	955.0	11.62	7.5	87.8	163.7
129	1	MCA	0.005	1,051.7	7.24	15.7	235.0	255.4
	2	MKI	0.008	1,001.0	8.06	20.1	171.8	181.5
	3	MCA-A	0.006	1,168.4	12.00	2.8	162.5	319.9
	4	MCA-B	0.008	867.4	8.58	7.4	215.9	375.0

FIGURE 6. PLOT OF MODEL-BASED AUC AGAINST EMPIRICAL AUC FOR ATROPINE

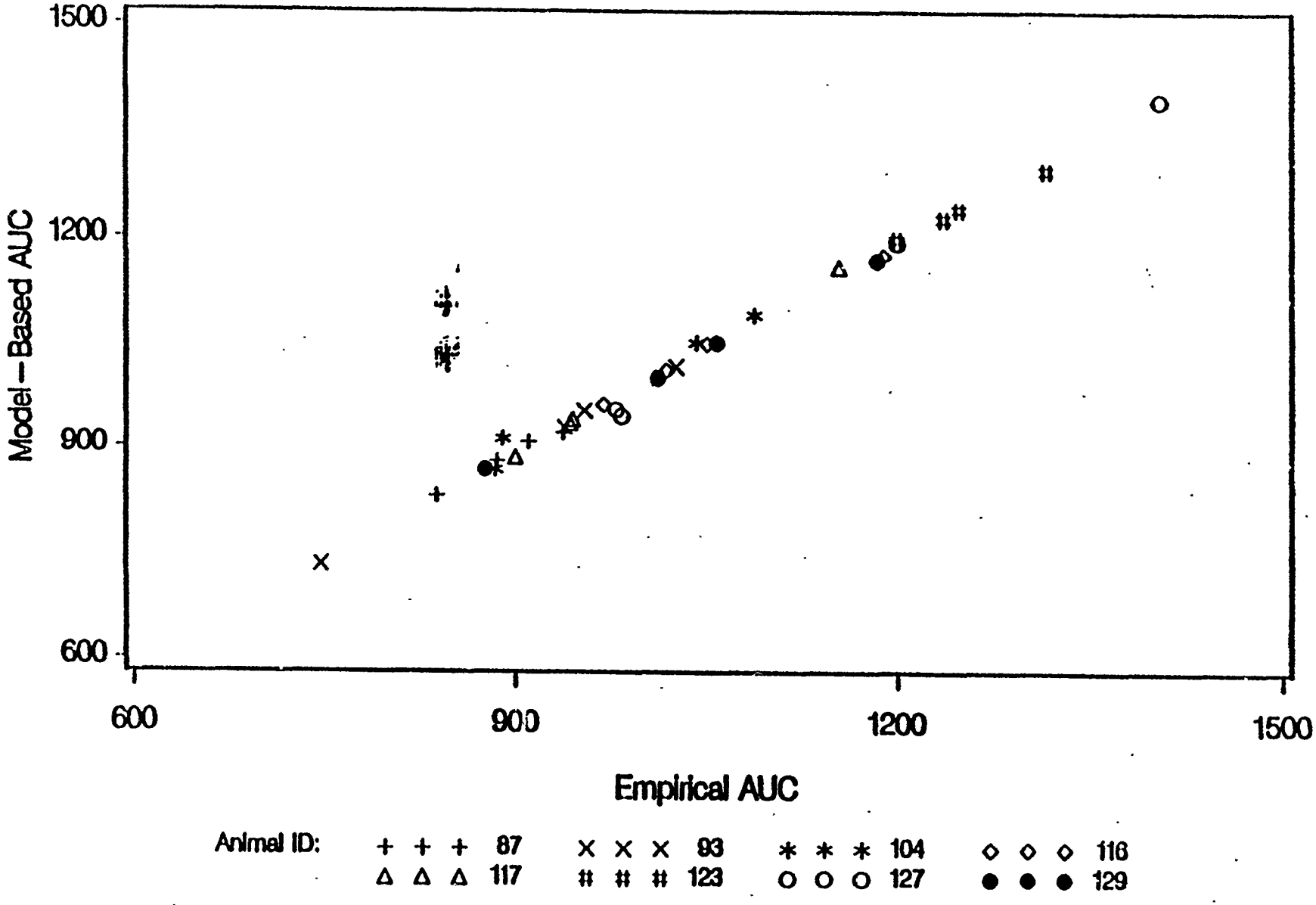


FIGURE 7. PLOT OF MODEL-BASED Cmax AGAINST EMPIRICAL Cmax FOR ATROPINE

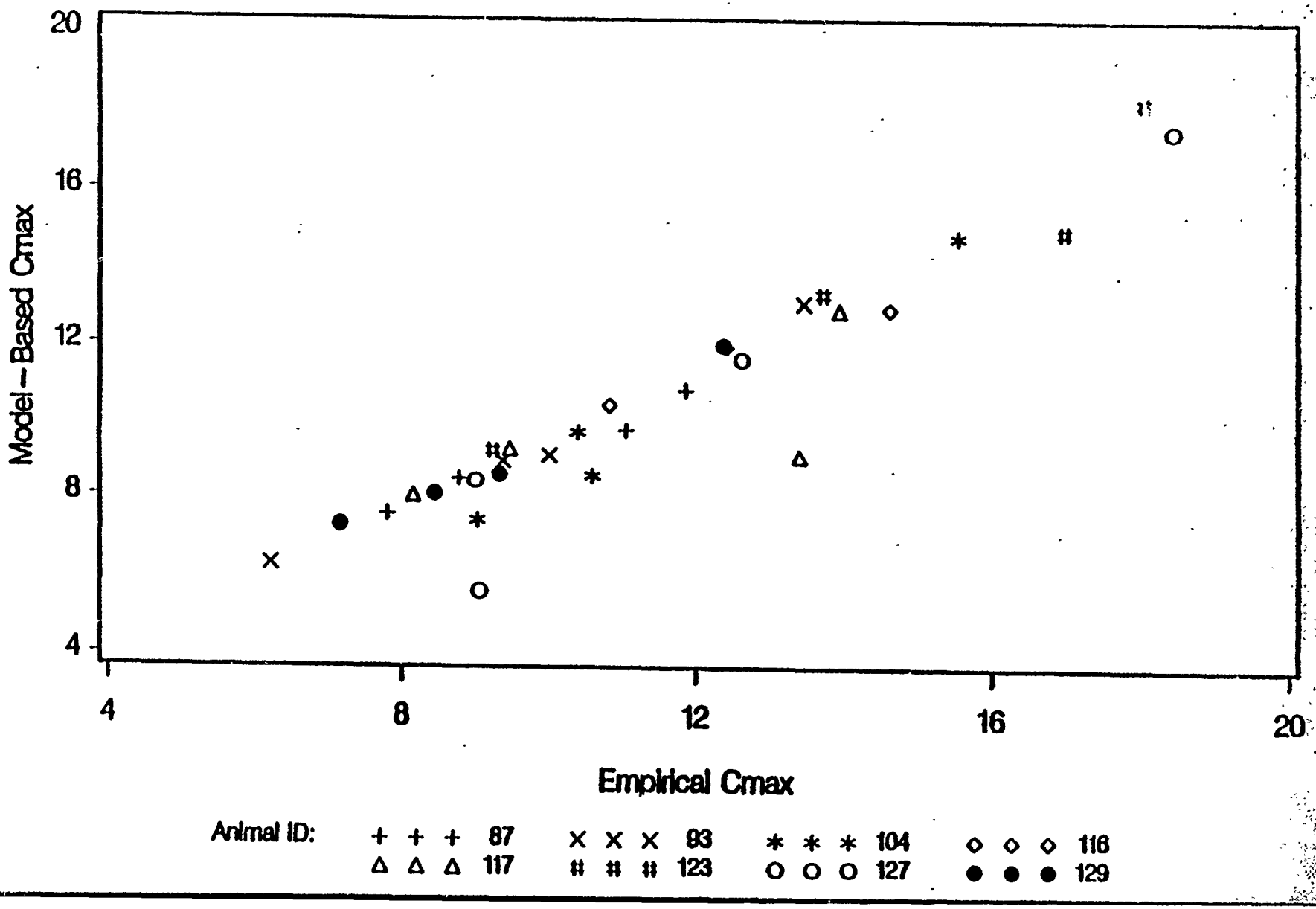
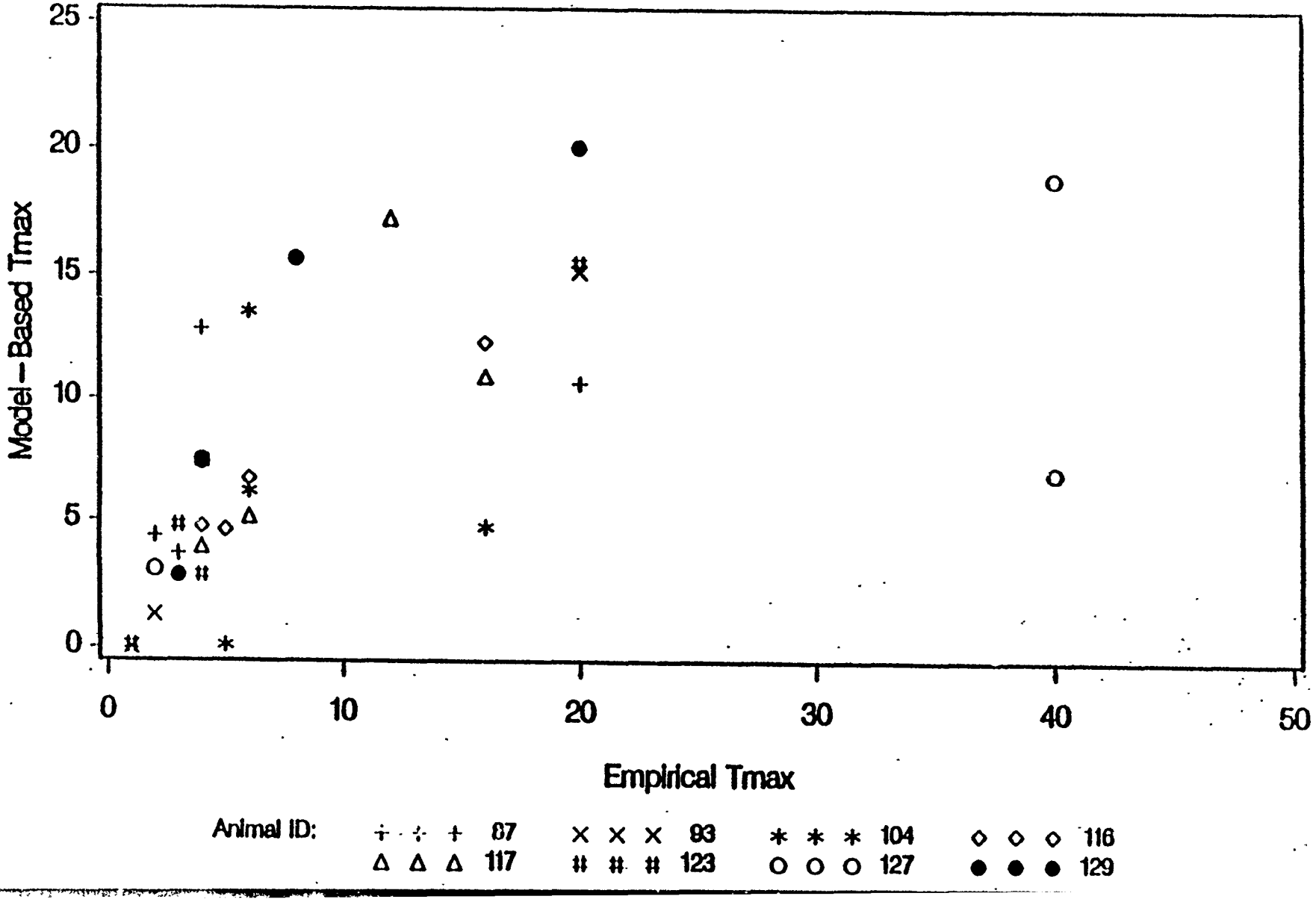


FIGURE 8. PLOT OF MODEL-BASED Tmax AGAINST EMPIRICAL Tmax FOR ATROPINE



Statistical procedures utilized to analyze the atropine pharmacokinetic parameters are analogous to those employed to analyze 2-PAM for all parameters except k_a. The three high, unquantifiable values estimated for k_a were treated as right censored at 5.5 min^{-1}. This means that the k_a values were not known, but would have been greater than or equal to the assigned value if they had been estimated. This approach allowed an analysis of variance on 32 k_a values, three of which were treated as right-censored. The presence of right-censored data, however, required specialized programs employing maximum likelihood methods rather than least squares techniques to perform the analysis of variance. Therefore, test statistics assessing the significance of effects on the k_a parameter have an approximate chi-square distribution instead of the F distribution employed for the other parameters. The analysis of variance included terms for autoinjector system, week of testing, animal effects, and residual effects.

A hypothesis test was conducted for each pharmacokinetic parameter to assess the statistical significance of any residual effects; results are shown in Table 11. Residual effects were determined to be statistically insignificant (at the 5 percent level) for all 10 parameters analyzed. Therefore, residual effects were dropped from the model and a second analysis of variance was carried out to assess the effects of autoinjector, animal-to-animal variability, and week of testing.

Table 12 summarizes the results of the statistical analyses and hypothesis testing for autoinjector, animal-to-animal, and week of testing variability. Autoinjector effects were statistically significant (at the 5 percent level) for the empirically estimated parameters C_{max} and t_{max} and for the model-based parameters k_a, C_{max}, t_{max} and V_{dp}. Multiple comparisons were performed (at the 5 percent significance level) for these parameters to determine which pairs of autoinjector group means were statistically different using Tukey's Studentized Range Test. Pairs of autoinjector group means determined to be significantly different are:

(1) For empirically estimated C_{max}, the MCA-A group mean was statistically greater than the group means estimated for MKI, MCA, and MCA-B.

TABLE 11. ASSESSMENT OF CARRY-OVER EFFECTS FOR ATROPINE PHARMACOKINETIC PARAMETERS

Parameter	F-Value	P-Value
	Empirically[a] Derived Parameters	
$AUC_{0-24\theta}$	1.13	0.37
C_{max}	0.66	0.59
t_{max}	0.70	0.56
	Model[b] Based Parameters	
k_a[c]	7.75	0.05[d]
k_{el}	1.29	0.32
$AUC_{0-24\theta}$	1.23	0.33
C_{max}	2.11	0.14
t_{max}	2.14	0.14
V_1	1.16	0.36
$V_{d\beta}$	1.24	0.33

(a) Derived from observed atropine concentration-time curve.
(b) Two-compartment model.
(c) Because it was not possible to estimate k_a for three animals, log-likelihood procedures were used to assess the statistical significance of carry-over effects for k_a. Therefore, the test statistic follows a chi-square distribution rather than a F distribution.
(d) Actual significance level calculated was 0.05159.

TABLE 12. SUMMARY OF STATISTICAL ANALYSIS OF AUTOINJECTOR, ANIMAL TO ANIMAL, AND WEEK OF TESTING VARIABILITY FOR ATROPINE PHARMACOKINETIC PARAMETERS

Pharmacokinetic Parameter (units)	Effect of Autoinjector: Model Predicted Average MCA	MCA-A	MCA-B	MKI	SE[a] of Average	F-Value	P-Value[b]	Animal Variability[c]: σ^2_A	σ^2_A/σ^2_e	P-Value	Week of Dosing: F-Value	P-Value
Empirical Parameters												
AUC_{0-24} (ng·min/mL)	1,655.2	1,663.8	1,632.6	974.66	33.67	1.41	0.273	11,758	1.297	0.001	4.66	0.015
C_{max} (ng/mL)	18.54	14.79	18.78	9.66	0.66	14.66	0.000	1.947	0.557	0.021	1.29	0.309
t_{max} (min)	12.37	2.67	5.37	17.75	2.53	7.17	0.002	11.234	0.228	0.133	3.19	0.049
Model-Based Parameters												
k_a (min^{-1})[d]	0.388	2.905	1.336	0.383	(e)	81.32	0.000	(f)	(f)	0.004	1.23	0.748
k_{el} (min^{-1})	0.008	0.012	0.008	0.008	0.001	1.78	0.187	0.000	0.000	0.007	2.67	0.079
AUC_{0-24} (ng·min/mL)	1,649.1	1,658.2	1,623.9	978.31	35.28	1.13	0.364	10,975	1.187	0.002	4.47	0.016
C_{max} (ng/mL)	9.58	14.66	9.92	8.66	0.57	28.73	0.000	1.965	0.789	0.008	2.23	0.128
t_{max} (min)	12.17	2.31	4.74	12.17	1.36	13.96	0.000	0.299	0.028	0.415	0.48	0.757
V_1 (L)	179.83	133.66	185.65	227.16	36.24	1.13	0.363	0.000	0.000	0.520	1.23	0.328
$V_{d\beta}$ (L)	248.18	258.38	343.48	188.35	23.38	7.65	0.002	997.35	0.238	0.128	0.29	0.833

(a) Standard error of the estimated average value of the pharmacokinetic parameter for each autoinjector. Because the experimental design was balanced across delivery system, the standard errors are the same for each delivery system.

(b) Observed significance level for the effect of autoinjector.

(c) σ^2_A = Estimate of the animal to animal variance component.

σ^2_A/σ^2_e = Ratio of the variance components estimated for animals to the variance component estimated for uncontrolled error.

(d) P-value = Observed significance level for the animal to animal variance component.

(e) Because it was not possible to estimate k_a for three combinations of animal and autoinjector, log-likelihood procedures were used to statistically analyze the k_a data. Therefore, test statistics follow a chi-square rather than a F-distribution.

(f) The standard errors are 0.052, 0.424, 0.186, and 0.049 for MCA, MCA-A, MCA-B, and MKI, respectively.

Animal to animal variability was not estimated for k_a.

(2) For empirically estimated t_{max}, the MCA-A and MCA-B group means were both determined to be statistically less than the group mean estimated for MKI; the MCA-A group t_{max} mean was determined to be statistically less than the group mean estimated for MCA.

(3) For the model-based k_a, both the MCA-A and MCA-B group means were statistically greater than those estimated for MKI and MCA.

(4) For the model-based C_{max}, the MCA-A group mean was determined to be statistically greater than those estimated for MKI, MCA and MCA-B.

(5) For the model-based t_{max}, both the MCA-A and MCA-B group means were determined to be statistically less than those estimated for MKI and MCA.

(6) For the model-based $V_{d\beta}$, both the MCA-A and MCA-B group means were greater than that estimated for MKI; the MCA-B group mean was determined to be statistically greater than that estimated for MCA.

The variations in the pharmacokinetic parameters over the four weeks of testing were determined to be statistically significant for empirically estimated parameters AUC_{0-240} and t_{max}, and for model-based AUC_{0-240}. As with 2-PAM, however, there was not a consistent increase or decrease in average weekly values, and there did not appear to be a relationship between high or low atropine and 2-PAM values. The between animal variance component was determined to be statistically significant for the empirically estimated parameters AUC_{0-240} and C_{max}, and for the model-based parameters k_a, AUC_{0-240}, and C_{max}.

Analyses of variance and multiple test comparison results for atropine pharmacokinetic parameters may be summarized as follows:

(1) Autoinjector effects were statistically significant for six of ten parameters, namely empirically estimated parameters C_{max} and t_{max}, and model-based parameters k_a, C_{max}, t_{max}, and $V_{d\beta}$.

(2) Atropine appeared to be absorbed more rapidly when delivered via MCA-A and MCA-B autoinjectors compared to MKI and MCA autoinjectors.

(3) The empirically estimated and model-based C_{max} mean values for MCA-A were statistically greater than those for MKI, MCA, and MCA-B.

(4) Effects of test week were significant for three parameters: empirically estimated AUC_{0-240} and t_{max}, and model-based AUC_{0-240}. Animal-to-animal variation was determined to be significant for five parameters: empirically estimated AUC_{0-240} and C_{max}, and model-based k_a, AUC_{0-240}, and C_{max}.

4.0 CONCLUSIONS

The pharmacokinetic parameters of 2-PAM and atropine following delivery by four different autoinjector systems were estimated using the same eight sheep injected with each system with a minimum of one week between injections. For 2-PAM, residual, or carry over, effects were determined to be statistically insignificant for all but one of the parameters analyzed, empirically estimated C_{max}. Considering the number of parameters analyzed, the marginal significance of one of ten parameters is compatible with what may result from random chance. The variations in 2-PAM pharmacokinetic parameters due to week of testing were insignificant except for empiric and model-derived AUC_{0-240}. There was no observable trend in AUC_{0-240}, however, over the weeks of testing, i.e., average weekly 2-PAM AUC_{0-240} neither consistently increased nor decreased. Animal to animal variability in 2-PAM pharmacokinetic parameters was statistically significant for both empirically estimated and model-based AUC_{0-240} and C_{max}. These animal effects were strongly influenced by one sheep which achieved higher plasma concentrations than other animals with all four autoinjector systems.

Mean 2-PAM pharmacokinetic parameter differences determined to be due to autoinjector systems were empirically estimated C_{max} and t_{max}, and model-based t_{max} and k_a. Multiple comparison tests identified significant differences only in t_{max}, with the MCA-A group mean t_{max} being significantly less than that of the MCA group mean, and in k_a, with the MCA-B group mean k_a being significantly greater than the MCA group k_a mean.

For atropine, all residual effects on pharmacokinetic parameters were statistically insignificant. The effects of week of testing were

statistically significant for empirically estimated $AUC_{0-24\,0}$ and t_{max}, and for model-based $AUC_{0-24\,0}$. As with 2-PAM, however, there was not a consistent increase or decrease in average weekly values, and there did not appear to be a relationship in high or low values between 2-PAM and atropine. Effects of animal to animal variation were significant for empiric and model-based $AUC_{0-24\,0}$ and C_{max}, and for model-based k_a.

Statistically significant differences in atropine pharmacokinetic parameters due to autoinjector system were empiric and model-based C_{max} and t_{max}, and model-based k_a and $V_{d\beta}$. For both empiric and model-based C_{max}, the MCA-A group mean was statistically greater than those estimated for MKI, MCA, and MCA-B autoinjector systems. For empirically estimated t_{max}, MCA-A and MCA-B group means were statistically less than the MKI group mean, and the MCA-A group mean t_{max} was also statistically less than the MCA group mean. Model-based t_{max} for both MCA-A and MCA-B autoinjector systems was statistically less than the t_{max} estimated for MKI and MCA systems. For k_a, both the MCA-A and MCA-B group means were statistically greater than those estimated for MKI and MCA systems. Both MCA-A and MCA-B group V_β means were statistically greater than that estimated for the MKI; the MCA-B group $V_{d\beta}$ mean was also statistically greater than that estimated for the MCA system.

Due to differences in the measured amounts of atropine and 2-PAM contained in different autoinjector systems, it could be argued that it is not valid to compare blood concentrations reached after injections with these systems. The amount of 2-PAM contained in the three MCA autoinjectors sampled was statistically greater than that contained in samples of other systems, and yet the pharmacokinetic evaluations did not reflect this. For atropine, the amount contained in the three MKI atropens sampled was statistically less than that contained in samples of the three other systems. Therefore, C_{max} and $AUC_{0-24\,0}$ were normalized by the average measured atropine dose in the three sampled autoinjectors of each system, and an analysis of variance was carried out on the normalized pharmacokinetic parameters C_{max}/D and $AUC_{0-24\,0}/D$. Results from the statistical analysis of the model-based and empirically estimated C_{max}/D agreed with those shown in Table 12 for C_{max}, i.e., effects of autoinjector and animal-to-animal variation were statistically significant, and the effects of test week were insignificant. For the empirically

estimated C_{max}/D, autoinjector group means were calculated to be 5.41, 7.08, 5.07, and 5.20 $(1,000\ L)^{-1}$ for MCA, MCA-A, MCA-B, and MKI, respectively, and the MCA-A group mean was determined to be statistically greater than those calculated for MKI, MCA, and MCA-B. Results from the statistical analysis of the model-based and empirically estimated $AUC_{0-24\theta}/D$ agreed with those shown in Table 12 for $AUC_{0-24\theta}$ for animal-to-animal and test week variation (both were determined to be statistically significant). The results for autoinjector effects were different: autoinjector effects were determined to be statistically significant. For empirically estimated $AUC_{0-24\theta}/D$, autoinjector group means were calculated to be 541, 509, 487 and 563 min/(1,000 L) for MCA, MCA-A, MCA-B, and MKI, respectively, and the MKI group mean was determined to be statistically greater than that calculated for the MCA-B system.

Overall, both 2-PAM and atropine appear to be absorbed more rapidly when delivered by MCA-A or MCA-B autoinjectors than when delivered by MCA or MKI autoinjectors. Also, maximum 2-PAM concentrations were numerically larger when delivered by MCA-A or MCA-B autoinjectors compared to MKI and MCA autoinjectors. Maximum atropine concentrations reached following use of MCA-A autoinjectors were statistically greater than those calculated for MKI, MCA, and MCA-B autoinjectors.

5.0 RECORD ARCHIVES

The eight sheep used in this study arrived at Battelle on April 10, 1990. Pharmacokinetic studies were conducted between July 16 and August 15, 1990. Records pertaining to the conduct of this study are contained in Battelle laboratory record books which are specific for this task. These record books are clearly labeled as to contents of each volume. These records and the final report will be maintained at the MREF until acceptance of the final report by the U.S. Army. At that time, records will be forwarded to the U.S. Army or archived at Battelle. Autoinjectors have been returned to their manufacturers.

6.0 ACKNOWLEDGMENTS

The names, titles and degrees of the principal contributors to this study are listed below:

Name	Title	Degree
Dr. Garrett S. Dill	Principal Investigator	D.V.M.
Dr. Carl T. Olson	Study Director	D.V.M., Ph.D.
Dr. Ronald G. Menton	Study Statistician	Ph.D.
Ms. Robyn C. Kiser	Study Supervisor	B.S.
Mr. Thomas H. Snider	Pharmacokinetics Modeler	B.S.
Ms. M. Claire Matthews	Statistician	M.A.
Mr. Timothy L. Hayes	Study Chemist	B.A.
Dr. Larry S. Miller	Immunochemist	Ph.D.
Dr. Peter L. Jepsen	Study Veterinarian	D.V.M.

There are a number of people who made performance of this task possible. Their invaluable assistance is gratefully acknowledged by the authors. Among the many are: James Arp and Sheri Moore for chemical analyses; Dr. Ashok Sawhney and Victor Moore for performance of RIAs; Linda Adams, Stephen Calver, Rebecca Geer, William Hart, Pamela Kinney, Jonathon Kohne, Jean Ostovich, Cynthia Pelley, and Jack Waugh for preparation of the sheep, drawing of blood, and obtaining plasma and serum samples; and Charlotte Hirst and Tami Kay for preparation of the report.

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APPENDIX A

Protocols

Comparison of the Pharmacokinetics of Atropine and Pralidoxime Chloride in Sheep Using Four Autoinjector Systems

Study performed by Battelle
505 King Avenue, Columbus, Ohio 43201-2693

1. MREF Manager: Garrett S. Dill, D,V.M.

2. Study Director: Carl T. Olson, D.V.M., Ph.D.

3. Study Veterinarian: Peter L. Jepsen, D.V.M.

4. Statistician: Ronald G. Menton, Ph.D.

5. Sponsor: U.S. Army Medical Research and Development Command (USAMRDC)

6. COR: LTC Don W. Korte, Jr., U.S. Army Medical Research Institute of Chemical Defense (USAMRICD)

7. Objective: The U.S. Army Medical Materiel Development Activity (USAMMDA) is currently evaluating candidate multichambered autoinjector antidote systems in order to select a system with which to replace the Mark I. Information is needed on the pharmacokinetics of atropine and pralidoxime chloride (2-PAM) when delivered by the different autoinjector systems to select the optimal system for further development. The objective of this Task is to compare the pharmacokinetics of atropine and 2-PAM when delivered by the Mark I (MKI) or three different candidate systems. The Task is performed by measuring blood levels of atropine and 2-PAM in sheep after intramuscular (IM) injection of the compounds, at similar dose levels, using each of the systems in each sheep. This study is conducted under the requirements of the U.S. Food and Drug Administration's (FDA) Good Laboratory Practices (GLP) regulations.

8. Experimental Design:

   A. Test System

      (1) Animals - Sheep are used for this study because of previous work measuring blood levels of atropine and 2-PAM in this species, and because of similarities with man in body weight. Sheep (Ovine) are yearling wethers of mixed breeding.

      Protocols of all experiments using animals are reviewed and approved by Battelle's Institutional Animal Care and Use ommittee (IACUC) prior to initiation of the study. The Program

Manager accepts responsibility for the proper care and use of animals in the conduct of research described in the protocols. Sheep are Q-Fever negative, mature wethers obtained from Thomas D. Morris, Inc. (Reistertown, MD) or another similar, approved source of research animals. Sheep are shorn, as necessary, to improve their comfort in an indoor environment or to increase ease of injections, blood sampling, and physiologic monitoring.

(2) Weight - Initial weight of sheep will be 60-80 kilograms.

(3) Quarantine - Sheep are examined by a veterinarian upon arrival. Blood samples are drawn for complete blood counts and fecal samples are obtained for parasite infestation evaluation. Sheep are held in isolation and observed for signs of clinical illness for at least 7 days prior to use in a study.

(4) Selection - Animals selected after quarantine are in good physical condition. Eight sheep are used in this pharmacokinetic study.

(5) Animal Identification - All animals are tagged in the ear to retain positive identification during handling and observation.

(6) Housing - Sheep are group housed in an outdoor fenced area with available shelter until they are used in experimentation. At the time of experimentation, they are placed in slings to which they have been acclimated.

(7) Lighting - Sheep are group housed in an outdoor fenced area prior to experimentation. When they are moved into experimental areas, fluorescent lighting with a light/dark cycle of 12 hr each per day is used.

(8) Temperature - Maintained at 65 ± 15 F in indoor areas.

(9) Humidity - Maintained at 50 ± 20 percent in indoor areas.

(10) Diet - Sheep are fed Purina Rumilab® Chow with limited quantities of locally-purchased hay and commercially available higher energy feeds, as needed, to maintain or increase weight. No contaminants that would interfere with the results of the study are known to be present in the feed.

(11) Water Supply - Water is supplied from the Battelle West Jefferson water system and given ad libitum during quarantine and holding. No contaminants that would affect the results of

the study are known to be present in the water. Water is analyzed for impurities on an annual basis.

(12) Battelle's Animal Resources Facilities have been registered with the U.S. Department of Agriculture (USDA) as a Research Facility (Number 31-21) since August 14, 1967, and are periodically inspected in accordance with the provisions of the Federal Animal Welfare Act. In addition, animals for use in research are obtained only from laboratory animal suppliers duly licensed by the USDA. Battelle's most recent statement of assurance regarding the Department of Health and Human Services (DHHS) policy on humane care of laboratory animals was accepted by the Office of Protection from Research Risks, National Institutes of Health on July 29, 1986. Animals at Battelle are cared for in accordance with the guidelines set forth in the "Guide for the Care and Use of Laboratory Animals" (DHHS Publication No. (NIH) 85-23) and/or in the regulations and standards as promulgated by the Agricultural Research Service, USDA, pursuant to the Laboratory Animal Welfare Act of August 24, 1966 as amended (P.L. 89-544 and P.L. 91-579).

(13) On January 31, 1978, Battelle received full accreditation of its animal care programs and facilities from the American Association for Accreditation of Laboratory Animal Care (AAALAC). Battelle's full accreditation status has been renewed after every inspection since the original accreditation. The MREF is a part of the facilities granted full accreditation.

B. Test Material

Atropine (CAS 51-55-8) and 2-PAM (CAS 51-15-0) contained in injection systems are provided by USAMRICD. Sufficient numbers of each system from the same lot are provided so that analyses can be done on representative samples to confirm identity and quantitate the amount of atropine (MREF SOP-89-55) and 2-PAM (MREF SOP-88-39) delivered by each system and so that sufficient numbers of sheep can be injected to perform this pharmacokinetic study.

C. Test Groups

Sheep are given atropine/2-PAM IM, using each of the injection systems on each day of testing. At times after injection of approximately 1, 2, 3, 4, 5, 6, 8, 12, 16, 20, 40, 60, 80, 120, 180, and 240 min, blood samples are taken from the jugular vein, either through an indwelling catheter or by using a syringe and needle.

Blood samples are analyzed for atropine and 2-PAM (MREF SOP-89-57) concentrations. 2-PAM concentration is measured by Battelle using spectrophotometric analyses and a standard 2-PAM curve prepared from known concentrations. Atropine analyses are conducted at Battelle's Columbus Laboratory using radioimmunoassay. After a minimum one-week washout period, the same sheep are used again, but each sheep is given atropine/2-PAM using an injection system not used the first time. This is repeated until each of the eight sheep are given atropine/2-PAM using each injection system.

When atropine and 2-PAM analyses are completed, blood concentrations as a function of time, maximum concentrations, times to maximum concentrations, area under the blood concentration curves from time 0 to 4 hr, absorption and elimination rate constants, and volumes of distribution are estimated. Statistical analyses, as described in Section 9, are performed to determine if any significant differences exist between values as a function of the injection system.

D. Study Preparations

Animals are held in a pen and acclimated to a sling at the MREF prior to use. Each sheep is weighed within 24 hr of intended use.

9. Statistical Approach: Pharmacokinetic parameters measured for atropine/2-PAM administered by each system are compared to those obtained for atropine/2-PAM administered by the other systems to determine any significant ($P < 0.05$) difference. Responses will be analyzed using crossover design analysis of variance techniques or t-tests.

10. Records to be Maintained:

A. Analyses of atropine and 2-PAM in injection systems;

B. Analyses of atropine and 2-PAM in blood;

C. Experimental parameters and test conditions.

11. Reports: A draft final report will be prepared and submitted for review to the USAMRDC COR within 30 working days after completion of the task. It will include the following:

A Experiment design;

B. Animal supplier;

C. Test animal selection criteria;

D. Pharmacokinetic estimates;

E. Statistical methodology;

F. Discussion of findings.

A final report that addresses the review comments of USAMRDC will be prepared and submitted within 30 working days of receipt of comments.

12. Approval Signatures:

Carl T. Olson, D.V.M., Ph.D.
Study Director — Date: 3-22-90

Garrett S. Dill, D.V.M.
Program Manager — Date: 3/23/90

Peter L. Jepsen, D.V.M.
Study Veterinarian — Date: 3/29/90

Ronald G. Menton, Ph.D.
Statistician — Date: 3/30/90

Don W. Korte, Jr., D.V.M.
USAMRICD COR — Date: 4/5/90

Richard A. Stewart
Quality Assurance Unit
Health and Environment Group

4-9-90
Date

Chas K. Burdick
Charles K. Burdick, Director
Total Quality Program
Health and Environment Group

4/9/90
Date

CTO/cah

Comparison of the Pharmacokinetics of Atropine and Pralidoxime Chloride in Sheep Using Four Autoinjector Systems

Protocol Amendment No. 1

Change: Page 4, Section 8.D.
"Each Sheep is weighed within 24 hr of intended use." is deleted.

Reason: Weighing each sheep immediately prior to each study is unnecessary since each animal will be given each treatment in a four-way crossover design. Injections will be given in a random fashion as designed by a statistician in order to preclude effects of day of injection on pharmacokinetic parameters.

Impact on Study: None.

Carl T. Olson — 6-28-90

Carl T. Olson, D.V.M., Ph.D.
Study Director

Date

Don W. Korte, Jr. — 25 Jun 90

LTC Don W. Korte, JR.
USAMRICD COR

Date

Comparison of the Pharmacokinetics of Atropine and Pralidoxime Chloride in Sheep Using Four Autoinjector Systems

Deviation: This protocol specifies sheep will be held in rooms with a temperature range of 50-80 F and a relative humidity of 30-70 percent. Conditions in animal rooms are recorded twice daily using a hand-held combination thermometer/hygrometer to obtain temperature and relative humidity readings. The relative humidity recorded in rooms in which sheep were held during this experiment were as high as 81 percent. Excursions above the relative humidity range specified in the protocol were reported to a maintenance engineer and adjustments of humidistats made.

Impact on Study: Temperature and relative humidity ranges recommended for sheep are not specified by the National Institutes of Health in their Guide for the Care and Use of Laboratory Animals (NIH Publication No. 86-23, Revised 1985). The short-lived excursions above the relative humidity specifications stated in the protocol should have no impact on the validity of the study.

Carl T. Olson

Carl T. Olson, D.V.M., Ph.D.
Study Director

10-18-90
Date

Don W. Korte

LTC Don W. Korte, Jr., M.S.
USAMRICD COR

18 OCT 90
Date

MREF Protocol 59
Medical Research and
Evaluation Facility
July 18, 1990

Comparison of the Pharmacokinetics of Atropine and Pralidoxime Chloride in Sheep Using Four Autoinjector Systems

Protocol Amendment No. 2

Change: Page 3, Section 8.C.
"At times after injection of approximately 1, 2, 3, 4, 5, 6, 8, 12, 16, 20, 40, 60, 80, 120, 180, and 240 min, blood samples are taken from the jugular vein, either through an indwelling catheter or by using a syringe and needle." is replaced with "Prior to the injection of atropine and 2-PAM and at times after injection of approximately 1, 2, 3, 4, 5, 6, 8, 12, 16, 20, 40, 60, 80, 120, 180, and 240 min, blood samples are taken from the jugular vein, either through an indwelling catheter or by using a syringe and needle."

Reason: Blood samples are taken prior to injection of atropine and 2-PAM for determination of control, baseline values for any interference in atropine or 2-PAM analyses.

Impact on Study: None.

Carl T. Olson

Carl T. Olson, D.V.M., Ph.D.
Study Director

7-18-90
Date

Don W. Korte

LTC Don W. Korte, JR.
USAMRICD COR

18 July 90
Date

MREF Protocol 59
Medical Research and
Evaluation Facility
July 31, 1990

Comparison of the Pharmacokinetics of Atropine and Pralidoxime Chloride in Sheep Using Four Autoinjector Systems

Protocol Amendment No. 3

Change: Page 4, Section 8.C.
"Blood samples are analyzed for atropine and 2-PAM (MREF SOP 89-57) concentrations." is changed to read "Blood samples are analyzed for atropine and 2-PAM (MREF SOP 85-19) concentrations."

Reason: The Technicon spectrophotometric method for determining concentrations of 2-PAM in plasma is to be used rather than the HPLC technique. Both techniques were used in a previous study and gave comparable results, but the Technicon method was faster and less laborious.

Impact on Study: None.

Carl T. Olson
Carl T. Olson, D.V.M., Ph.D.
Study Director

July 31, 1990
Date

Don W. Korte
LTC Don W. Korte, JR.
USAMRICD COR

31 July 90
Date

APPENDIX B

SOPS

STANDARD OPERATING PROCEDURE
MREF SOP-88-39

TITLE: Analysis and Structural Verification of Pralidoxime Chloride

LABORATORY: MREF, HML, or King Ave. SOP APPROVAL DATE: 02/26/90

PLACE OF OPERATION OR TEST: Any safety approved laboratory within the approved facilities

This Standard Operating Procedure (SOP) has been prepared as prescribed by Contract DAMD17-89-C-9050 and will be effective for one year from date of approval unless sooner rescinded or superseded.

No deviation from this SOP will be permitted. Whenever the approved method is changed, the SOP will be revised.

Supervisory personnel will assure that all personnel involved with this SOP have been properly trained and instructed in its provisions and attest to this requirement by affixing their signatures on page 3.

A copy of this SOP will be posted at the job site whenever the operation is being performed.

Submitted By: Timothy L Hayes 2/20/90
Signature/Date

Timothy L. Hayes, Principal Research Scientist
Printed Name/Title

Approved By: [signature] 2/22/90
Signature/Date

Garrett S. Dill, D.V.M., Manager
Printed Name/Title

Approved By: [signature] 2/26/90
Signature/Date

David L. Stitcher, CIH, Safety/Surety Officer
Printed Name/Title

Revised February 20, 1990

APPROVED

Approved By: Richard A. Shank 2.27.90
Signature/Date

Quality Assurance Unit
Health and Environment Group
Printed Name/Title

Approved By: Ronald K. Jamison for 2/27/90
Signature/Date

Charles K. Burdick, Director
Total Quality Program
Health and Environment Group
Printed Name/Title

Revised February 20, 1990

SIGNATURES

I have read and understand the contents of MREF SOP-88-39.

Signature	Date	Signature	Date
[signature]	3 '5		
[signature]	3/30/90		
[signature]	4-9-90		
[signature]	4-20-90		
[signature]	5/16/90		
[signature]	5/16/90		
[signature]	6/25-90		

Revised February 20, 1990

APPROVED
[signature]

STANDARD OPERATING PROCEDURE 88-39

Analysis and Structural Verification of Pralidoxime Chloride

A. Statement of Work: This SOP describes the procedures for verification of identity and quantitative measurement of pralidoxime chloride (2-PAM Cl) by high performance liquid chromatography (HPLC). The procedures for structural verification by nuclear magnetic resonance (NMR) of 2-PAM Cl present in drug formulations are also described. The HPLC effort can be conducted at either the MREF, HML or King Avenue but the NMR requires the facilities at King Avenue.

B. Responsibility:

1. Personnel Qualifications: Technical staff will consist of individuals designated by the Chemistry Coordinator to perform structural verification of the drug used in this task; i.e., 2-PAM Cl.

2. Leaders: Leaders of each operation will be designated by the Study Director for that operation. Each leader will insure that the following are observed:

a. Only authorized personnel meeting requirements set forth in Section B.1 are allowed in the room during operations.

b. Adequate, approved, protective equipment is available at all times to personnel at their work site.

c. All leader and technical staff responsibilities specified in the MREF FSSP are followed when work is conducted at the MREF.

d. Each MREF or HML employee has been trained in the techniques of administering first aid and self aid.

e. Work under this SOP is performed only in the area(s) or room(s) designated by this SOP.

f. No food, beverage, or tobacco product is consumed, used, or brought into the laboratory. The wearing of contact lenses is prohibited in the laboratory.

g. The safety requirements of this SOP, as well as normal laboratory safety, are maintained.

Revised February 20, 1990

h. All applicable SOPs are read and signed by all technical staff involved in the operation.

3. Technical Staff: Technical staff will be responsible for abiding by requirements set forth in Section 8.2. In addition, they must use personal, protective equipment provided and develop safe work habits to protect themselves and fellow workers from injury and to prevent damage to material, equipment, and facilities.

4. Research Organization: The organization involved in this research is the MREF of Battelle Memorial Institute, 505 King Avenue, Columbus, Ohio 43201-2693.

C. Materials To Be Used: The 2-PAM Cl used on this program will be provided by the U.S. Army Medical Research and Development Command (USAMRDC) or purchased from a traceable source of purity. Upon receipt, the 2-PAM Cl will be stored in a desiccator at -10 C or as directed by the supplier. NMR spectra will be obtained on dilute solutions of the drug dissolved in > 99.8 percent deuterium oxide (Stohler Isotope Chemicals or equivalent). NMR tubes will be the Stohler Isotope Chemicals "Ultra Precision" model or the equivalent model from other manufacturers.

Other materials will include acetonitrile (Burdick and Jackson HPLC grade or equivalent), deionized water or millipore water, acetic acid, glacial (Baker reagent grade Cat. No. 9508-03), tetrabutylammonium chloride (Aldrich 28,888-8), benzophenone (Aldrich 23,985-2), tetrabutylammonium nitrate (Kodak 9664), sodium lauryl sulfate (dodecyl sulfide, sodium salt) (Aldrich 86-201-0), helium gas, and nitrogen gas.

D. Equipment: Proton NMR spectra will be obtained on Battelle's Varian CFT-20 Fourier transform NMR spectrometer located in Room 7237-A of the King Avenue facility.

The HPLC analytical system, to be used consists of the following: HPLC pump, HPLC ultraviolet (UV) detector, HPLC injection system (autosampler), HPLC reverse-phase column, strip-chart recorder (optional), and electronic data system. Any equivalent system may be used once confirmation of performance has been established.

Other equipment includes: glass bottles, glass vials, Teflon cap liners, microsyringes, pipettes, volumetric flasks, graduated cylinders, autosampler vials, refrigerator, Teflon wash bottles, gas tight syringes, filter flask system, Pasteur pipettes, dropper bulbs, chart paper, spherisorb ODS 2 analytical HPLC column or equivalent, recorder pens, weighing paper, pipettes, pipette bulbs, and spatula.

Revised February 20, 1990

E. Hazards Involved:

1. Solvents: The solvents used in preparing the dilute material may have hazards associated with their use. A copy of the Material Safety Data Sheets (MSDS) is available in the administrative area of the MREF or through Battelle's Safety Office at 505 King Avenue. A brief listing of hazards associated with handling the more commonly used solvents has been included:

   a. Acetonitrile: Acetonitrile is a flammable liquid that must be handled as a solvent with a dangerous fire risk. The flash point of acetonitrile is 5.56 C. The 1988-1989 ACGIH TLV for acetonitrile is 40 parts per million (ppm) as an 8-hr TWA and 60 ppm as a 15-min STEL. Skin contact may also represent a significant route of exposure.

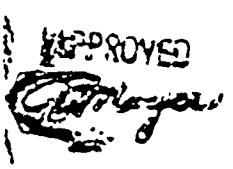
   b. Methanol: Methanol is a flammable liquid that must be handled as a solvent with a dangerous fire risk. The flash point (open cup) of methanol is 12.2 C, with an autoignition temperature of 464 C. The 1988-1989 ACGIH TLV for methanol is 200 ppm as an 8-hr TWA and 250 ppm as a 15-min STEL. Also, skin contact may represent a significant route of exposure.

   c. Benzene: Benzene is a flammable liquid that must be handled as a solvent with a dangerous fire risk. Benzene is toxic by ingestion, inhalation, and skin absorption. Benzene is regulated as a carcinogen by the Occupational Safety and Health Administration (OSHA) resulting in excess leukemia. Containers must say "DANGER CONTAINS BENZENE CANCER HAZARD." OSHA 8-hr permissible exposure limit (PEL) = 1 ppm, Action Level = 0.5 ppm.

F. Safety Requirements:

1. Hoods: Hood face velocity must average 100 ± 20 lfpm. The average is computed from individual readings taken in approximately each square foot of hood face (usually nine readings). No equipment will be within 20 cm of the face of the hood.

Revised February 20, 1990



2. Protective Equipment: When working in the MREF laboratory, the following clothing and protective gear are required as a minimum for all personnel. This equipment must be used as directed in the FSSP.

   lab coat
   latex gloves (as needed)
   protective eyewear

   All provisions of the FSSP apply to the checking and testing of gloves, aprons, respirators, and other protective equipment.

3. First Aid: The location of the nearest eye-wash fountain shower, and fire extinguisher will be known to all workers before work begins.

G. Procedures:

1. MREF Entry: Before entering the secured facility, note the status of the "Agent-in-Use" light at the turnstile. If the "Agent-in-Use" lights are turned on, note the room location and be sure that upon entry to the laboratory area all safety equipment and procedures described in FSSP SOP MREF-18 are in place. Upon entry of the room, confirm that there are no audible alarms. No operations can be initiated in a room with audible alarms. After entry, personnel will observe the magnehelic gauge on the hood. If inspection reveals that the hood has failed, is marginal in flow, or operates outside the guidelines of FSSP SOP MREF-21, the problem is reported to the MREF Manager and the operation does not begin.

2. Hood Set Up: The operation hood area must be prepared with all materials necessary to perform the operation prior to starting the operation. All materials will be kept behind the 8-inch line in the hood.

   Plastic-backed, absorbent paper must be used to protect the work surface of the hood.

3. Sample Preparation: The drug formulation samples provided by the USAMRDC are manipulated so that the interference of solvents and other components associated with the samples is minimized to provide relatively pure drug samples for NMR analysis.

   HPLC analyses may be performed on either the dosing formulation as received, dilutions of the parent materials, or on reference standard solutions of known concentration.

Revised February 20, 1990

a. Analytical Reference Standard: 2-PAM Cl solid reference standard supplied by the USAMRDC is dried at 100 C, 0.4-mm Hg for 3 hr prior to use. This is performed by placing the solid material contained in its original container which has had its cap removed into a pre-heated oven. The oven is sealed and the vacuum adjusted to 0.4 mm Hg.

b. NMR Analysis: Approximately 2.0 mL of the 2-PAM Cl formulation is transferred to a 9.5 dram vial and frozen therein by partially immersing in dry ice/acetone after the vial is capped. This vial is placed in a chamber of a lyophilization apparatus and subjected to high vacuum until the sample reaches a state of dryness.

NMR samples are prepared by dissolution of several mg of the dried samples in deuterium oxide and are transferred into an NMR tube (tube capped after transfer) for NMR analysis.

c. HPLC Analysis: Samples are diluted with mobile phase so that the expected concentration range is between 0.01 and 0.10 mg/mL. Samples are refrigerated until analysis.

4. Preparation of Standard Solutions: Standard solutions of 2-PAM Cl are prepared for an NMR reference spectrum and HPLC standard curve determinations.

a. NMR: Within a glove bag thoroughly flushed with dry nitrogen or argon, weigh 10 mg ± 0.1 of 2-PAM Cl onto weighing table. Transfer the sample into a screw-capped bottle and close tightly. Outside the bag, dissolve the sample in an accurately measured volume of 10.0 mL of deuterium oxide and recap the bottle to minimize the contamination of the sample with undeuterated moisture.

b. HPLC: Accurately weigh 50 mg ± 0.1 mg of 2-PAM Cl onto weighing paper. Quantitatively transfer the 2-PAM Cl into a 50-mL volumetric flask containing approximately 40 mL of mobile phase (see Section G.5.b.). Mix the solution thoroughly. Dilute to 50 mL with water and remix the solution. The resulting concentration of the 2-PAM Cl stock will be approximately 1 mg/mL.

Weigh out 10 g ± 0.1 g of benzophenone, the internal standard (IS), and quantitatively transfer the material into a 25-mL volumetric flask containing approximately 20 mL of acetonitrile. Mix well until dissolved. Dilute to 25.0 mL with acetonitrile and remix the solution.

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The resulting concentration of the benzophenone internal standard stock is 400 mg/mL.

Mix and dilute the 2-PAM Cl stock solution with mobile phase (see Section G.4.b) in 10 mL volumetric flask as follows:

1.0-mL stock + 9.0-mL mobile phase
0.50-mL stock + 9.5-mL mobile phase
0.25-mL stock + 9.75-mL mobile phase
0.10-mL stock + 9.90-mL mobile phase
0.0-mL stock + 10.0-mL mobile phase

After the standards have been prepared, each level is the spiked 5 µL of the internal standard solution. The final standard concentrations are 0.10, 0.050, 0.025, 0.010, and 0.0 mg per mL.

Diluted standard solutions are kept refrigerated until used. Standards may be stored refrigerated for up to 30 days.

5. Analysis Start-Up: NMR is performed to verify the structure of the 2-PAM Cl. HPLC is performed to quantitatively determine the concentration of 2-PAM in the samples and identity confirmation of 2-PAM in the dosing solution by retention indices comparison.

   a. NMR: Calibrate the NMR instrument and data system according to instructions in the operator's manual. When properly calibrated against the standard reference solutions identified in the manual, proceed with the analysis.

   b. Quantitative HPLC: Prepare HPLC mobile phase buffer for quantitative analysis by dissolving 2.7 g of tetramethylammonium chloride in approximately 900 mL of deionized water. Add 1.0 mL of glacial acetic acid and dilute to 1 L and mix. Store in a clean, 1-L glass bottle. Use within 30 days.

   The mobile phase may be established using a gradient system with a 40 percent buffer : 60 percent acetonitrile ratio or mixed prior to analysis. To mix the mobile prior to analysis, add 400 mL of the buffer prepared above to a 1-L glass bottle and add 600 mL of acetonitrile and mix. Once the buffer has been prepared, it must be filtered and used within 30 days.

   Insure the appropriate analytical column has been installed in the analytical system and that the injector is equipped with at least a 20-µL sample injection loop.

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All mobile phase must be degassed for at least 5 min with nitrogen, or helium prior to use.

The detector and the pump must be turned on for a warm-up period of at least 15 min prior to system evaluation. The pump flow must be set at 1.2 mL/min during the warm-up period. After approximately 15 min, measure the flow for 5 min with a 10-mL graduated cylinder. The flow rate must be set at 1.2 ± 0.1 mL/min. Adjust the flow rate setting on the pump if necessary to obtain an actual flow rate within these limits and re-check.

After the pump has been on for about 30 min, adjust the detector zero with the balance control with the detector set at 0.064 AUFS. Adjust the recorder to electrical zero at "0" chart units. Adjust the detector zero to slightly above the electrical zero position with the recorder balance control.

c. HPLC Identity Confirmation: Prepare HPLC mobile phase buffer for the initial identity confirmation using a Supelco LC-1 column by dissolving 6.0 g of sodium lauryl sulfate and 1.0 g of tetrabutylammonium nitrate in 1,000 mL of deionized water. Add 20 mL of glacial acetic acid to the solution and mix. Filter the solution with a 5 μm filter and store in a clean glass bottle. Use within 30 days.

The mobile phase may be established using a gradient system with a 60 percent buffer:40 percent acetonitrile ratio or mixed prior to analysis. To mix the mobile prior to analysis, add 600 mL of the buffer prepared above to a 1-L glass bottle and add 400 mL of acetonitrile and mix. Once the buffer has been prepared, it must be used within 30 days.

Insure the appropriate analytical column has been connected to the injector and detector, and that the injector is equipped with a 20-μL sample injection loop.

All mobile phase must be degassed for at least 5 min with nitrogen, or helium prior to use.

The detector and the pump must be turned on for a warm-up period of at least 15 min prior to system evaluation. The pump flow must be set at 1.0 mL/min during the warm-up period. After approximately 15 min, measure the flow for 5 min with a 10-mL graduated cylinder. The flow rate must be set at

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For every ten samples to be analyzed, at least one blank sample and one standard must be analyzed. All samples must be analyzed under the same conditions used for the standards.

c. HPLC Identity Confirmation: For confirmation of the identity of 2-PAM Cl by HPLC, a second set of HPLC conditions is employed. The following is a set of HPLC conditions found to be satisfactory for the confirmation of 2-PAM Cl:

Column: Supelco LC-1 (Stock No. 5-8296) 250 x 4.6 mm, 5 micron and Supelco LC-1 guard column (Stock No. 5-9551).

Mobile Phase: See Section G.4.c

Detector: UV @ 254 nm

Flow Rate: 1.0 mL/min

Injection Volume: 20 µL

For confirmation purposes, analyze a 2-PAM Cl standard and a formulation sample under these HPLC conditions.

7. Instrument Shut-Down:

a. When the instrument is not to be used for extended periods of time, the system must be shut down following manufacturer's instructions to ensure column life and instrument stability.

b. For overnight shut-down, turn off the UV detector, chart recorder, and pump controller.

c. For weekend shut-down, follow the same procedure as for overnight shut-down but also cap off the analytical column to prevent the solid phase from drying.

8. Data Reduction: The NMR spectrum obtained in Section G.5.a is compared with the reference spectrum to verify structural identity. HPLC samples analyzed in Section G.5.b are compared with results obtained from standards to determine concentration.

a. NMR: Compare the NMR spectrum for the sample with the spectrum obtained for the 2-PAM Cl reference standard. Verify correspondence of chemical shifts, multiplicities, and intensities for structural verification in conjunction with HPLC findings.

Revised February 20, 1990

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1.0 ± 0.1 mL/min. Adjust the flow rate setting on the pump if necessary to obtain an actual flow rate within these limits and re-check.

After the pump has been on for about 30 min, adjust the detector zero with the balance control with the detector set at 0.064 AUFS. Adjust the recorder to electrical zero at "0" chart units. Adjust the detector zero to slightly above the electrical zero position with the recorder balance control.

6. Analysis of Samples: NMR is performed for structural confirmation. HPLC standards and collected samples are analyzed to determine concentration and identify confirmation.

a. NMR: Multiple acquisitions (> 100 transients) are generally required. Spectra will be printed on standard NMR paper and computer referenced to the chemical shift of sodium 2,2-dimethyl-2-silapentane-5-sulfonate determined on the interpretation.

b. Quantitative HPLC: The following is a set of HPLC conditions that have been found to be satisfactory for quantitative analysis of 2-PAM Cl:

Column: Alltech Spherisorb-ODS 2 (Stock No. 8736) and Supelco LC-18 Guard Column (Stock No. 5-8232).

Mobile Phase: See Section G.4.b

Detector: UV @ 298 nm

Flow Rate: 1.2 mL/min

Injection Volume: 20 µL

For quantitative analysis of 2-PAM Cl samples, transfer 1-mL duplicate aliquots of each 2-PAM Cl standard to autosampler vials and place the vials in the autosampler in ascending concentration order. Set up the data system to acquire data for each standard as described in the data system instruction manual. Transfer 1-mL duplicate aliquots of each sample to autosampler vials and place the vials in the autosampler.

Revised February 20, 1990

APPROVED

b. Quantitative HPLC: Obtain printouts of the peak area ratios for each standard and sample as described in the instruction manual. Prepare a standard curve from the peak area ratios versus concentration of the standards.

Determine the 2-PAM Cl concentration in the samples and control standards using the standard curve. If necessary, correct for any dilution made to the samples prior to analysis.

If the response for any of the control standards varies from the predicted response by more than ± 10 percent, then the samples associated with that standard are reanalyzed.

c. HPLC Identity Confirmation: Compare the retention times and relative responses of the 2-PAM Cl standard and sample peak for structural confirmation.

H. Emergency Procedures: All personnel involved in the HML or MREF laboratory operations, must be familiar with the respective laboratory's FSSP, and the emergency procedures detailed within this document. All personnel involved in the King Avenue operation must be familiar with HEG H/SP B-01 and the emergency procedures detailed within this document.

I. First Aid Procedures: First aid and self aid at the MREF are to be conducted as specified in the FSSP.

TLH:cah

Revised February 20, 1990

APPROV

STANDARD OPERATING PROCEDURE
MREF SOP 88-50

TITLE: Analysis of Pralidoxime Chloride (2-PAM) in Whole Blood Using an Ultraviolet (UV) Spectrophotometer

LABORATORY: MREF or HML SOP Approval Date: May 19, 1989

EXPIRATION DATE: August 10, 1991

PLACE OF OPERATION OR TEST: Throughout the MREF and HML

This standard operating procedure (SOP) has been prepared as prescribed by Contract DAMD17-89-C-9050 and will be effective for one year from date of approval unless sooner rescinded or superseded.

No deviation from this SOP will be permitted. Whenever the approved method is changed, the SOP will be revised.

Supervisory personnel will assure that all personnel involved with this SOP have been trained properly and instructed in its provisions.

A copy of this SOP will be posted at the job site at all times.

Approved by: David Stitcher 10 Aug 90
Signature/Date

David L. Stitcher, CIH, Safety and Surety Officer
Printed Name/Title

STANDARD OPERATING PROCEDURE
MREF SOP-88-50

TITLE: Analysis of Pralidoxime Chloride (2-PAM) in Whole Blood Using an Ultraviolet (UV) Spectrophotometer

LABORATORY: MREF or HML SOP APPROVAL DATE: May 19, 1989

PLACE OF OPERATION OR TEST: Throughout the MREF and HML

This Standard Operating Procedure (SOP) has been prepared as prescribed by Contract DAMD17-89-C-9050 and will be effective for one year from date of approval unless sooner rescinded or superseded.

No deviation from this SOP will be permitted. Whenever the approved method is changed, the SOP will be revised.

Supervisory personnel will assure that all personnel involved with this SOP have been properly trained and instructed in its provisions and attest to this requirement by affixing their signatures on page 3.

A copy of this SOP will be posted at the Medical Research and Evaluation Facility (MREF) or Hazardous Materials Laboratory (HML) job site at all times.

Submitted By: Timothy L Hayes 5/15/89
Signature/Date

Timothy Hayes, Research Scientist
Printed Name/Title

Approved By: 5/15/89
Signature/Date

Garrett S. Dill, D.V.M., Manager
Printed Name/Title

Approved By: 5/19/89
Signature/Date

Donald W. Cagle, CIH, Safety/Surety Officer
Printed Name/Title

APPROVED

Approved By:

Ramona Mayer 5/19/89
Signature/Date

Ramona A. Mayer, Manager, QA Unit
Printed Name/Title

Approved By:

for 5/24/89
Signature/Date

Anna D. Barker, Ph.D.
Group Vice President and General Manager
Health and Environment
Printed Name/Title

APPROVED

SIGNATURES

I have read and understand the contents of MREF SOP-88-50.

Signature	Date
[illegible]	5-25-89
[illegible]	5-25-89
[illegible]	5-30-89
[illegible]	5/31/89
[illegible]	6-1-89
[illegible]	6-2-89
[illegible]	6/2/89
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[illegible]	6/12/89
[illegible]	6-12-89
[illegible]	[illegible]

Signature	Date
[illegible]	6/15/89
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[illegible]	6-30-89
[illegible]	7/5/89
[illegible]	7-31-89
[illegible]	10-9-89

[illegible]

STANDARD OPERATING PROCEDURE 88-50

Analysis of Pralidoxime Chloride (2-PAM) in Whole Blood
Using an Ultraviolet (UV) Spectrophotometer

A. Statement of Work: The following SOP describes a procedure for the determination of pralidoxime chloride (2-PAM) content in whole blood using an ultraviolet (UV) spectrophotometer. The method is based upon a direct UV absorption analysis procedure to measure the 2-PAM content in the prepared samples. To perform this analysis, a sample preparation must first be performed on the whole blood samples. This sample preparation requires three separate processes. The first consists of hemolyzing the blood with water and barium hydroxide solution, and the second de-proteinates the blood by addition of zinc sulfate and sodium chloride. The third precipitates the solid materials from the solution through centrifugation producing a sample ready for direct UV analysis. The prepared sample is analyzed for absorbance of light at 300 nm. A control (system blank) is used to correct for absorption by the cuvettes and reagents.

B. Responsibility:

1. Personnel Qualifications: All technical staff will be familiar with the handling of biological samples within the MREF laboratory. They must know the requirements of the Buddy System.

2. Leaders: Leaders of each operation will be designated by the Study Director for that operation. Each leader will insure that the following are observed:

   a. Only authorized personnel meeting requirements set forth in Section B.1 are allowed in the room during XCSM operations.

   b. XCSM are issued exclusively to personnel who have been designated in writing from the Manager, MREF, as authorized to receive XCSM.

   c. XCSM control and accountability are maintained.

   d. Adequate, approved, protective equipment is available at all times to personnel at their work site.

   e. All leader and technical staff responsibilities specified in the MREF FSSP are followed.

   f. Each employee has been trained in the techniques of administering first aid and self aid.

g. Work under this SOP is performed only in the area(s) or room(s) designated by this SOP.

h. No food, beverage, or tobacco product is consumed, used, or brought into the laboratory. The wearing of contact lenses is prohibited in the laboratory.

i. The safety requirements of this SOP, as well as normal laboratory safety, are maintained.

j. All applicable SOPs are read and signed by all technical staff involved in the operation.

3. Technical Staff: Technical staff will be responsible for abiding by requirements set forth in Section 8.2. In addition, they must use personal, protective equipment provided and develop safe work habits to protect themselves and fellow workers from injury and to prevent damage to material, equipment, and facilities.

4. Research Organization: The organization involved in this research is the MREF of Battelle Memorial Institute, 505 King Avenue, Columbus, OH 43201-2693.

C. Materials To Be Used:

1. Solvents and Chemicals: 2-PAM, barium hydroxide octahydrate, zinc sulfate heptahydrate, and sodium chloride.

D. Tools and Equipment: Freezer, refrigerator, labels, first-aid kit, plastic-backed, absorbent paper, brown paper, squirt bottles, wiping tissues, beakers, bottles, maxi-vials, pipettes, pipette bulbs, tissue paper, laboratory coat, safety shoes, safety glasses, spatula, stainless-steel pans, glass stir rods, syringes, needles, forceps, scrub suit, latex gloves, 16 x 100-mm culture tubes, disposable cuvettes, vortex mixer, UV spectrophotometer, and centrifuge.

E. Hazards Involved:

1. Chemicals: The reagents used in this process may have hazards associated with their use. A copy of the Material Safety Data Sheets (MSDS) is available from the manufacturer or through Battelle's Safety Office at 505 King Avenue. A brief listing of hazards associated with handling these chemicals has been included:

a. 2-PAM: 2-PAM is a harmful powder which is readily absorbed through the skin.

b. Barium Hydroxide: Barium hydroxide is highly toxic by ingestion. Corrosive to tissue in presence of moisture; strong irritant to tissue.

c. Zinc Sulfate: Zinc sulfate is an irritant to tissue. It is low in toxicity. Hygroscopic.

2. Gloves and aprons made of butyl rubber are flammable and have no self-extinguishing capability; therefore, care must be taken to avoid open flame or heat that may ignite them.

F. Safety Requirements:

1. Hoods: Hood face velocity must average 100 ± 10 lfpm. The average is computed from individual readings taken in approximately each square foot of hood face (usually nine readings). In addition, no individual reading will vary more than 20 percent from the average. No equipment will be within 20 cm of the face of the hood.

2. Protective Equipment: The following clothing and protective gear are required as a minimum for all personnel.

scrub suit
safety shoes
latex gloves
safety glasses

All provisions of the MREF FSSP apply to the checking and testing of gloves, aprons, and other protective equipment.

3. First Aid: The location of the nearest eye-wash fountain, deluge shower, and fire extinguisher will be known to all workers before work begins.

G. Procedures:

1. Entry: Before entering the secured facility, note the status of the "Agent-in-Use" light at the turnstile. If the "Agent-in-Use" lights are turned on, note the room location and be sure that upon entry to the laboratory area that all safety equipment and procedures described in FSSP SOP MREF-18 are in place. Upon entry of the room, confirm that there are no audible alarms. No operations can be initiated in a room with audible alarms. After entry, personnel will observe the magnehelic gauge on the hood. If inspection reveals that the hood has failed, is marginal in flow, or operates outside the guidelines of FSSP SOP MREF-21, the problem is reported to the MREF Manager and the operation does not begin.

2. Hood Set Up: The operation hood area must be prepared with all materials necessary to perform the operation prior to starting the operation. All materials will be kept behind the 8-inch line in the hood.

Plastic-backed, absorbent paper must be used to protect the work surface of the hood.

3. Equipment Preparation:

   a. Glassware: All glassware shall be cleaned and silanized with hexamethyl disilizane (HMDS) prior to use. This serves two purposes. First, it minimizes adsorption of chemicals on otherwise active glass surfaces, and secondly facilitates cleaner separation of solid and liquid layers due to a smoother surface of the glass wall.

      Wash three times each with 5 percent alconox solution, followed by methanol, and finally acetone, then dry in a drying oven. Place clean glassware in a vacuum oven and pull vacuum via an aspirator or vacuum pump to 20 to 25-mm Hg. Heat the oven to approximately 180 C and inject 1-mℓ HMDS. Continue to heat the oven for 2-3 hr. Still under vacuum, allow the oven to cool to room temperature (overnight), then vent the oven. Glassware treated in this manner is now ready for use.

   b. Instrument Preparation: The UV spectrophotometer is prepared with the following settings:

      (1) Wavelength - 300 nm

      (2) Read sample observance every 1 sec for 4 sec starting at time 0. (This gives five absorbance readings which allow for the approximation of error due to drift of the wavelength setting.)

4. Solution Preparation:

   a. Preparation of 2-PAM Analytical Standards and Spiking Solutions:

      (1) 500-μg/mℓ 2-PAM Stock Solution: Dispense a 0.0505-g sample of 2-PAM into a 100-mℓ volumetric flask containing approximately 40 mℓ of deionized water. Dilute to volume with deionized water and mix well before transferring to storage vials.

      (2) 50-μg/mℓ 2-PAM Spiking Solution: Into a 50-mℓ volumetric flask containing approximately 20 mℓ of deionized water, deliver 5.0 mℓ of the 500-μg/mℓ 2-PAM stock solution (using a 5,000-μℓ syringe). Dilute to volume with deionized water and mix on a vortex mixer.

      (3) 30-μg/mℓ Analytical Standard: Into a 5-mℓ volumetric flask containing approximately 1 mℓ of deionized water, deliver 3.0 mℓ of the 50-μg/mℓ 2-PAM spiking solution (using a 5,000-μℓ syringe). Dilute to volume with deionized water and mix on a vortex mixer.

(4) 25-µg/mℓ Analytical Standard: Into a 5-mℓ volumetric flask containing approximately 1 mℓ of deionized water, deliver 2.5 mℓ of the 50-µg/mℓ 2-PAM spiking solution (using a 2,500-µℓ syringe). Dilute to volume with deionized water and mix on a vortex mixer.

(5) 20-µg/mℓ Analytical Standard: Into a 5-mℓ volumetric flask containing approximately 1 mℓ of deionized water, deliver 2.0 mℓ of the 50-µg/mℓ 2-PAM spiking solution (using a 2,500-µℓ syringe). Dilute to volume with deionized water and mix on a vortex mixer.

(6) 15-µg/mℓ Analytical Standard: Into a 5-mℓ volumetric flask containing approximately 1 mℓ of deionized water, deliver 1.5 mℓ of the 50-µg/mℓ 2-PAM spiking solution (using a 2,500-µℓ syringe). Dilute to volume with deionized water and mix on a vortex mixer.

(7) 10-µg/mℓ Analytical Standard: Into a 5-mℓ volumetric flask containing approximately 1 mℓ of deionized water, deliver 1.0 mℓ of the 50-µg/mℓ 2-PAM spiking solution (using a 1,000-µℓ syringe). Dilute to volume with deionized water and mix on a vortex mixer.

(8) 8.0-µg/mℓ Analytical Standard: Into a 5-mℓ volumetric flask containing approximately 1 mℓ of deionized water, deliver 0.8 mℓ of the 50-µg/mℓ 2-PAM spiking solution (using a 1,000-µℓ syringe). Dilute to volume with deionized water and mix on a vortex mixer.

(9) 5.0-µg/mℓ Analytical Standard: Into a 5-mℓ volumetric flask containing approximately 1 mℓ of deionized water, deliver 0.5 mℓ of the 50-µg/mℓ 2-PAM spiking solution (using a 500-µℓ syringe). Dilute to volume with deionized water and mix on a vortex mixer.

(10) 4.0-µg/mℓ Analytical Standard: Into a 5-mℓ volumetric flask containing approximately 1 mℓ of deionized water, deliver 0.4 mℓ of the 50-µg/mℓ 2-PAM spiking solution (using a 500-µℓ syringe). Dilute to volume with deionized water and mix on a vortex mixer.

(11) 2.5-µg/mℓ Analytical Standard: Into a 5-mℓ volumetric flask containing approximately 1 mℓ of deionized water, deliver 0.25 mℓ of the 50-µg/mℓ 2-PAM spiking solution (using a 250-µℓ syringe). Dilute to volume with deionized water and mix on a vortex mixer.

b. All stock solutions should be kept refrigerated when not in use. Stock solutions made from neat material for the purpose of making further diluted standard/spiking solutions must be discarded and new preparations made monthly.

Standard/spiking solutions made from stock solutions must be remade for each day of analysis.

5. Sample Preparation:

a. 2.0 mℓ of each whole blood sample is removed and measured into a 16 x 100-mm screw cap culture tube using a 2-mℓ volumetric pipette.

b. To the 2.0-mℓ whole blood samples measured in Section G.5.a, add 3.8 mℓ of deionized water using a 5.0-mℓ syringe and 1.0 mℓ of barium hydroxide using a 1.0-mℓ volumetric pipette. The solution is mixed on the vortex mixer for 30 sec.

c. To the mixed sample solution, add 1.0-mℓ 0.33 m zinc sulfate using a 1.0-mℓ volumetric pipette and 0.2-mℓ sodium chloride using a 250-μℓ syringe. The solution is mixed on a vortex mixer for 10 sec.

d. The sample plus reagents contained in the culture tube is then placed in a centrifuge and the solids precipitated at 1,500 g's for 10 min.

e. The "clear" top layer is removed and transferred to another 2 mℓ centrifuge tube and re-centrifuged at 10,000 g's for 3 min.

f. Transfer the sample to labeled cuvettes for analysis.

6. Calibration:

a. Instrument calibration must be performed for quantitation of 2-PAM in the samples using the blank deionized water (blank) and the calibration standards prepared in Section G.4.a. A complete set of calibration standards must be analyzed prior to analysis of any sample extracts. All analyses of standard sets must be within 10 percent relative standard deviation. If any standard analysis value is outside this limit, the analysis of unknowns is stopped until the problem is resolved.

b. Once the calibration of the instrument has been checked, the sample extracts are analyzed in a sequence with a calibration check standard being analyzed after every fifth sample. A calibration check standard can be any solution of 2-PAM within the calibration range and of known concentration.

c. A complete set of calibration standards is analyzed following the last sample each day. All calibration standards analyzed throughout the analysis are used to develop a complete calibration curve for quantitation of the sample extracts.

d. Only concentration values that fall inside the range of the calibration standards will be reported. Samples that yield responses less than the calibration range will be reported as less than the lower quantitation limit. Any sample response that exceeds the largest calibration standard will be reported as greater than the highest calibration standard and must be diluted to within the calibration range.

e. Detection limit determination is performed by analyzing a series of extraction recovery samples in the range of interest. If a peak area response is observed with greater than a three to one signal to noise ratio, the method detection limit can be determined as that concentration. The detection limit must be verified by extraction for each sample set.

7. Analysis of Samples: Samples and calibration standards are analyzed using the same procedures and conditions. Following every fifth analysis a system check standard must be analyzed.

8. Calculations:

a. The calibration data are analyzed using a linear regression analysis and the quantitative measurements made based upon and external standard procedure.

b. Using a linear regression program, generate the slope, intercept, and correlation coefficient for 2-PAM in the calibration data. The resulting calibration parameters will be used to calculate the observed concentration of 2-PAM in the unknown samples.

c. Enter the absorbance as the ordinate (y-value) and the corresponding standard concentration as the abscissa (x-value).

d. Enter each data point obtained from the calibration standards, and calculate percent relative standard deviation (%RSD) between replicate standards.

e. If a regression program is not available, program the following calculations:

$$b = \frac{[(\Sigma y)(\Sigma x^2)-(\Sigma x)(\Sigma xy)]}{[n(\Sigma x^2)-(\Sigma x)^2]}$$

$$a = \frac{[n(\Sigma xy)-(\Sigma x)(\Sigma y)]}{[n(\Sigma x^2)-(\Sigma x)^2]}$$

$$r = \frac{[n(\Sigma xy-(\Sigma x)(\Sigma y)]}{[(n(\Sigma x^2)-(\Sigma x)^2)^{1/2}(n\Sigma(y^2)-(\Sigma y)^2)^{1/2}]}$$

where: $y = ax + b$
a = slope
b = y-intercept
r = correlation coefficient
x = peak area
y = concentration of 2-PAM in $\mu g/m\ell$
n = number of replicates

f. To obtain actual concentration of 2-PAM in the samples, the observed absorbance should be adjusted by subtracting the average absorbance of extraction blanks. This value is used to calculate the 2-PAM concentration from the regression.

For example, if the following values were obtained for 2-PAM in a sample extract,

Observed absorbance response = 1.5020
Average absorbance response of extraction blanks = 0.02
Corrected absorbance response = 1.482

TLH:tsh

STANDARD OPERATING PROCEDURE
MREF SOP-89-55

TITLE: Analysis and Structural Verification of Atropine in Citrate Buffer

LABORATORY: MREF, HML, or King Ave. SOP APPROVAL DATE: 02/26/90

PLACE OF OPERATION OR TEST: Any safety approved laboratory within the facilities

This Standard Operating Procedure (SOP) has been prepared as prescribed by Contract DAMD17-89-C-9050 and will be effective for one year from date of approval unless sooner rescinded or superseded.

No deviation from this SOP will be permitted. Whenever the approved method is changed, the SOP will be revised.

Supervisory personnel will assure that all personnel involved with this SOP have been properly trained and instructed in its provisions and attest to this requirement by affixing their signatures on page 3.

A copy of this SOP will be posted at the job site whenever the operation is being performed.

Submitted By: Timothy L Hayes 2/20/90
Signature/Date

Timothy L. Hayes, Research Scientist
Printed Name/Title

Approved By: [signature] 2/21/90
Signature/Date

Garrett S. Dill, D.V.M., Manager
Printed Name/Title

Approved By: David Stitcher 2/26/90
Signature/Date

David L. Stitcher, CIH, Safety/Surety Officer
Printed Name/Title

Revised February 20, 1990

Approved By:

Richard A. Stewart 2-27-90
Signature/Date

Quality Assurance Unit
Health and Environment Group
Printed Name/Title

Signature/Date 2/27/90

Charles K. Burdick, Director
Total Quality Program
Health and Environmental Group
Printed Name/Title

Revised February 20, 1990

SIGNATURES

I have read and understand the contents of MREF SOP-89-55.

Signature	Date	Signature	Date
Malcolm J. Reeves	3-14-90		
Melissa J. Hayes	3/19/90		
Sheri D. Moore	3/30/90		
James A. Beck	4/2/90		
James Clapp	4/2/90		
Raymond L. Cunningham	6/26/90		

Revised February 20, 1990

APPROVED

STANDARD OPERATING PROCEDURE 89-55

Analysis and Structural Verification of
Atropine Base in Citrate Buffer

A. Statement of Work: This SOP describes the entire procedures for verification of identity and quantitative measurement of atropine free base by high performance liquid chromatography (HPLC). The procedures for structural verification by nuclear magnetic resonance (NMR) of atropine present in drug formulations are also described. The HPLC effort can be conducted at either the MREF, HML or King Avenue, but the NMR requires the facilities at King Avenue.

B. Responsibility:

1. Personnel Qualifications:

All technical staff will be familiar with handling hazardous materials within the laboratory. Personnel performing the following procedures must read and sign this SOP.

2. Leaders: Leaders of each operation will be designated by the Study Director for that operation. Each leader will insure that the following are observed:

a. Only authorized personnel meeting requirements set forth in Section 8.1 are allowed in the room during operations.

b. Adequate, approved, protective equipment is available at all times to personnel at their work site.

c. All leader and technical staff responsibilities specified in the MREF or HML FSSP are followed when work is conducted at the respective laboratories.

d. Each MREF and HML employee has been trained in the techniques of administering first aid and self aid.

e. Work under this SOP is performed only in the area(s) or room(s) designated by this SOP.

Revised February 20, 1990

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f. No food, beverage, or tobacco product is consumed, used, or brought into the laboratory. The wearing of contact lenses is prohibited in the laboratory.

g. The safety requirements of this SOP, as well as normal laboratory safety, are maintained.

h. All applicable SOPs are read and signed by all technical staff involved in the operation.

3. Technical Staff: Technical staff will be responsible for abiding by requirements set forth in Section B.2. In addition, they must use personal, protective equipment provided and develop safe work habits to protect themselves and fellow workers from injury and to prevent damage to material, equipment, and facilities.

4. Research Organization: The organization involved in this research is the MREF of Battelle Memorial Institute, 505 King Avenue, Columbus, Ohio 43201-2693.

C. Materials To Be Used:

1. Solvents and Chemicals: The atropine sulfate solid which will be used on this program for preparation of analytical standards will be provided by the U.S. Army Medical Research and Development Command (USAMRDC) or a source which can provide an established purity.

If the atropine dosing solution is not received in a pre-packaged form upon receipt, the atropine dosing solution in citrate buffer will be stored in subdued lighting at 4 C. If a pre-packaged form has been received, it will be stored as directed by the supplier.

NMR spectra will be obtained on dilute solutions of the drug dissolved in > 99.8 percent deuterium oxide (Stohler Isotope Chemicals or equivalent). NMR tubes will be the Stohler Isotope Chemicals "Ultra Precision" model or the equivalent model from other manufacturers.

Other materials will include acetonitrile (Burdick and Jackson HPLC Grade), methanol (Burdick and Jackson HPLC Grade), benzene (Burdick and Jackson HPLC Grade), deionized water or millipore water, glacial acetic acid (Baker Reagent Grade), tetrabutylammonium chloride (Aldrich 98+ percent), sodium lauryl sulfate (Aldrich 98 percent), sodium heptane sulfonate (1-heptane sulfonic acid, sodium salt) (Aldrich 98+ percent), tetramethylammonium chloride (Aldrich 98+ percent), and helium or nitrogen gas.

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D. Equipment: Freezer, refrigerator, labels, first-aid kit, plastic-backed, absorbent paper, squirt bottles, wiping tissues, beakers, bottles, maxi-vials, pipettes, pipette bulbs, tissue paper, laboratory coat, protective eyewear, spatula, stainless-steel pans, glass stir rods, syringes, needles, forceps, and latex gloves.

Proton NMR spectra will be obtained on Battelle's Varian CFT-20 Fourier transform NMR spectrometer located in Room 7237-A of the King Avenue facility.

The HPLC analytical system, to be used consists of the following: HPLC pump, HPLC ultra violet (UV) detector, HPLC injection system (autosampler), analytical column, strip-chart recorder (optional), electronic data system. Any equivalent system may be used once confirmation of performance has been established.

E. Hazards Involved:

1. Solvents: The solvents used in preparing the dilute material may have hazards associated with their use. A copy of the Material Safety Data Sheets (MSDS) is available from the manufacturer or through Battelle's Safety Office at 505 King Avenue. A brief listing of hazards associated with handling the more commonly used solvents has been included:

   a. Acetonitrile: Acetonitrile is a flammable liquid that must be handled as a solvent with a dangerous fire risk. The flash point of acetonitrile is 5.56 C. The 1988-1989 ACGIH TLV for acetonitrile is 40 parts per million (ppm) as an 8-hr TWA and 60 ppm as a 15-min STEL. Skin contact may also represent a significant route of exposure.

   b. Methanol: Methanol is a flammable liquid that must be handled as a solvent with a dangerous fire risk. The flash point (open cup) of methanol is 12.2 C, with an autoignition temperature of 464 C. The 1988-1989 ACGIH TLV for methanol is 200 ppm as an 8-hr TWA and 250 ppm as a 15-min STEL. Also, skin contact may represent a significant route of exposure.

   c. Benzene: Benzene is a flammable liquid that must be handled as a solvent with a dangerous fire risk. Benzene is toxic by ingestion, inhalation, and skin absorption. Benzene is regulated as a carcinogen by the Occupational Safety and Health Administration (OSHA) resulting in excess leukemia. Containers must say "DANGER CONTAINS BENZENE CANCER HAZARD." OSHA 8-hr permissible exposure limit (PEL) = 1 ppm, Action Level = 0.5 ppm.

Revised February 20, 1990

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F. Safety Requirements:

1. Hoods: Hood face velocity must average 100 ± 20 lfpm. The average is computed from individual readings taken in approximately each square foot of hood face (usually nine readings). No equipment will be within 20 cm of the face of the hood.

2. Protective Equipment: When working in the laboratory, the following clothing and protective gear are required as a minimum for all personnel. This equipment must be used as directed in the FSSP.

   lab coat
   latex gloves (as needed)
   protective eyewear

   All provisions of the FSSP apply to the checking and testing of gloves, aprons, and other protective equipment.

3. First Aid: The location of the nearest eye-wash fountain, shower, and fire extinguisher will be known to all workers before work begins.

G. Procedures:

1. MREF Entry: Before entering the secured facility, note the status of the "Agent-in-Use" light at the turnstile. If the "Agent-in-Use" lights are turned on, note the room location and be sure that upon entry to the laboratory area all safety equipment and procedures described in FSSP SOP MREF-18 are in place. Upon entry of the room, confirm that there are no audible alarms. No operations can be initiated in a room with audible alarms. After entry, personnel will observe the magnehelic gauge on the hood. If inspection reveals that the hood has failed, is marginal in flow, or operates outside the guidelines of FSSP SOP MREF-21, the problem is reported to the MREF Manager and the operation does not begin.

2. Hood Set Up: The operation hood area must be prepared with all materials necessary to perform the operation prior to starting the operation. All materials will be kept behind the 8-inch line in the hood.

3. Sample Preparation: The drug formulation samples provided for analysis will be manipulated so that the interference of solvents and other components associated with the samples is minimized to provide relatively pure drug samples for NMR analysis.

Revised February 20, 1990

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HPLC analyses may be performed on either the dosing formulations as received, dilutions of the parent materials, or on reference standard solutions of known concentration.

a. Analytical Reference Standard: Solid atropine sulfate standard used as a reference material is dried at 100 C, 0.4 mm Hg for 3 hr prior to use in a vacuum oven. This is performed by placing the solid material contained in its original container which has had its cap removed into a pre-heated oven. The oven is sealed and the vacuum adjusted to 0.4 mm Hg.

b. NMR: For the NMR sample preparation, 1 mL of test sample is made basic with 2.0 mL of 0.1 M sodium hydroxide to reach a pH of approximately 13 (verified by color pHast paper). This solution is stirred rapidly with benzene (5.0 mL) for 15 min and then poured through Whatman lps phase separation paper (with 1.0-mL benzene rinse). The filtrate is stirred for 1 min with 2.0-mL deionized water and this mixture is passed again through a fresh phase separation paper (with 1.0-mL benzene rinse). The benzene filtrate is evaporated in a rotary evaporator to yield atropine as its free base. The sulfate is reformed by adding a slight molar excess of dilute D_2SO_4 in D_2O to the free base.

   NMR samples are prepared by transfer of the deuterium oxide solution and transferred into an NMR tube (tube capped after transfer) for NMR analysis.

c. HPLC Analysis: Samples are either analyzed directly or can be diluted so that the expected concentration range is between 0.1 and 1.0 mg/mL.

4. Preparation of Standard Solutions: Standard solutions of atropine sulfate are prepared for NMR reference spectrum and HPLC standard curve determinations.

   a. NMR: Within a glove bag thoroughly flushed with dry nitrogen or argon, weigh 10 ± 0.1 mg of atropine sulfate onto weighing paper. Transfer the sample into a screw-capped bottle and close tightly. Outside the bag, dissolve the sample in an accurately measured volume of 10.0 mL of deuterium oxide and recap the bottle to minimize the contamination of the sample with undeuterated moisture.

   b. HPLC: Weigh 50 ± 0.1 mg of atropine sulfate onto weighing paper. Quantitatively, transfer the sample into a 50-mL volumetric flask containing approximately 40 mL of mobile phase (see Section G.6.b)

INITIATED

Mix the solution thoroughly on a vortex mixer. Dilute to 50.0 mL with the mobile phase and remix the solution. The resulting concentration of the atropine sulfate will be approximately 1 mg/mL.

Mix and dilute the atropine sulfate stock solution with the mobile phase as follows:

10.0-mL stock + 0.0-mL mobile phase
5.0-mL stock + 5.0-mL mobile phase
2.5-mL stock + 7.5-mL mobile phase
1.0-mL stock + 9.0-mL mobile phase
0.0-mL stock + 10.0-mL mobile phase

The atropine sulfate concentrations obtained are 1.00, 0.50, 0.25, 0.10, and 0.0 mg per mL.

Diluted standard solutions are kept refrigerated until use. Standards may be kept refrigerated for up to 30 days.

5. Analysis Start-Up: NMR is performed to verify the structure of atropine sulfate. HPLC is performed to quantitatively determine the concentration of atropine sulfate and confirm the identity of the atropine in the samples.

   a. NMR: Calibrate the NMR instrument and data system according to instructions in the operator's manual. When properly calibrated against the standard reference solutions identified in the manual, proceed with the analysis Section G.7.a.

   b. Quantitative HPLC: Prepare HPLC mobile phase for quantitative analysis by dissolving 2.2 g of sodium heptane sulfonate (1-heptane sulfonic acid sodium salt) and 2.7 g of tetramethylammonium chloride in approximately 90 mL of deionized water. Add 1.0 mL of glacial acetic acid and dilute to 1 L and mix. Filter buffer solution before using.

   The mobile phase may be established using a gradient system with a 78 percent buffer : 2 percent methanol : 20 percent acetonitrile ratio or mixed prior to analysis. To mix the mobile prior to analysis, add 780 mL of the buffer prepared above to a 1-L glass bottle, add 20 mL of methanol and 200 mL of acetonitrile and mix. Once the buffer has been prepared, it must be filtered and used within 30 days.

APPROVED

Insure that the appropriate analytical column has been installed in the analytical system, and that the injector is equipped with at least a 20 µL sample injection loop.

All mobile phase must be filtered and degassed for at least 5 min with nitrogen or helium, prior to use.

The detector and the pump must be turned on for a warm-up period of at least 15 min prior to system evaluation. The pump flow must be set at 1.0 mL/min during the warm-up period. After approximately 15 min, measure the flow for 5 min with a 10-mL graduated cylinder. The flow rate must be set at 1.0 ± 0.1 mL/min. Adjust the flow rate setting on the pump controller if necessary to obtain an actual flow rate within these limits and re-check flow.

After the pump has been on for 30 min, adjust the detector zero with the balance control with the detector attenuation set at the appropriate attenuation. Adjust the recorder to electrical zero at "0" chart units. Adjust the detector zero to slightly above the electrical zero position with the recorder balance control.

c. HPLC Identity Confirmation: Prepare HPLC mobile phase for identity confirmation by adding 6.0 g of sodium lauryl sulfate and 1.0 g of tetrabutylammonium nitrate to a 1-L volumetric flask and dissolve the reagents in approximately 500 ml of deionized water. Add 20 mL of glacial acetic acid to the solution and mix. The volumetric flask is filled to the 1-L mark and the solution re-mixed. Filter the solution with a 5 µm filter and store in a clean glass bottle. Use within 30 days.

The mobile phase may be established using a gradient system with a 60 percent buffer : 40 percent acetonitrile ratio or mixed prior to analysis. To mix the mobile prior to analysis, add 600 mL of the buffer prepared above to a 1-L glass bottle and add 400 mL of acetonitrile and mix. Once the buffer has been prepared it must be used within 30 days.

Insure that a Supelco LC-1 column or equivalent has been connected to the injector and detector and the injector is equipped with a 20 µL sample injection loop.

All mobile phase must be degassed for at least 5 min with helium or nitrogen prior to use.

The detector and the pump must be turned on for a warm-up period of at least 15 min prior to system evaluation. The pump flow must be set at 1.0 mL/min during the warm-up period. After approximately 15 min, measure the flow for 5 min with a 10-mL graduated cylinder. The flow rate should be 1.0 ± 0.1 mL/min. Adjust the flow rate setting on the pump if necessary to obtain an actual flow rate within these limits and re-check.

After the pump has been on for 30 min, adjust the detector zero with the balance control with the detector set at the appropriate attenuation. Adjust the recorder to electrical zero at "0" chart units. Adjust the detector zero to slightly above the electrical zero position with the recorder balance control.

6. Analysis of Samples: NMR is performed for structural confirmation. HPLC standards and collected samples are analyzed to determine concentration and identity confirmation.

   a. NMR: Multiple acquisitions (> 100 transients) are generally required. Spectra will be printed on standard NMR paper and computer referenced to the chemical shift of sodium 2,2-dimethyl-2-silapentane-5-sulfonate determined on the same day to facilitate interpretation.

   b. Quantitative HPLC: The following is a set of HPLC conditions that have been found to be satisfactory for quantitative analysis of atropine sulfate by HPLC (reference 1):

      Column: C18 u-Bondapak or equivalent, 250-mm long x 4.6-mm inner diameter with 5 micron particle size.

      Mobile Phase: See Section G.6.b

      Detector: UV @ 260 nm

      Flow Rate: 1.8 mL/min

      Injection Volume: 20 µL

      For quantitative analysis of atropine sulfate samples, transfer 1-mL duplicate aliquots of each atropine sulfate standard to autosampler vials and place the vials in the autosampler in ascending concentration order. Set up the data system to acquire data for each standard as described in the instruction manual. Transfer 1-mL duplicate aliquots of each sample to autosampler vials and place the vials in the autosampler.

For every ten samples to be analyzed, one blank sample and one standard must be analyzed as a minimum. All samples must be analyzed under the same conditions as used for the standards.

c. HPLC Identity Confirmation: For confirmation of the identity of atropine sulfate by HPLC, a second set of HPLC conditions is employed. The following is a set of HPLC conditions found to be satisfactory for the confirmation of atropine.

Column: Supelco LC-1, 250-mm long x 4.6-mm inner diameter, with 5 micron particle size.

Mobile Phase: See Section G.6.c

Detector: UV @ 254 nm

Flow Rate: 1 mL/min

Injection Volume: 20 µL

For confirmation purposes, analyze an atropine sulfate standard and a sample from the formulation under these HPLC conditions.

7. Instrument Shut-Down:

a. When the instrument is not to be used for extended periods of time, the system must be shut down following manufacturer's instructions to ensure column life and instrument stability.

b. For overnight shut-down, turn off the UV detector, chart recorder, and pump controller.

c. For weekend shut-down, follow the same procedure as for overnight shut-down but also cap off the analytical column to prevent the solid phase from drying.

8. Data Reduction: The NMR spectra obtained in Section G.7 are compared to reference NMR spectra for atropine to verify structural identity. The HPLC samples analyzed in Section G.7 are compared with results obtained from known reference standards to determine concentration.

a. NMR: Compare the NMR spectrum for the sample with the spectrum obtained for the atropine sulfate reference standard. Verify correspondence of chemical shifts, multiplicities, and intensities for structural verification in conjunction with HPLC findings.

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APPROVED

b. Quantitative HPLC: Obtain printouts of the peak areas for each standard and sample as described in the data system instruction manual. Prepare a standard curve from the peak areas versus concentration of the standards.

Determine the atropine sulfate concentration in the samples and control standards using the standard curve. If necessary, correct any dilution made to the samples prior to analysis.

If the response for any of the control standards varies from the predicted response by more than ± 10 percent, then the samples associated with that standard are reanalyzed.

c. HPLC Identity Confirmation: HPLC confirmation of the identity of atropine sulfate is performed by analysis under a second set of HPLC conditions. Compare the retention times and relative responses of the atropine sulfate reference standard and sample peak for structural confirmation in conjunction with the first set of HPLC results and NMR conclusions.

H. Emergency Procedures: All personnel involved in the HML or MREF laboratory operations, must be familiar with the respective laboratory's FSSP, and the emergency procedures detailed within this document. All personnel involved in the King Avenue operation must be familiar with HEG H/SP 8-01 and the emergency procedures detailed within this document.

I. First Aid Procedures: First aid and self aid at the MREF are to be conducted as specified in the FSSP.

J. References:

1. "Assay of Formulated Atropine Solution, WR-6241AK, B107753, Lot No. RU7144," Report No. 527, Contract No. DAMD17-85-C-5141, SRI International Project No. 8504, December 10, 1985.

TLH:cah

Revised February 20, 1990

APPROVED

Manual Number:

Battelle SOP Number: TOX VI-014-00
Effective Date: December 28, 1990
Page 1 of 10

Key Words: ATROPINE, RADIOIMMUNOASSAY, RIA

Standard Operating Procedure (SOP)

THE DETERMINATION OF SERUM ATROPINE SULFATE CONCENTRATIONS BY RADIOIMMUNOASSAY (RIA)

Originated by: Victor D. Myers Date 12-17-90

Approved by: J. M. Kellinger Date 12/20/90
Manager
Toxicology and Pharmacology Dept.

Approved by: Richard A. Shaffer for Date 12/21/90
Executive Secretary
Total Quality Council
Health and Environment Group

Reviewed and Registered by QA: Kathleen Reed Date 12-21-90

Distribution List:

Quality Assurance Unit
SOP Manuals

Battelle
Health and Environment Group
505 King Avenue
Columbus, Ohio 43201

I/II. SCOPE/PURPOSE:

The purpose of this Standard Operating Procedure (SOP) is to describe a Radioimmunoassay method employed in the determination of serum atropine sulfate concentrations.

III. REFERENCES:

1. Wurzburger, R. J., Miller, R. L., Boxenbaum, H. G., and J. Spector. 1977. Radioimmunoassay of Atropine In Plasma. J. Pharmacol Exp Therap 203: 435.

2. Kradjan, W. A., Smallridge, R. C., Davis, P. J., and P. Verma. 1985. Atropine Serum Concentrations After Multiple Inhaled Doses of Atropine Sulfate. Clin Pharmacol Therap 38: 12.

IV. DEFINITIONS: None

V. PROCEDURES:

Preliminary Tasks

A. Preparation of Phosphate Buffered Saline (PBS), pH 7.5

1. Combine the following components to prepare 1 liter PBS (10 mM Na_2HPO_4, 150 mM NaCl), pH 7.5:

Na_2HPO_4	1.[illegible]0 grams
NaCl	8.[illegible]5 grams
distilled water	9[illegible].0 ml

2. Adjust the pH to [illegible] with 0.1 N HCl. Bring the volume to 1000 ml with distilled water.

3. Store PBS at 1-9°C. The PBS is stable for a period of one month from the date of preparation.

B. Preparation of Saturated Ammonium Sulfate

1. Combine the following reagents to prepare 500 ml saturated ammonium sulfate:

$(NH_4)_2SO_4$	257.6 grams
distilled water	500.0 ml

2. Do not adjust pH. Store at 1-9°C. This reagent is stable for a period of one month from date of preparation. Prepare at least 24 hours prior to use.

C. Preparation of 50 percent Saturated Ammonium Sulfate

1. Combined the following reagents to prepare 500 ml of 50 percent saturated ammonium sulfate:

$(NH_4)_2SO_4$	128.8 grams
distilled water	500.0 ml

2. Do not adjust pH. Store at 1-9°C. This reagent is stable for a period of one month from the date of preparation. Prepare at least 24 hours prior to use.

D. Preparation of ^{3}H-Atropine Stock Solution

1. ^{3}H-Atropine is prepared in PBS, pH 7.5, at a concentration of approximately 4000 CPM/20 µl. This material is aliquoted and stored at -70 (± 5)°C. The labeled atropine is stable for a period of one year.

2. Thaw a fresh aliquot daily. Dispose of the leftover material at the conclusion of the experiment according to Battelle SOP for disposal of radioactive materials.

Preparation of Primary Atropine Stock Solution

1. Prepare a 1.0 mg/ml solution of atropine sulfate in PBS, pH 7.5. Weigh a minimum of 10.0 mg atropine sulfate. Mix thoroughly and aliquot. Store at -70 (± 5)°C. The material is stable for a period of one year from the date of preparation.

F. Preparation of Rabbit Anti-Atropine Antisera Stock

1. The correct concentration of rabbit anti-atropine antisera will be determined in preliminary testing. The stock antisera is stored as 30 µl aliquots at -70 (± 5)°C. Dilute the antisera to the proper concentration in PBS, pH 7.5. Prepare the diluted antibody fresh daily. Leftover material may be frozen and used for repeat analyses performed within a period of five days. Thereafter, dispose of the diluted material.

G. Normal Serum

1. A stock of normal serum obtained from the same species as that of the serum samples being analyzed will be aliquoted and

stored at -70 (± 5)°C. The frozen stock is stable for a period of one year.

2. Aliquot(s) of normal serum are thawed freshly on the assay day. The serum is used undiluted in the assay. Unused material may be frozen and used on a subsequent test day.

H. Test Samples

1. Test samples are stored at -70 (± 5)°C.

RIA Set Up (Day 1)

1. Prepare atropine sulfate Stocks A and B fresh daily from freshly thawed aliquot of the Primary Atropine Stock solution as follows:

   a) Combine 10 µl Primary Atropine Stock + 990 µl PBS (Dilution a)
   b) Combine 10 µl Dilution a + 990 µl PBS (Dilution b)
   c) Combine 250 µl Dilution b + 750 µl PBS (Stock A)
   d) Combine 10 µl Dilution b + 990 µl PBS (Stock B)

2. Dispose of the leftover Primary Atropine Stock as well as leftover atropine Stocks A and B and dilutions a and b at the conclusion of the RIA set up.

3. Prepare Stock C by combining 1.8 ml Stock A with 1.5 ml of normal serum derived from the same species as the sera under analyses. The volumes may be modified proportionately in order to produce the correct volumes for larger or smaller experiments. Dispose of the unused material at the end of the day.

4. Prepare Stock D by combining 200 µl Stock A with 2.3 ml normal sera derived from the same species as the sera under analyses. The volumes may be modified proportionately in order to prepare the correct volumes for larger or smaller experiments. Dispose of the unused material at the end of the test day.

5. The RIA procedure is set up as described on the attached form entitled "Atropine Sulfate Radioimmunoassay Tube Setup". Reagents are aliquoted to 12 x 75 mm polystyrene RIA tubes in order from left to right as indicated in this form.

6. Upon adding all reagents, vortex each tube 5-10 seconds.

7. Incubate the tubes 20 (± 1) hours at 1-9°C.

8. Prepare the total counts control by adding 20 µl ^{3}H-atropine to each of two 20 ml scintillation vials. Add 10.0 ml Hydrofluor and 1.0 ml distilled water to each vial and mix.

Completion of RIA (Day 2):

1. Add 0.5 ml 100 percent saturated ammonium sulfate to each RIA tube. Vortex for 5-10 seconds. Incubate for 30 minutes at 1-9°C. Centrifuge at approximately 2800 RPM (1550 x g) for 30 minutes at room temperature (RT). Carefully aspirate the supernate with a pasteur pipet and transfer to a container for radioactive liquid waste.

2. Add 1.0 ml 50 percent saturated ammonium sulfate to each tube. Vortex for 5-10 seconds. Centrifuge at approximately 2800 RPM (1550 x g) for 30 minutes at RT. Aspirate the supernate with a pasteur pipet and transfer to a container for radioactive liquid waste.

3. Add 1.0 ml distilled water to each tube to dissolve the pellet. Vortex for 5-10 seconds.

4. Transfer the contents of each RIA tube to a separate scintillation vial by carefully pouring. Rinse the RIA tubes with 2.0 ml Hydrofluor and transfer the fluid to the respective vial.

5. Add 8.0 ml Hydrofluor to each scintillation vial and mix.

6. Count the vials for 10 minutes or to a preset error of 2.0 percent in a liquid scintillation counter.

Data Analysis:

1. Data analysis is performed using RiaCalc DM, Version 2.65 (Pharmacia Wallac). Data is reported as ng/ml.

VI. QUALITY CONTROL

1. All equipment and instruments will be operated, calibrated, and maintained according to their respective SOPs.

2. The study director or his designee will review all raw data, completed data forms and other pertinent study records.

3. The form entitled "Atropine Sulfate Radioimmunoassay Tube Setup" details the contents of each standard, control, and sample tube and will be employed daily during assay set up to insure correct distribution of reagents.

4. The form entitled "Record For Instruments, Equipment, Reagents Used For Radioimmunoassay" will be used to document all reagents and equipment used in an assay.

5. The form entitled "Atropine Sulfate RIA Worklist" will be utilized to record the identification and assay sequence for controls and samples for an assay.

6. Preparation of buffers and other reagents will be recorded on the attached form entitled "Buffer/Reagent Preparation".

7. A series of low, medium, and high controls are included in each experiment to assess the quality of each experiment. Control data will be tabulated for each run and will be reviewed by the study director.

8. Additional control parameters such as B/I, B/T, the slope and intercept of the regression curve and other parameters are computed by RiaCalc DM. These will be tabulated for each experiment and reviewed by the study director.

BUFFER/REAGENT PREPARATION

Study: _____

Project: _____

Buffer/Reagent: _____

Buffer Storage Conditions: _____ Buffer Expir. Date: _____

Constituents:

Reagent	Supplier	Lot	Receipt Date	Expiration Date	Amount Used

Balance: Description: _____

BCD ID: _____ Location: _____

Standard Weights: BCD ID: _____

Determination No.	Actual Wt.	Wt. Read
1		
2		
3		
4		

pH Adjustment (Reagent and Volume): _____

pH Meter: BCD ID: _____ Final pH: _____

Comments:

Prepared By: _____ Date: _____

Reviewed By: _____ Date: _____

ATROPINE SULFATE RADIOIMMUNOASSAY TUBE SETUP

STUDY CONTROL No._____ PROJECT No._____

DATE:_____ RUN No._____ PAGE No:_____

Tube No.	Conc.	Standard	Sample	Buffer	Normal Serum	Antibody	3H-Atropine Sulphate
	Standard Curve						
1	T. Tube	None					20 uL
2	T. Tube	None					20 uL
3	NSB	None		430 uL			
4	NSB	None		430 uL	50		
5	0 pg	None		330 uL	50 uL	100 uL	20 uL
6	0 pg	None		330 uL	50 uL	100 uL	20 uL
7	0 pg	None		330 uL	50 uL	100 uL	20 uL
8	0 pg	None		330 uL	50 uL	100 uL	20 uL
9	25 pg	25 uL Stock B		305 uL	50 uL	100 uL	20 uL
10	25 pg	25 uL Stock B		305 uL	50 uL	100 uL	20 uL
11	50 pg	50 uL Stock B		280 uL	50 uL	100 uL	20 uL
12	50 pg	50 uL Stock B		280 uL	50 uL	100 uL	20 uL
13	75 pg	75 uL Stock B		255 uL	50 uL	100 uL	20 uL
14	75 pg	75 uL Stock B		255 uL	50 uL	100 uL	20 uL
15	100 pg	100 uL Stock B		230 uL	50 uL	100 uL	20 uL
16	100 pg	100 uL Stock B		230 uL	50 uL	100 uL	20 uL
17	150 pg	150 uL Stock B		180 uL	50 uL	100 uL	20 uL
18	150 pg	150 uL Stock B		180 uL	50 uL	100 uL	20 uL
19	250 pg	10 uL Stock A		320 uL	50 uL	100 uL	20 uL
20	250 pg	10 uL Stock A		320 uL	50 uL	100 uL	20 uL
21	500 pg	20 uL Stock A		310 uL	50 uL	100 uL	20 uL
22	500 pg	20 uL Stock A		310 uL	50 uL	100 uL	20 uL
23	750 pg	30 uL Stock A		300 uL	50 uL	100 uL	20 uL
24	750 pg	30 uL Stock A		300 uL	50 uL	100 uL	20 uL
25	1000 pg	40 uL Stock A		290 uL	50 uL	100 uL	20 uL
26	1000 pg	40 uL Stock A		290 uL	50 uL	100 uL	20 uL
	Quality Control						
27	100 pg	50 uL Stock D		330 uL		100 uL	20 uL
28	100 pg	50 uL Stock D		330 uL		100 uL	20 uL
29	250 pg	25 uL Stock C		330 uL	25 uL	100 uL	20 uL
30	250 pg	25 uL Stock C		330 uL	25 uL	100 uL	20 uL
31	500 pg	50 uL Stock C		330 uL		100 uL	20 uL
32	500 pg	50 uL Stock C		330 uL		100 uL	20 uL
	Samples (Atropine Run List)		50 uL	330 uL		100 uL	20 uL
	Samples (Atropine Run List)		50 uL	330 uL		100 uL	20 uL

Technician Signature: _____ Date: _____

Reviewed By: _____ Date: _____

Atropine Sulfate RIA Run List

(Radioimmunoassay Laboratory)
Battelle, 505 King Avenue, Columbus, OH 43

Date: _____ Run No._____ Page No._____
Study Control No:_____ Project No._____

Tube No.	Sample ID or Code (Source)	Draw Time	Draw Date	Concentration		Comments
				pg/50µL	ng/mL	

Operator Signature: _____ Date: _____
Reviewed By: _____ Date: _____

RECORD FOR INSTRUMENTS, EQUIPMENT, REAGENTS USED FOR RADIOIMMUNOASSAY

Project:	Assay:	Project No SC No:

LIST OF INSTRUMENTS/ EQUIPMENT USED

SN	Instrument/ Equipment	Model	Battelle ID	Location
1	Gamma Counter			
2	Scintillation Counter			
3	Water Bath (Temp.)			
4	Heating Blocks/Dry Bath (Temp.)			
5	Incubator (Temp.)			
6	Refrigerator (Temp.)			
7	Freezer (Temp.)			
8				
Other: Incubation Time		In:		
		Out Time:		

LIST OF CHEMICALS, SOLVENTS, AND REAGENTS USED

SN		Cat. #	Lot No.	Exp. Date
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				

Comments:

Technician Signature:	Date:
Reviewed By:	Date:

APPENDIX C

Pharmacokinetic Analysis Data for Individual Animals

TABLE 1. RESULTS OF RIA ANALYSIS FOR SERUM ATROPINE CONCENTRATION (ng/mL)[a] FOLLOWING INJECTION WITH THE MKI SYSTEM

Animal Number	87	93	104	116	117	123	127	129	Mean	Standard Deviation
Body Weight (kg)	80.0	77.7	76.4	70.0	75.5	65.0	81.4	81.4	75.9	5.8
Time in Minutes After Injection										
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	1.14	3.00	1.03	2.29	0.00	0.00	0.00	1.36	1.10	1.12
2	4.86	6.61	4.23	4.80	4.22	2.99	2.76	2.82	4.16	1.31
3	7.31	8.31	6.28[b]	6.41	6.24	6.20	4.59	3.68	6.13	1.45
4	6.27	9.38	6.77	6.73	6.72[c]	5.98	4.97	5.11	6.49	1.36
5	5.56	7.39	7.51	6.88	7.27	7.24	4.64	5.04	6.44	1.17
6	7.00	8.56	10.59	7.64	6.96	7.58	5.36	5.45	7.39	1.69
8	6.45	8.20	9.46	7.28	7.56	8.38	5.63	6.35	7.41	1.25
12	7.29	7.80	8.30[d]	8.44	7.49	8.44	5.76	6.78	7.54	0.93
16	7.47	9.30	8.04	9.31	8.16	8.93	7.25	7.74	8.27	0.81
20	7.80	8.57	7.56	8.47	8.12	9.24	8.68	8.45	8.36	0.53
40	6.27	5.83	7.03	6.96	7.02	8.53	9.06	7.87	7.32	1.09
60	4.70[e]	4.12	5.38	5.85	5.25	7.25	7.26	5.94	5.72	1.12
80	3.78[f]	2.81	4.44	4.34	4.31	6.03	6.16	4.98	4.61	1.11
120	2.75	1.78	3.05	3.09	3.32	4.49	4.33	3.53	3.29	0.87
180	1.98[g]	1.52	2.15	2.47	2.29	2.99	3.42	2.66	2.44	0.60
240	1.30	1.34	0.00	1.57	1.56	1.99	2.28	1.53	1.45	0.67

[a]The minimum quantifiable concentration is 1 ng/mL.
[b]Actual time of blood sampling was 3.33 min.
[c]Actual time of blood sampling was 4.25 min.
[d]Actual time of blood sampling was 13 min.
[e]Actual time of blood sampling was 61 min.
[f]Actual time of blood sampling was 80.33 min.
[g]Actual time of blood sampling was 180.75 min.

TABLE 2. RESULTS OF RIA ANALYSIS FOR SERUM ATROPINE CONCENTRATION (ng/mL)[a] FOLLOWING INJECTION WITH THE MCA SYSTEM

Animal Number	87	93	104	116	117	123	127	129	Mean	Standard Deviation
Body Weight (kg)	80.0	77.7	76.4	70.0	75.5	65.0	81.4	81.4	75.9	5.8
Time in Minutes After Injection										
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	2.99[b]	0.00	5.61	5.70	0.00	10.15	0.00	0.00	3.06	3.80
2	6.36	1.49	10.06	10.49	3.13	14.57	2.36	2.26	6.34	4.85
3	2.58	4.46	10.12	11.29	6.28	16.98	3.27	4.99	7.50	4.93
4	8.78	4.99	9.94	11.34	7.39	14.52	4.37	5.15	8.31	3.55
5	8.38	4.97	10.15	12.25	7.24	14.69	5.13	5.50	8.54	3.58
6	8.51	5.88	10.39	12.42	7.44	14.33	5.70	6.22	8.86	3.23
8	8.36	5.78	8.55	11.12	7.68	12.21	5.91	7.16	8.35	2.30
12	8.56	5.78	7.39	11.15	13.41	11.62	7.59[c]	6.89	9.05	2.69
16	8.12	6.07	7.51	9.74	8.25	10.43	8.25	7.09	8.18	1.39
20	6.90	6.21	7.32	9.15	7.62	9.62	8.41[d]	7.03	7.78	1.18
40	6.00	5.81	6.00	6.56	6.03	7.32	9.00	6.75	6.68	1.06
60	4.59	5.17	4.16	5.45	4.89	6.19	8.42[e]	5.62	5.56	1.31
80	3.92	4.23	3.80	4.48	4.20	5.14	7.67	4.85	4.79	1.25
120	3.04	3.45	2.87	3.27	3.19	4.18	5.92	4.13	3.76	1.00
180	2.59	3.36	2.28	2.51	2.55	3.27	3.81[f]	3.41	2.97	0.56
240	1.91	2.31	1.73	2.18	1.72	2.45	2.53[g]	2.19	2.13	0.31

[a]The minimum quantifiable concentration is 1 ng/mL.
[b]Actual time of blood sampling was 1.25 min.
[c]Actual time of blood sampling was 12.33 min.
[d]Actual time of blood sampling was 20.33 min.
[e]Actual time of blood sampling was 61.5 min.
[f]Actual time of blood sampling was 183 min.
[g]Actual time of blood sampling was 244.25 min.

TABLE 3. RESULTS OF RIA ANALYSIS FOR SERUM ATROPINE CONCENTRATION (ng/mL)[a] FOLLOWING INJECTION WITH THE MCA-A SYSTEM

Animal Number	87	93	104	116	117	123	127	129	Mean	Standard Deviation
Body Weight (kg)	80.0	77.7	76.4	70.0	75.5	65.0	81.4	81.4	75.9	5.8
Time in Minutes After Injection										
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	8.79	13.46	14.22[b]	10.14	9.99	18.05	15.99	11.11	12.72	3.25
2	11.32	13.38	13.99	12.11	13.50	16.78	18.45	11.26	13.85	2.57
3	11.86	11.30[c]	12.48	12.29	10.08	16.49	17.99	12.38	13.11	2.69
4	11.12	12.11	11.84	14.63	13.95	15.92	16.41	11.79	13.47	2.04
5	8.53	10.86	15.55	12.96	13.80	16.47	14.05	11.34	12.94	2.61
6	9.52	10.97	11.82	12.74	12.19	15.04	13.90	11.46	12.20	1.72
8	9.05	9.92	11.79	11.07	11.26	13.81	12.30	10.53	11.21	1.47
12	7.69	10.96	10.40	9.89	10.05	12.15	9.64	10.32	10.14	1.26
16	7.12	8.45[d]	8.39[e]	9.76	8.48	11.30	8.71	9.16	8.92	1.22
20	6.75	8.46	8.97	9.33	7.61	10.61	8.80	8.76	8.66	1.14
40	5.29	6.42	6.94	8.16	5.36	7.73	6.33	6.04	6.54	1.03
60	4.31	5.18[f]	5.54	6.42	4.39	6.38	4.73	5.37	5.29	0.81
80	3.76	4.34	5.04	5.45	3.27	5.16	3.96	5.26	4.53	0.81
120	2.86	3.33	3.53	3.59	3.34	3.75	2.96	4.02	3.42	0.39
180	2.45	2.54[g]	2.49	3.17	2.04	3.12	2.24	3.88	2.74	0.60
240	1.91	1.87[h]	2.09	1.98	1.28	2.25	1.35	2.50	1.90	0.42

[a] The minimum quantifiable concentration is 1 ng/mL.
[b] Actual time of blood sampling was 1.75 min.
[c] Actual time of blood sampling was 3.5 min.
[d] Actual time of blood sampling was 16.33 min.
[e] Actual time of blood sampling was 16.25 min.
[f] Actual time of blood sampling was 60.25 min.
[g] Actual time of blood sampling was 180.67 min.
[h] Actual time of blood sampling was 240.5 min.

TABLE 4. RESULTS OF RIA ANALYSIS FOR SERUM ATROPINE CONCENTRATION (ng/mL)[a] FOLLOWING INJECTION WITH THE MCA-B SYSTEM

Animal Number	87	93	104	116	117	123	127	129	Mean	Standard Deviation
Body Weight (kg)	80.0	77.7	76.4	70.0	75.5	65.0	81.4	81.4	75.9	5.8
Time in Minutes After Injection										
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	8.52	9.04	3.88	6.98	6.85	12.08	5.17	6.72	7.40	2.51
2	11.05	10.01	8.91	10.33	7.45	12.71	10.20	2.37	9.13	3.13
3	10.68	9.12	7.74	10.70	8.66	12.61	12.40	9.25	10.15	1.75
4	10.01	8.42	7.69	10.16	9.23	13.73	12.63	9.33	10.15	2.05
5	9.41	8.65	6.64	10.82	9.12	13.35	11.49	8.59	9.76	2.07
6	8.14	7.97	6.09	9.8[illegible]	9.47	13.18	12.28	8.76	9.47	2.32
8	8.37	7.87	5.40	9.41	9.17	12.59	11.26	8.62	9.09	2.17
12	7.38	8.05	4.82[b]	8.73	8.15	11.98	9.62	7.33	8.26	2.05
16	7.03[c]	8.23	9.02	9.38	9.32[d]	11.11[e]	8.99	7.39	8.[illegible]	1.28
20	6.80	8.03	8.75	8.48	8.66	10.39	8.81	7.09	8.38	1.12
40	5.57	6.65	6.81	6.13	7.65	8.99	6.61	6.16	6.82	1.06
60	4.76	5.24	5.65	5.20	6.32	6.74	5.10	4.61	5.45	0.74
80	3.97	4.08	4.59	4.34	5.18	5.53	4.20	3.66	4.45	0.63
120	3.08	3.08	4.00	3.29	3.78	4.29	3.20	2.85	3.45	0.51
180	2.94	2.18	2.70	2.80	3.31	3.50[f]	2.15	2.29	2.73	0.51
240	1.82	1.70	2.45	2.09	2.52	2.34[g]	1.52	1.68[h]	2.02	0.39

[a]The minimum quantifiable concentration is 1 ng/mL.
[b]Actual time of blood sampling was 12.25 min.
[c]Actual time of blood sampling was 16.33 min.
[d]Actual time of blood sampling was 16.25 min.
[e]Actual time of blood sampling was 16.5 min.
[f]Actual time of blood sampling was 180.25 min.
[g]Actual time of blood sampling was 240.25 min.
[h]Actual time of blood sampling was 240.5 min.

TABLE 5. PHARMACOKINETIC PARAMETERS FOR ATROPINE ESTIMATED FROM TWO COMPARTMENT MODELS FOLLOWING INJECTION WITH THE MKI SYSTEM[a]

Animal Number Body Weight (kg)	87 80.0	93 77.7	104 76.4	116 70.0	117 75.5	123 65.9	127 81.4	129 81.4	Mean 75.9	Standard Deviation 5.8
C_{max} (ng/mL)	7.52	8.92	8.54	8.64	8.01	9.21	5.56	8.06	8.1	1.1
t_{max} (min)	10.6	7.4	13.5	12.3	10.9	15.5	7.0	20.1	12.2	4.3
$AUC_{0-\infty}$ (ng*min/mL)	967	790	1,057	1,145	1,114	1,496	1,460	1,202	1,154	237
K_a (min^{-1})	0.359	0.514	0.235	0.298	0.357	0.235	0.852	0.157	0.376	0.221
K_{el} (min^{-1})	0.009	0.004	0.014	0.009	0.012	0.012	0.004	0.005	0.009	0.004
$V_{d\beta}$ (L)	215	215	136	197	199	168	196	182	188	26
$V_{d\beta}$/BW (L/kg)	2.68	2.77	1.78	2.81	2.64	2.56	2.41	2.23	2.49	0.34
A	0.72	4.09	8.64	2.03	0.463	-9.24	-9.24	0.96		
B	7.85	6.08	8.89	8.05	8.49	10.54	11.94	9.11		
Alpha	0.033	0.021	0.138	0.022	0.019	0.223	0.068	0.019		
Beta	0.008	0.010	0.008	0.007	0.008	0.007	0.008	0.008		

[a]Atropine dose approximately 1.73 mg.

TABLE 6. PHARMACOKINETIC PARAMETERS FOR ATROPINE ESTIMATED FROM TWO COMPARTMENT MODELS FOLLOWING INJECTION WITH THE MCA SYSTEM[a]

Animal Number Body Weight (kg)	87 80.0	93 77.7	104 76.4	116 70.0	117 75.5	123 65.0	127 81.4	129 81.4	Mean 75.9	Standard Deviation 5.8
C_{max} (ng/mL)	8.46	5.24	9.69	11.96	9.09	14.96	8.41	7.24	9.5	2.8
T_{max} (min)	12.8	15.1	6.2	6.7	17.3	4.8	18.8	15.7	12.2	5.5
$AUC_{0-\infty}$ (ng*min/mL)	1,288	1,567	1,029	1,514	1,287	1,601	1,757	1,538	1,448	231
K_a (min^{-1})	0.278	0.279	0.638	0.648	0.166	0.925	0.216	0.253	0.425	0.275
K_{el} (min^{-1})	0.009	0.004	0.014	0.009	0.012	0.012	0.004	0.005	0.009	0.004
$V_{d\beta}$/BW (L)	273	315	206	286	205	195	186	255	240	48
$V_{d\beta}$/BW (L/kg)	3.41	4.06	2.69	4.08	2.71	3.00	2.29	3.14	3.17	0.65
A	7.14	1.02	9.09	8.55	15.33	10.79	-8.13	0.86		
B	5.89	5.97	7.69	5.70	6.14	8.87	14.01	7.43		
Alpha	0.060	0.024	0.198	0.039	0.069	0.103	0.041	0.034		
Beta	0.005	0.004	0.008	0.004	0.005	0.006	0.007	0.005		

[a]Atropine dose approximately 1.95 mg.

TABLE 7. PHARMACOKINETIC PARAMATERS FOR ATROPINE ESTIMATED FROM TWO COMPARTMENT MODELS FOLLOWING INJECTION WITH THE MCA-A SYSTEM[a]

Animal Number Body Weight (kg)	87 80.0	93 77.7	104 76.4	116 70.0	117 75.5	123 65.0	127 81.4	129 81.4	Mean 75.9	Standard Deviation 5.8
C_{max} (ng/mL)	10.81	13.12	14.82	12.97	12.94	18.26	17.53	12.00	14.1	2.6
t_{max} (min)	3.7	0.0	0.1	4.8	4.0	0.1	3.1	2.8	2.3	2.0
$AUC_{0-\infty}$ (ng*min/mL)	1,149	1,336	1,400	1,401	1,158	1,612	1,048	2,088	1,399	331
K_a (min^{-1})	1.306	[b]	[b]	0.931	1.153	[b]	1.359	2.064		
K_{el} (min^{-1})	0.011	0.010	0.011	0.012	0.013	0.011	0.022	0.006	0.012	0.005
V_{dp} (L)	294	277	246	181	319	225	1892	320	256	55
V_{dp}/BW (L/kg)	3.67	3.56	3.22	2.58	4.23	3.47	2.32	3.93	3.37	0.65
A	7.22	6.24	7.01	7.50	9.79	10.00	16.31	6.89		
B	6.47	6.88	7.86	10.40	5.50	8.31	9.39	5.97		
Alpha	0.123	0.053	0.068	0.183	0.060	0.060	0.200	0.042		
Beta	0.006	0.006	0.006	0.008	0.006	0.006	0.0010	0.003		

[a]Atropine dose approximately 2.09 mg.
[b]Meaningful values of K_a could not be obtained due to the extremely rapid absorption observed.

TABLE 8. PHARMACOKINETIC PARAMETERS ESTIMATED FROM TWO COMPARTMENT MODELS OF SERUM ATROPINE CONCENTRATIONS AS A RESULT OF INJECTION WITH THE MCA-B SYSTEM [a]

Animal Number Body Weight (kg)	87 80.0	93 77.7	104 76.4	116 70.0	117 75.5	123 65.0	127 81.4	129 81.4	Mean 75.9	Standard Deviation 5.8
C_{max} (ng/mL)	9.75	9.08	7.36	10.42	9.25	13.34	11.62	8.58	9.9	1.9
t_{max} (min)	4.4	1.3	4.8	4.6	5.1	2.9	7.5	7.4	4.7	2.1
$AUC_{0-\infty}$ (ng*min/mL)	1,156	1,234	1,646	1,688	1,755	2,239	1,079	1,246	1,505	396
K_a (min^{-1})	1.001	5.039	1.171	1.116	1.022	2.032	0.462	0.601	1.556	1.483
K_{el} (min^{-1})	0.013	0.007	0.005	0.007	0.006	0.006	0.017	0.008	0.008	0.004
$V_{d\beta}$ (L)	223	561	331	389	343	363	164	375	343	118
$V_{d\beta}$/BW (L/kg)	2.78	7.22	4.33	5.55	4.54	5.58	2.01	4.61	4.58	1.64
A	11.28	7.65	1.88	6.73	5.08	9.38	14.74	5.01		
B	7.52	1.55	5.70	4.62	4.58	4.52	9.39	4.81		
Alpha	0.328	0.011	0.010	0.027	0.011	0.019	0.189	0.029		
Beta	0.007	0.003	0.004	0.003	0.004	0.003	0.009	0.004		

[a]Atropine dose approximately 2.12 mg.

TABLE 9. MODEL-DERIVED AREAS UNDER THE SERUM ATROPINE CONCENTRATION-TIME CURVES TO EACH SAMPLING TIME FOLLOWING INJECTION WITH THE MKI SYSTEM

Animal	AUC_{0-1}	AUC_{0-2}	AUC_{0-3}	AUC_{0-4}	AUC_{0-5}	AUC_{0-6}	AUC_{0-8}	AUC_{0-12}
87	2.5	6.7	12.1	18.3	25.0	32.1	46.9	76.9
93	3.9	10.2	17.7	26.0	34.7	43.6	61.4	96.3
104	2.5	6.8	12.5	19.2	26.7	34.6	51.4	86.0
116	2.5	6.8	12.5	19.1	26.4	34.2	50.7	85.0
117	2.6	7.0	12.7	19.2	26.3	33.8	49.4	81.4
123	2.1	5.9	11.1	17.2	24.1	31.7	48.1	83.6
127	2.1	5.3	9.2	13.6	18.5	23.7	35.2	61.1
129	1.4	3.9	7.4	11.8	16.8	22.5	35.1	64.1
Mean	2.4	6.6	11.9	18.1	24.8	32.0	47.3	79.3
Standard Deviation	0.7	1.8	3.0	4.3	5.5	6.6	8.7	11.7

Animal	AUC_{0-16}	AUC_{0-20}	AUC_{0-48}	AUC_{0-60}	AUC_{0-80}	AUC_{0-120}	AUC_{0-180}	AUC_{0-240}
87	106.4	135.0	263.1	370.3	460.6	601.4	742.2	828.5
93	129.4	160.7	294.2	396.6	475.9	586.9	681.6	730.9
104	119.8	152.1	293.1	410.3	509.4	664.4	818.6	912.3
116	119.3	152.6	302.2	426.7	531.0	693.9	858.4	962.0
117	113.0	143.7	283.8	403.1	504.9	666.4	831.5	935.4
123	120.4	156.9	327.9	477.2	607.5	820.2	1,047.7	1,198.6
127	90.1	121.0	286.1	443.5	583.0	808.8	1,043.7	1,193.8
129	95.5	127.7	281.2	413.5	526.1	704.3	886.2	1,001.0
Mean	111.7	143.7	291.4	417.6	524.8	693.3	863.7	970.3
Standard Deviation	13.5	14.4	18.7	32.2	49.9	85.3	129.7	162.6

TABLE 10. MODEL DERIVED AREAS UNDER THE SERUM ATROPINE CONCENTRATION-TIME CURVES TO EACH SAMPLING TIME FOLLOWING INJECTION WITH THE MCA SYSTEM

Animal	AUC_{0-1}	AUC_{0-2}	AUC_{0-3}	AUC_{0-4}	AUC_{0-5}	AUC_{0-6}	AUC_{0-8}	AUC_{0-12}
87	2.7	7.4	13.5	20.6	28.4	36.6	53.8	88.4
93	1.7	4.6	8.4	12.9	17.9	23.3	34.9	59.4
104	6.2	15.2	25.3	35.6	45.7	55.4	73.8	106.7
116	6.4	16.1	27.3	39.2	51.2	63.3	86.9	131.4
117	2.2	6.3	11.7	18.3	25.8	34.0	51.7	89.6
123	10.7	25.2	40.6	55.9	70.8	85.1	111.9	160.0
127	1.4	3.9	7.3	11.6	16.5	21.9	34.2	62.3
129	1.8	5.0	9.2	14.2	19.8	26.0	39.1	67.4
Mean	4.1	10.4	17.9	26.0	34.5	43.2	60.8	95.7
Standard Deviation	3.3	7.6	11.9	15.9	19.4	22.6	27.9	35.5

Animal	AUC_{0-16}	AUC_{0-28}	AUC_{0-48}	AUC_{0-80}	AUC_{0-88}	AUC_{0-128}	AUC_{0-168}	AUC_{0-248}
87	121.3	151.9	277.5	377.0	462.9	608.7	779.9	907.9
93	84.4	109.1	225.1	329.6	424.4	590.5	793.4	953.4
104	136.4	164.0	286.8	391.6	481.6	625.2	773.1	866.8
116	172.4	210.2	364.1	480.6	576.1	731.6	912.1	1,050.0
117	127.3	162.8	304.6	409.6	497.6	645.1	815.6	940.7
123	202.3	240.6	400.0	533.5	651.2	848.9	1,070.9	1,227.3
127	94.1	127.6	305.4	480.6	641.7	912.2	1,203.3	1,394.6
129	96.3	125.1	259.6	379.4	486.9	671.8	889.1	1,051.7
Mean	129.3	161.4	302.9	422.7	527.8	704.3	904.7	1,049.1
Standard Deviation	40.8	44.6	56.1	68.4	84.7	118.2	156.0	179.4

TABLE 11. MODEL-DERIVED AREAS UNDER THE SERUM ATROPINE CONCENTRATION-TIME CURVES TO EACH SAMPLING TIME FOLLOWING INJECTION WITH THE MCA-A SYSTEM

Animal	AUC_{0-1}	AUC_{0-2}	AUC_{0-3}	AUC_{0-4}	AUC_{0-5}	AUC_{0-6}	AUC_{0-8}	AUC_{0-12}
87	9.1	20.1	31.2	41.9	52.0	61.7	79.9	112.2
93	12.8	25.2	37.3	49.0	60.5	71.7	93.3	133.6
104	14.4	28.2	41.7	54.7	67.3	79.6	103.0	146.3
116	9.5	22.2	35.6	48.9	61.7	74.1	97.6	140.5
117	9.9	22.5	35.6	48.5	61.0	73.2	96.2	137.9
123	17.7	34.8	51.3	67.3	82.8	97.8	126.5	179.1
127	16.0	34.5	52.1	68.4	83.3	97.1	121.9	164.0
129	10.9	23.0	35.0	46.7	58.1	69.3	91.1	131.9
Mean	12.5	26.3	40.0	53.2	65.9	78.1	101.2	143.2
Standard Deviation	3.2	5.7	7.8	9.7	11.4	13.0	15.7	20.5

Animal	AUC_{0-16}	AUC_{0-20}	AUC_{0-40}	AUC_{0-60}	AUC_{0-80}	AUC_{0-120}	AUC_{0-180}	AUC_{0-240}
87	140.8	167.0	279.5	376.1	461.6	605.2	766.3	879.5
93	170.6	204.8	346.8	459.4	555.0	712.8	890.2	1,016.5
104	185.6	221.8	371.6	492.4	596.2	768.0	958.4	1,090.7
116	180.0	217.2	383.6	525.9	648.2	844.0	1,046.3	1,175.0
117	174.6	207.4	333.8	427.4	505.3	633.9	779.9	885.1
123	226.5	269.6	443.3	578.0	692.0	880.2	1,091.3	1,240.7
127	200.4	233.6	375.5	491.8	587.9	732.8	868.9	945.6
129	169.7	205.0	352.9	472.0	575.4	755.0	981.2	1,168.4
Mean	181.0	215.8	360.9	477.9	577.7	741.5	922.8	1,050.2
Standard Deviation	25.0	29.1	46.7	61.0	73.4	93.8	117.8	139.4

TABLE 12. MODEL-DERIVED AREAS UNDER THE SERUM ATROPINE CONCENTRATION-TIME CURVES TO EACH SAMPLING TIME FOLLOWING INJECTION WITH THE MCA-B SYSTEM

Animal	AUC_{0-1}	AUC_{0-2}	AUC_{0-3}	AUC_{0-4}	AUC_{0-5}	AUC_{0-6}	AUC_{0-8}	AUC_{0-12}
87	8.7	19.4	30.1	40.1	49.5	58.2	74.5	104.1
93	9.1	18.1	27.0	35.9	44.7	53.4	70.6	104.0
104	5.2	12.0	19.2	26.5	33.9	41.2	55.8	84.4
116	7.4	17.2	27.6	38.0	48.4	58.6	78.6	116.7
117	6.1	14.4	23.4	32.6	41.8	51.1	69.3	105.1
123	11.9	25.2	38.5	51.7	64.7	77.5	102.7	151.2
127	6.3	16.1	27.5	39.7	52.0	64.2	87.4	129.0
129	4.3	10.8	18.5	26.8	35.4	44.0	61.1	94.1
Mean	7.4	16.6	26.5	36.4	46.3	56.0	75.0	111.1
Standard Deviation	2.5	4.5	6.4	8.2	9.9	11.6	14.9	21.0

Animal	AUC_{0-16}	AUC_{0-20}	AUC_{0-40}	AUC_{0-60}	AUC_{0-96}	AUC_{0-120}	AUC_{0-180}	AUC_{0-240}
87	131.8	158.6	281.7	389.6	484.2	639.7	808.1	921.5
93	136.2	167.3	306.8	423.6	521.6	674.5	829.3	929.4
104	112.5	139.9	268.8	384.9	489.9	671.4	887.0	1,051.7
116	152.5	186.1	329.3	442.3	536.0	688.5	867.8	1,011.3
117	139.8	173.5	328.3	463.0	581.0	776.7	997.2	1,158.7
123	197.2	241.0	431.3	584.0	709.9	908.0	1,127.4	1,296.3
127	165.8	199.5	344.3	464.3	564.6	718.7	867.8	955.1
129	125.3	154.7	280.4	380.6	464.0	598.9	752.2	867.4
Mean	145.1	177.6	321.4	441.5	543.9	709.6	892.1	1,023.9
Standard Deviation	26.6	31.6	52.0	66.8	78.2	95.8	118.4	142.4

TABLE 13. RESULTS OF CHEMICAL ANALYSIS FOR PLASMA 2-PAM CONCENTRATION (μg/mL)[a] FOLLOWING INJECTION WITH THE MKI SYSTEM

Animal Number	87	117	104	129	127	123	93	116	Mean	Standard Deviation
Body Weight (kg)	80.0	75.5	76.4	81.4	81.4	65.0	77.7	70.0	75.9	5.8
Time in Minutes After Injection										
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	3.46	2.45	0.36	2.27	1.11	1.46	1.94	1.37	1.80	0.95
2	5.79	5.84	1.29	5.58	3.26	5.74	3.97	3.05	4.32	1.69
3	6.34	6.74	1.74[b]	6.34	5.10	9.99	5.26	4.32	5.73	2.34
4	5.31	6.77[c]	1.87	7.08	5.89	11.07	5.97	5.33	6.16	2.54
5	6.08	6.99	2.30	7.13	5.51	13.95	6.30	6.44	6.84	3.25
6	6.69	6.99	3.07	7.62	5.97	14.22	7.24	7.03	7.35	3.12
8	5.82	7.20	3.96	7.38	6.43	13.97	7.76	7.22	7.47	2.89
12	6.08	6.20	4.21[d]	6.72	5.71	11.92	8.09	7.69	7.08	2.29
16	6.27	6.58	4.32	6.72	6.58	10.61	7.81	7.89	7.16	1.80
20	4.83	6.53	4.72	6.43	6.09	9.34	7.19	7.00	6.52	1.46
40	4.69	4.23	3.83	5.03	3.77	5.36	5.19	4.67	4.60	0.60
60	3.04[e]	2.95	2.99	4.02	2.50	3.66	3.43	3.10	3.21	0.47
80	2.65[f]	2.27	2.46	3.02	1.71	2.71	2.52	2.15	2.44	0.40
120	1.82	1.44	1.80	1.94	1.25	1.53	1.38	1.20	1.54	0.28
180	0.78[g]	0.95	0.93	1.03	0.57	0.79	0.63	0.60	0.79	0.17
240	0.00	0.66	0.54	0.60	0.00	0.44	0.39	0.37	0.38	0.25

(a) The minimum quantifiable concentration is 0.3 μg/mL.
(b) Actual time of blood sampling was 3.33 min.
(c) Actual time of blood sampling was 4.25 min.
(d) Actual time of blood sampling was 13 min.
(e) Actual time of blood sampling was 61 min.
(f) Actual time of blood sampling was 80.33 min.
(g) Actual time of blood sampling was 180.75 min.

TABLE 14. RESULTS OF CHEMICAL ANALYSIS FOR PLASMA 2-PAM CONCENTRATION (μg/mL)[a] FOLLOWING INJECTION WITH THE MCA SYSTEM

Animal Number Body Weight (kg)	127 81.4	129 81.4	87 80.0	116 70.0	93 77.0	117 75.5	104 76.4	123 65.0	Mean 75.9	Standard Deviation 5.8
Time in Minutes After Injection										
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	0.00	0.00	1.95[b]	2.13	0.00	0.00	1.16	3.44	1.08	1.32
2	0.00	0.82	3.95	4.49	0.00	2.18	3.54	9.09	3.01	3.02
3	0.00	1.78	6.13	6.48	0.35	4.44	3.99	12.78	4.49	4.14
4	0.00	2.05	6.56	7.54	0.66	5.06	4.97	12.68	4.94	4.15
5	0.00	2.43	7.47	9.45	0.86	5.37	5.36	15.03	5.74	4.94
6	0.00	3.39	7.59	9.39	1.43	6.24	5.58	14.69	6.04	4.68
8	0.61	3.90	7.54	9.48	2.00	6.28	5.97	13.84	6.20	4.23
12	1.39[c]	4.17	7.44	9.71	2.60	6.70	6.05	13.02	6.38	3.79
16	1.71	4.49	6.80	8.78	3.33	6.36	6.25	11.39	6.14	3.05
20	2.25[d]	4.66	6.11	7.90	3.62	5.77	5.96	9.89	5.77	2.39
40	3.31	4.22	4.18	4.50	4.18	4.17	4.66	5.89	4.39	0.72
60	3.57[e]	3.86	3.02	3.01	3.64	3.13	3.36	4.20	3.47	0.42
80	3.39	3.36	2.13	2.17	2.88	2.40	2.50	2.80	2.70	0.49
120	2.71	2.29	1.28	1.26	1.96	1.48	1.48	1.62	1.76	0.52
180	2.07[f]	1.32	0.65	0.69	1.18	0.94	0.66	0.85	1.05	0.48
240	1.20[g]	0.83	0.00	0.36	0.56	0.46	0.39	0.44	0.53	0.35

(a) The minimum quantifiable concentration is 0.3 μg/mL.
(b) Actual time of blood sampling was 1.25 min.
(c) Actual time of blood sampling was 12.33 min.
(d) Actual time of blood sampling was 20.33 min.
(e) Actual time of blood sampling was 61.5 min.
(f) Actual time of blood sampling was 183 min.
(g) Actual time of blood sampling was 244.25 min.

TABLE 15. RESULTS OF CHEMICAL ANALYSIS FOR PLASMA 2-PAM CONCENTRATION (μg/mL)[a] FOLLOWING INJECTION WITH THE MCA-A SYSTEM

Animal Number	93	116	127	123	104	129	87	117	Mean	Standard Deviation
Body Weight (kg)	77.7	70.0	81.4	65.0	76.4	81.4	80.0	75.5	75.9	5.8
Time in Minutes After Injection										
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	4.17	3.19	4.02	6.25	3.22[b]	2.16	2.93	3.10	3.63	1.23
2	6.49	5.11	8.04	7.69	3.58	4.87	5.55	5.87	5.90	1.48
3	7.58[c]	6.69	9.93	10.09	4.35	7.35	6.95	6.01	7.37	1.91
4	8.67	8.92	10.09	11.49	4.95	8.71	7.87	8.87	8.70	1.87
5	9.14	8.94	9.78	13.05	5.92	9.89	8.07	9.40	9.27	1.99
6	9.02	8.96	10.61	13.09	5.43	10.02	7.87	9.18	9.27	2.20
8	9.06	8.51	9.64	12.98	5.90	10.64	7.82	9.32	9.23	2.07
12	8.78	7.58	8.54	11.49	5.32	10.16	6.52	8.24	8.33	1.94
16	7.75[d]	6.88	7.31	9.93	4.65[e]	8.99	5.74	7.13	7.30	1.67
20	8.88	6.20	6.82	9.02	4.96	8.21	5.32	6.50	6.99	1.56
40	5.23	4.07	3.92	5.53	3.85	5.26	3.37	4.28	4.44	0.79
60	3.52[f]	2.68	2.64	3.79	2.83	3.86	2.32	3.20	3.10	0.58
80	2.65	1.90	1.85	2.68	2.05	2.72	1.70	2.21	2.22	0.41
120	1.54	1.07	1.04	1.55	1.42	1.66	0.89	1.37	1.32	0.28
180	0.85[g]	0.65	0.51	1.04	0.56	0.83	0.41	0.96	0.73	0.23
240	0.57[h]	0.42	0.00	0.72	0.33	0.44	0.00	0.39	0.36	0.25

(a) The minimum quantifiable concentration is 0.3 μg/mL.
(b) Actual time of blood sampling was 1.75 min.
(c) Actual time of blood sampling was 3.5 min.
(d) Actual time of blood sampling was 16.33 min.
(e) Actual time of blood sampling was 16.25 min.
(f) Actual time of blood sampling was 60.25 min.
(g) Actual time of blood sampling was 180.67 min.
(h) Actual time of blood sampling was 240.5 min.

TABLE 16. RESULTS OF CHEMICAL ANALYSIS FOR PLASMA 2-PAM CONCENTRATION (μg/mL)[a] FOLLOWING INJECTION WITH THE MCA-B SYSTEM

Animal Number	104	123	93	117	87	116	127	129	Mean	Standard Deviation
Body Weight (kg)	76.4	65.0	77.7	75.5	80.0	70.0	81.4	81.4	75.9	5.8
Time in Minutes After Injection										
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	0.77	6.62	4.57	4.64	2.75	1.66	2.39	1.13	3.07	2.03
2	4.35	8.63	8.49	7.38	6.73	4.44	7.03	3.18	6.28	2.04
3	4.58	10.06	9.44	8.75	7.81	5.89	9.94	3.63	7.51	2.51
4	5.08	13.53	9.73	9.41	8.55	6.52	11.06	4.11	8.50	3.14
5	5.10	14.03	8.27	9.64	8.61	7.27	10.59	4.19	8.46	3.11
6	4.93	14.97	10.15	10.08	8.34	7.32	10.46	4.83	8.89	3.32
8	4.93	14.70	10.36	10.40	7.72	7.77	9.91	5.32	8.89	3.17
12	4.77[b]	13.26	9.81	9.55	7.52	7.12	8.39	5.21	8.20	2.73
16	4.52	11.95[c]	9.11	8.95[d]	6.18[e]	6.85	7.52	5.42	7.56	2.38
20	4.62	10.85	8.55	8.08	5.58	6.18	6.73	6.00	7.07	1.99
40	3.86	6.11	5.28	5.28	3.83	3.85	3.64	4.95	4.60	0.92
60	3.07	4.24	3.64	3.36	2.65	2.73	2.36	3.64	3.21	0.62
80	2.39	3.08	2.48	2.43	1.83	1.86	1.63	2.89	2.33	0.52
120	1.68	2.05	1.43	1.51	1.00	1.03	0.95	1.71	1.42	0.40
180	0.82	1.09[f]	0.72	0.84	0.56	0.55	0.42	0.79	0.72	0.21
240	0.00	0.65[g]	0.44	0.54	0.00	0.00	0.00	0.44[h]	0.26	0.29

(a) The minimum quantifiable concentration is 0.3 μg/mL.
(b) Actual time of blood sampling was 12.25 min.
(c) Actual time of blood sampling was 16.5 min.
(d) Actual time of blood sampling was 16.25 min.
(e) Actual time of blood sampling was 16.33 min.
(f) Actual time of blood sampling was 180.25 min.
(g) Actual time of blood sampling was 240.25 min.
(h) Actual time of blood sampling was 12.25 min.

TABLE 17. PHARMACOKINETIC PARAMETERS FOR 2-PAM ESTIMATED FROM TWO COMPARTMENT MODELS FOLLOWING INJECTION WITH THE MKI SYSTEM[a]

Animal Number Body Weight (kg)	87 80.0	93 77.7	104 76.4	116 70.0	117 75.5	123 65.0	127 81.4	129 81.4	Mean 75.9	Standard Deviation 5.8
C_{max} (μg/mL)	6.27	8.02	4.54	7.85	7.22	13.07	6.44	7.39	7.60	2.47
t_{max} (min)	5.2	12.1	25.0	13.7	7.1	11.4	13.0	8.7	12.0	6.0
AUC (μg*min/mL)	584	656	550	547	599	728	1027	678	671	157
K_a (min^{-1})	0.842	0.246	0.085	0.178	0.532	0.166	0.284	0.394	0.341	0.247
K_{el} (min^{-1})	0.011	0.015	0.015	0.021	0.014	0.039	0.008	0.015	0.017	0.010
$V_{d\beta}$ (L)	89	41	8	80	120	19	653	59	133	213
$V_{d\beta}$/BW (L/kg)	1.11	0.53	0.10	1.14	1.58	0.28	8.02	0.72	1.69	2.61
A	1.20	0.74	4.73	9.15	5.07	98.28	8.20	5.24		
B	5.55	10.08	13.98	4.60	3.38	7.30	0.58	7.82		
Alpha	0.011	0.004	0.009	0.042	0.026	0.129	0.023	0.227		
Beta	0.011	0.021	0.062	0.011	0.008	0.012	0.001	0.011		

[a] 2-PAM dose approximately 641 mg.

TABLE 18. PHARMACOKINETIC PARAMETERS FOR 2-PAM ESTIMATED FROM TWO COMPARTMENT MODELS FOLLOWING INJECTION WITH THE MCA SYSTEM[a]

Animal Number Body Weight (kg)	87 80.0	93 77.7	104 76.4	116 70.0	117 75.5	123 65.0	127 81.4	129 81.4	Mean 75.9	Standard Deviation 5.8
C_{max} (μg/mL)	7.62	4.04	6.30	9.76	6.53	14.34	3.47	4.72	7.10	3.56
T_{max} (min)	11.5	45.2	11.4	12	14.3	9.2	69.8	22.5	24.5	21.8
AUC (μg*min/mL)	512	645	541	579	560	750	743	682	626	92
K_a (min^{-1})	0.202	0.034	0.277	0.182	0.172	0.254	0.021	0.132	0.159	0.093
K_{el} (min^{-1})	0.027	0.014	0.014	0.029	0.020	0.033	0.009	0.008	0.019	0.010
$V_{d\beta}$ (L)	35	74	79	49	55	30	49	88	57	21
$V_{d\beta}$/BW (L/kg)	0.43	0.96	1.03	0.71	0.73	0.47	0.60	1.08	0.75	0.25
A	26.43	25.63	0.64	21.10	13.36	31.10	8.37	-2.31		
B	6.98	1.53	7.10	6.29	5.96	10.69	6.68	8.16		
Alpha	0.146	0.024	0.016	0.087	0.102	0.133	0.015	0.017		
Beta	0.014	0.004	0.013	0.013	0.011	0.016	0.007	0.010		

[a] 2-PAM dose approximately 684 mg.

TABLE 19. PHARMACOKINETIC PARAMETERS FOR 2-PAM ESTIMATED FROM TWO COMPARTMENT MODELS FOLLOWING INJECTION WITH THE MCA-A SYSTEM[a]

Animal Number	87	93	104	116	117	123	127	129	Mean	Standard Deviation
Body Weight (kg)	80.0	77.7	76.4	70.0	75.5	65.0	81.4	81.4	75.9	5.8
C_{max} (μg/mL)	7.70	9.28	5.60	8.58	8.89	12.78	10.26	10.21	9.16	2.09
T_{max} (min)	8.4	7.5	10.1	9.4	10.4	8.2	6.4	12.3	9.1	1.8
AUC (μg*min/mL)	408	659	474	485	575	760	494	678	567	122
K_e (min^{-1})	0.283	0.460	0.292	0.247	0.223	0.364	0.468	0.172	0.314	0.108
K_{el} (min^{-1})	0.036	0.016	0.019	0.033	0.030	0.022	0.027	0.030	0.027	0.007
$V_{d\beta}$ (L)	33	92	41	32	35	65	65	22	48	24
$V_{d\beta}$/BW (L/kg)	0.41	1.18	0.54	0.46	0.46	1.00	0.80	0.26	0.64	0.32
A	31.93	7.87	16.06	32.05	34.51	13.05	8.15	56.44		
B	6.66	3.23	5.96	7.31	6.67	6.40	7.13	8.17		
Alpha	0.209	0.023	0.235	0.176	0.153	0.061	0.089	0.133		
Beta	0.017	0.010	0.012	0.016	0.013	0.011	0.016	0.013		

[a] 2-PAM dose approximately 634 mg.

TABLE 20. PHARMACOKINETIC PARAMETERS FOR 2-PAM ESTIMATED FROM TWO COMPARTMENT MODELS FOLLOWING INJECTION WITH THE MCA-B SYSTEM[a]

Animal Number Body Weight (kg)	87 80.0	93 77.7	104 76.4	116 70.0	117 75.5	123 65.0	127 81.4	129 81.4	Mean 75.9	Standard Deviation 5.8
C_{max} (μg/mL)	8.31	10.18	5.11	7.68	10.18	14.56	10.36	5.25	9.00	3.09
T_{max} (min)	7.7	6.2	7.5	10.0	7.8	8.8	8.1	9.0	8.2	1.1
AUC (μg*min/mL)	452	637	519	462	983	890	452	569	620	207
K_a (min^{-1})	0.345	0.577	0.528	0.263	0.483	0.328	0.268	0.461	0.407	0.121
K_{el} (min^{-1})	0.031	0.018	0.011	0.024	0.012	0.022	0.044	0.008	0.021	0.012
$V_{d\beta}$ (L)	43	69	110	66	364	63	29	90	104	108
$V_{d\beta}$/BW (L/kg)	0.54	0.89	1.44	0.95	4.82	0.96	0.35	1.10	1.38	1.43
A	17.40	7.87	-0.06	7.06	11.61	16.13	37.94	-4.94		
B	7.55	3.95	5.70	6.81	0.83	6.16	8.17	8.72		
Alpha	0.211	0.022	0.002	0.091	0.024	0.053	0.183	0.080		
Beta	0.017	0.013	0.010	0.016	0.002	0.009	0.020	0.014		

[a] 2-PAM dose approximately 634 mg.

TABLE 21. MODEL-DERIVED AREAS UNDER THE PLASMA 2-PAM CONCENTRATION-TIME CURVES TO EACH SAMPLING FOLLOWING INJECTION WITH THE MKI SYSTEM

Animal	AUC_{0-1}	AUC_{0-2}	AUC_{0-3}	AUC_{0-4}	AUC_{0-5}	AUC_{0-6}	AUC_{0-8}	AUC_{0-12}
87	3.8	9.1	15.1	21.3	27.6	33.8	46.2	70.1
117	3.3	8.6	14.8	21.7	28.8	36.0	50.4	78.2
104	0.6	1.9	3.6	5.7	8.3	11.2	17.8	33.5
129	3.1	8.1	14.4	21.2	28.5	35.9	50.8	79.7
127	2.0	5.4	9.9	15.2	20.9	27.0	39.9	66.1
123	4.2	11.5	21.1	32.4	44.8	57.9	85.1	138.3
93	2.2	5.9	11.0	16.9	23.6	30.8	46.1	78.0
116	1.8	5.1	9.5	14.9	21.0	27.6	42.1	73.2

Animal	AUC_{0-16}	AUC_{0-20}	AUC_{0-48}	AUC_{0-68}	AUC_{0-96}	AUC_{0-120}	AUC_{0-168}	AUC_{0-240}
87	93.0	114.8	210.3	286.3	346.8	433.3	507.5	545.0
117	104.2	128.4	228.0	300.9	355.9	432.1	499.7	538.6
104	51.1	69.4	155.5	223.9	278.0	358.6	436.3	482.4
129	106.8	132.5	243.5	331.8	402.1	502.7	589.1	632.8
127	91.3	114.8	207.9	270.7	314.2	369.1	416.4	449.8
123	185.4	225.4	356.4	438.8	501.3	588.5	660.6	695.4
93	109.5	139.4	261.0	345.4	404.7	478.2	533.7	563.4
116	104.4	134.0	249.2	323.5	374.7	440.3	493.2	519.5

TABLE 22. MODEL DERIVED AREA UNDER THE PLASMA 2-PAM CONCENTRATION-TIME CURVES TO EACH SAMPLING TIME FOLLOWING INJECTION WITH THE MCA SYSTEM

Animal	AUC_{0-1}	AUC_{0-2}	AUC_{0-3}	AUC_{0-4}	AUC_{0-5}	AUC_{0-6}	AUC_{0-8}	AUC_{0-12}
87	2.4	6.7	12.2	18.7	25.8	33.3	48.9	79.7
117	1.7	4.7	8.8	13.6	19.1	25.0	37.7	64.3
104	1.8	4.9	8.9	13.7	19.0	24.7	36.7	61.8
129	0.7	2.0	3.8	6.0	8.6	11.6	18.4	34.3
127	0.1	0.4	0.8	1.4	2.0	2.8	4.7	9.8
123	5.3	14.4	26.0	39.1	53.3	67.9	97.3	152.6
93	0.3	0.8	1.7	2.7	4.0	5.5	9.1	18.4
116	2.7	7.6	14.0	21.7	30.3	39.4	58.8	98.4

Animal	AUC_{0-16}	AUC_{0-20}	AUC_{0-40}	AUC_{0-60}	AUC_{0-80}	AUC_{0-120}	AUC_{0-180}	AUC_{0-240}
87	108.1	133.6	230.9	300.1	352.1	421.0	472.9	495.3
117	90.4	114.6	210.8	281.1	336.3	415.8	485.3	521.2
104	86.6	110.5	212.8	290.9	350.3	430.0	491.5	518.8
129	52.0	70.6	162.8	244.5	314.5	424.5	532.9	596.5
127	16.5	24.4	78.4	144.8	214.0	341.9	487.6	582.4
123	200.5	241.5	387.7	485.9	557.0	646.7	709.4	733.8
93	30.0	43.2	121.6	200.2	267.6	365.2	449.3	498.0
116	135.3	168.3	283.5	354.9	406.6	476.1	531.3	556.6

TABLE 23. MODEL-DERIVED AREAS UNDER THE PLASMA 2-PAM CONCENTRATION-TIME CURVES TO EACH SAMPLING TIME FOLLOWING INJECTION WITH THE MCA-A SYSTEM

Animal	AUC_{0-1}	AUC_{0-2}	AUC_{0-3}	AUC_{0-4}	AUC_{0-5}	AUC_{0-6}	AUC_{0-8}	AUC_{0-12}
87	3.4	8.9	15.8	23.4	31.3	39.3	55.1	83.6
117	3.3	8.9	16.1	24.3	33.2	[illegible].4	61.1	96.4
104	2.1	5.7	10.2	15.3	20.7	26.4	37.8	60.0
129	3.1	8.5	15.7	24.2	33.6	43.6	64.5	106.4
127	4.9	12.6	21.9	31.9	42.3	52.6	72.6	109.0
123	5.1	13.5	24.0	35.8	48.4	61.2	86.9	135.4
93	3.9	10.1	17.8	26.4	35.4	44.6	63.1	99.2
116	3.3	8.9	16.1	24.1	32.7	41.6	59.4	92.6

Animal	AUC_{0-16}	AUC_{0-20}	AUC_{0-40}	AUC_{0-60}	AUC_{0-80}	AUC_{0-120}	AUC_{0-180}	AUC_{0-240}
87	108.1	129.4	210.8	267.6	308.0	357.3	389.6	401.3
117	127.3	154 [illegible]	253.0	324.6	379.9	456.6	519.0	548.4
104	80.8	100.2	182.5	246.4	296.2	365.5	422.1	449.0
129	145.1	179.5	305.2	391.7	457.4	546.9	618.0	650.5
127	140.4	167.7	266.9	331.6	377.1	433.2	471.0	485.2
123	178.4	216.1	351.5	438.9	503.1	592.9	671.6	713.1
93	132.9	164.4	291.8	381.9	446.6	529.3	592.4	623.0
116	121.5	146.7	241.2	307.4	355.5	416.2	458.4	474.7

TABLE 24. MODEL-DERIVED AREAS UNDER THE PLASMA 2-PAM CONCENTRATION-TIME CURVES TO EACH SAMPLING TIME FOLLOWING INJECTION WITH THE MCA-B SYSTEM

Animal	AUC_{0-1}	AUC_{0-2}	AUC_{0-3}	AUC_{0-4}	AUC_{0-5}	AUC_{0-6}	AUC_{0-8}	AUC_{0-12}
87	3.8	10.0	17.6	25.9	34.4	43.0	59.7	89.9
117	4.5	11.7	20.4	30.0	40.0	50.2	70.6	109.7
104	2.3	5.8	10.1	14.9	19.8	24.9	35.1	55.2
129	1.7	4.4	8.0	12.0	16.5	21.2	31.3	52.8
127	4.4	11.6	20.6	30.7	41.2	51.9	73.0	110.8
123	5.4	14.3	25.8	38.8	52.7	67.2	96.5	153.0
93	5.0	12.6	21.7	31.5	41.6	51.7	71.9	110.5
116	2.5	6.8	12.4	18.9	25.9	33.4	48.8	79.2

Animal	AUC_{0-16}	AUC_{0-20}	AUC_{0-40}	AUC_{0-60}	AUC_{0-80}	AUC_{0-120}	AUC_{0-180}	AUC_{0-240}
87	116.1	139.4	230.5	294.5	340.0	395.2	431.3	444.2
117	145.9	179.1	308.9	395.2	454.3	527.3	587.9	628.6
104	74.6	93.2	175.6	242.5	296.8	376.6	448.1	485.8
129	75.2	97.8	203.6	289.6	356.1	445.6	514.7	545.2
127	142.4	169.0	263.4	324.4	365.6	412.1	439.1	447.4
123	203.8	248.6	408.7	508.8	580.4	680.2	771.0	822.3
93	146.5	179.8	314.0	406.9	471.8	549.9	601.8	621.8
116	107.3	132.6	227.6	291.5	337.4	395.3	435.9	451.9

APPENDIX D

Sample Pharmacokinetic Modeling Program Used in Analyses

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LIBNAME REG '[TS.15.ATROPINE.MCA]';
OPTIONS LS=80;
DATA TRUNC;
  SET REG.MCARAW;
  IF ANIMAL=87 AND CONC NE 0;
PROC MEANS NOPRINT DATA=TRUNC;
  VAR T;
  ID ANIMAL;
  OUTPUT OUT=MAX MAX=MAXT;
DATA MAX2;
  SET MAX;
  _TYPE_='FINAL';
PROC SORT; BY _TYPE_;
PROC NLIN DATA=TRUNC CONVERGE=1E-2 MAXITER=100 METHOD=MARQUARDT OUTEST=ESTIM;
  PARMS A=4.44
        B=7.01
        ALPHA=0.049
        BETA=0.005
        KA=0.40;
  AEXP=EXP(-ALPHA*T);
  BEXP=EXP(-BETA*T);
  KEXP=EXP(-KA*T);
  MODEL CONC=A*(AEXP-KEXP)+B*(BEXP-KEXP);
  DER.A=AEXP-KEXP;
  DER.B=BEXP-KEXP;
  DER.ALPHA=-A*T*AEXP;
  DER.BETA=-B*T*BEXP;
  DER.KA=(A+B)*T*KEXP;
  TITLE 'TWO-COMPARTMENT ATROPINE PHARMACOKINETICS MODEL';
  TITLE2 'TASK 89-15 MCA AUTOINJECTOR';
  OUTPUT OUT=REG.P870UT P=CONCHAT L95M=LCL U95M=UCL;
PROC SORT DATA=ESTIM;
  BY _TYPE_;
PROC PRINT DATA=REG.P870UT;
DATA EST;
  SET ESTIM;
  IF _TYPE_='FINAL';
DATA REG.MCAAN87;
  MERGE EST MAX2; BY _TYPE_;
  D=1950000;
  DX=1;
  X=-1;
  SUMY=0;
  PART=1;
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   DO UNTIL (X GE 240);
   * ALTERNATIVELY, THE ABOVE STATEMENT COULD READ DO UNTIL (X GE MAXT);
     X=X+DX;
     Y=A*(EXP(-ALPHA*X)-EXP(-KA*X))+B*(EXP(-BETA*X)-EXP(-KA*X));
     SUMY=SUMY+Y;
     PART=Y/SUMY;
          IF X=1 THEN DO; SUMY1=SUMY; END;
     ELSE IF X=2 THEN DO; SUMY2=SUMY; END;
     ELSE IF X=3 THEN DO; SUMY3=SUMY; END;
     ELSE IF X=4 THEN DO; SUMY4=SUMY; END;
     ELSE IF X=5 THEN DO; SUMY5=SUMY; END;
     ELSE IF X=6 THEN DO; SUMY6=SUMY; END;
     ELSE IF X=8 THEN DO; SUMY8=SUMY; END;
     ELSE IF X=12 THEN DO; SUMY12=SUMY; END;
     ELSE IF X=16 THEN DO; SUMY16=SUMY; END;
     ELSE IF X=20 THEN DO; SUMY20=SUMY; END;
     ELSE IF X=40 THEN DO; SUMY40=SUMY; END;
     ELSE IF X=60 THEN DO; SUMY60=SUMY; END;
     ELSE IF X=80 THEN DO; SUMY80=SUMY; END;
     ELSE IF X=120 THEN DO; SUMY120=SUMY; END;
     ELSE IF X=180 THEN DO; SUMY180=SUMY; END;
     ELSE IF X=240 THEN DO; SUMY240=SUMY; END;
   END;
   INTAUC=SUMY*DX;
   CALCAUC=A/ALPHA+B/BETA-(A+B)/KA;
   D1=A*(KA-ALPHA)+B*(KA-BETA);
   K21=((A*BETA*KA)+(B*ALPHA*KA)-(A+B)*ALPHA*BETA)/D1;
   KEL=ALPHA*BETA/K21;
   K12=ALPHA+BETA-K21-KEL;
   V1=D/(A+B)/1000;
   Vdbeta=V1*KEL/BETA;
   TBETA=LOG(2)/BETA;
   TMAX=1/(KA-KEL)*LOG(KA/KEL);
   CMAX=A*(EXP(-ALPHA*TMAX)-EXP(-KA*TMAX))+B*(EXP(-BETA*TMAX)-EXP(-KA*TMAX));
   DROP _TYPE_ _NAME_ _ITER_ SUMY D1;
PROC PRINT;
   TITLE1 'TASK 89-15: TWO-COMPARTMENT PK MODEL FOR MCA ATROPINE
     AUTOINJECTOR';
   TITLE2 'PARAMETERS FOR ANIMAL 87';
   VAR _SSE_--Y INTAUC--CMAX;
PROC PRINT;
   VAR SUMY1--SUMY240;
```