Statistical Analysis Plan

Sponsor Protocol Number: ACH-CYT-02

Repeat-Dose Pharmacokinetic and Pharmacodynamic Evaluation of Cytisine in Healthy Smokers

[redacted]

Prepared for:

Achieve Life Sciences, Inc.

Prepared by:

[redacted]

CONFIDENTIAL

Version	Author
Final 1.0	[redacted]

[redacted]

[redacted]

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Simbec Protocol ID: RD 735/33716

Author: Kerry Williams

Version: Final 1.0

The undersigned have reviewed and revised this SAP and find it to be consistent with the study requirements:

[redacted]

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GLOSSARY OF ABBREVIATIONS

ADaM	Analysis Data Model
AE	Adverse Event
Ae	Amount Excreted
ANOVA	Analysis of Variance
AUC	Area Under the Plasma Concentration Curve
$AUC_{0-\infty}$	Area Under the Plasma Concentration-time Curve calculated from the time of dosing to infinity
AUC_{0-t}	Area Under the Plasma Concentration-time Curve calculated from the time of dosing to the last measurable concentration
BLQ	Below Limit of Quantification
BMI	Body Mass Index
CI	Confidence Interval
C_{max}	Maximum observed plasma concentration
C_{min}	Minimum observed plasma concentration
CO	Carbon Monoxide
CRF	Case Report Form
CS	Clinically Significant
CSR	Clinical Study Report
CV	Coefficient of Variation
DBL	Database Lock
DMP	Data Management Plan
DRM	Data Review Meeting
ECG	Electrocardiogram
FTND	Fagerström Test for Nicotine Dependence
GM	Geometric Mean
ICH	International Conference on Harmonisation
IMP	Investigational Medicinal Product
LLOQ	Lower Limit of Quantification
LSMean	Least Squares Mean
MedDRA	Medical Dictionary for Regulatory Activities

mg	Milligram
mL	Millilitre
NCS	Not Clinically Significant
PD	Pharmacodynamic
PK	Pharmacokinetics
PT	Preferred Term
QC	Quality Control
RBC	Red Blood Cell
SAP	Statistical Analysis Plan
SD	Standard Deviation
SDTM	Study Data Tabulation Model
SOC	System Organ Class
TCQ-SF	Tobacco Craving Questionnaire – Short Form
TEAE	Treatment Emergent Adverse Event
TFL	Table/Figure/Listing
T_{max}	Time from dosing to the maximum observed plasma concentration
WBC	White Blood Cell

1 INTRODUCTION

1.1 GENERAL

This statistical analysis plan (SAP) describes the statistical methods to be used during the reporting and analysis of data collected under the ACH-CYT-02 protocol dated 21st September 2017 and should be read in conjunction with the study protocol and case report form (CRF).

This version of the plan has been developed using the protocol version 2.0 dated 21st September 2017 and the annotated CRF dated 4th October 2017. Any further changes to the protocol or CRF will be reviewed for potential impact on the SAP, which will be amended if it is deemed necessary.

Draft versions of the SAP will undergo review by the statistical reviewer, Project Manager, PK Analyst, Medical Writer and the Sponsor/Sponsor representative. The analysis plan will be finalised and approved by the Sponsor prior to Database Lock (DBL).

1.2 CHANGES FROM PROTOCOL

- Change from baseline Tobacco Craving Questionnaire – Short Form scores will be included as a pharmacodynamic endpoint.
- The protocol identified reduction in smoking as a pharmacodynamic endpoint but did not refer to smoking cessation as an endpoint. Smoking cessation has been included in this SAP as a pharmacodynamic endpoint.
- Summaries of smoking cessation will be presented.
- Individual QT corrections (QTcI) will not be performed because it was not feasible for this study as a substantial amount of pre-treatment data across a large range of heart rates was required.
- Inclusion of an ECG analysis set and ECG-PK analysis set.
- The summary of Holter ECG QTcF categories will not include the categories QTcF ≤ 450 mSec and QTcF increase ≤ 30 mSec.
- Summaries of prior and concomitant medications will be included.
- Smoking history at baseline will be summarised.

1.3 CHANGES FROM PREVIOUS VERSIONS OF THE SAP

Not applicable.

2 STUDY OBJECTIVES

Primary Objectives:

The primary objectives of this study are:

- to evaluate the pharmacokinetic (PK) parameters during repeat dosing of 1.5 mg or 3.0 mg cytisine when administered as the commercial 25-day schedule;
- to evaluate the pharmacodynamic (PD) effects (e.g. reduction in smoking) with repeat dosing of 1.5 mg or 3.0 mg cytisine when administered as the commercial 25-day schedule.

Secondary Objectives:

The secondary objectives of this study are:

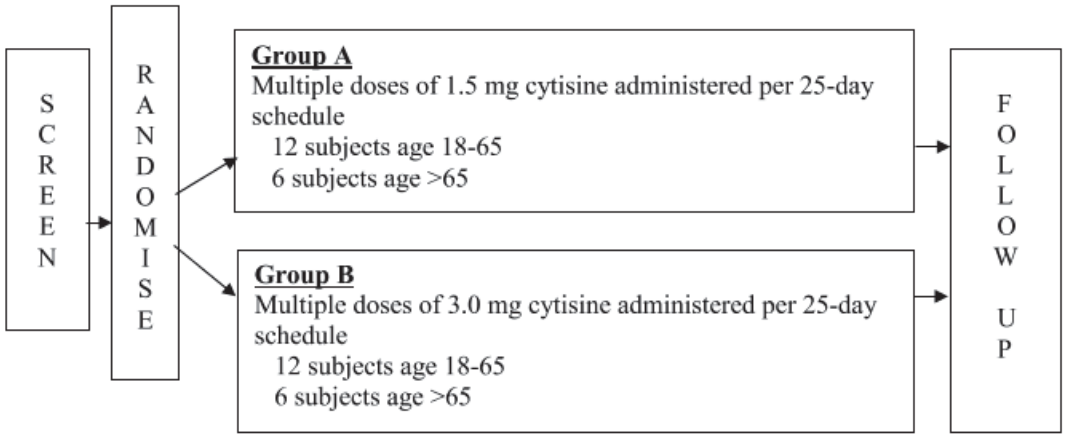
- to compare the PK parameters and tolerability for repeat dosing of 1.5 mg and 3.0 mg cytisine during the 25-day schedule in healthy smokers at 18-65 and >65 (elderly) years of age;
- to assess the renal elimination of cytisine via measurement of urinary concentrations of cytisine during treatment on Day 1 and Day 25;
- to evaluate for effects on QT/QTc interval prolongation and cardiac safety during treatment on Day 1 and Day 25 in healthy smokers at 18-65 and >65 (elderly) years of age.

3 STUDY DESIGN

3.1 OVERVIEW

This is an open-label, randomised, multiple-dose PK and PD study to evaluate the PK profile and PD effect of cytisine when administered at doses of 1.5 mg and 3.0 mg following the commercialised 25-day schedule. A total of 36 healthy adult smokers, such that 24 subjects are 18-65 years of age and 12 subjects are >65 years of age will be randomised to complete the study, as shown in Figure 1. An attempt will be made for 50% male and 50% female enrolment.

Figure 1: Study Design Overview



The study is comprised of a pre-study screen, followed by 25 days of treatment and a post-study follow-up. Screening assessments will be carried out within 28 days prior to first administration of cytisine. Eligible subjects will be asked to return on the evening prior to Day 1 (i.e. Day -1) for repeat of key entry testing, baseline questionnaires and an overview of the risks of smoking and quit advice according to the World Health Organisation (WHO) guidelines. Randomisation to dose groups will occur on the morning of Day 1 followed by initiation of study treatment. Eligible subjects will take cytisine (in the form of the commercial product [redacted] as outlined in Table 1. Group A subjects will take 1 tablet at each time point (1.5 mg) and Group B subjects will take 2 tablets at each time point (3.0 mg). A post-study follow-up visit will be conducted 6 to 8 days after the last dose of cytisine. The maximum duration of this study is estimated to be around 9 weeks (screening to last subject's last visit).

Table 1: Dose Timing and Total Daily Dose

Days	Regimen	Total Daily Dose		Approximate Interval
		1.5 mg Dose	3.0 mg Dose	
1-3	6 times daily	9.0 mg	18.0 mg	2 hours
4-12	5 times daily	7.5 mg	15.0 mg	2.5 hours
13-16	4 times daily	6.0 mg	12.0 mg	3 hours
17-20	3 times daily	4.5 mg	9.0 mg	4-5 hours
21-24	2 times daily	3.0 mg	6.0 mg	6 hours
25	Once daily	1.5 mg	3.0 mg	-

3.2 INCLUSION AND EXCLUSION CRITERIA

To be eligible for inclusion into this study, each subject must fulfil all inclusion criteria and not violate any exclusion criteria (for the protocol under which they are entered) during screening prior to randomisation. Details of the inclusion and exclusion criteria are presented within the protocol (sections 6.1, 6.2).

3.3 STUDY TREATMENT

Each subject will receive one of the following treatments in accordance with the randomisation schedule:

- Group A: 1.5 mg cytisine (1 x 1.5 mg tablet) administered per commercialised 25-day schedule;
- Group B: 3.0 mg cytisine (2 x 1.5 mg tablets) administered per commercialised 25-day schedule.

Cytisine should be administered orally according to the dose timing schedule (see Table 1) with each dose to be either one [redacted] and, when possible, with a meal or a snack.

3.4 SCHEDULE OF STUDY PROCEDURES

Study Day	Screening	Study Day										Post-Study
	-28 to -2	-1[1]	1-4[1]	4-11	12-13[2]	13-15	16-17[2]	17-19	20-21[2]	21-23	24-26[3]	6-8 days after last dose
Written informed consent	X											
Number of cigarettes smoked in previous 24 hours	X	X										
Daily Smoking and Study Medication Diary[4]		X	X	X	X	X	X	X	X	X	X	X
Demographic data	X											
Randomization			X[5]									
Vital signs	X	X[6]	X[6]		X[6]		X[6]		X[6]		X[6]	
Medical history	X	X[7]										
Prior and concomitant medication	X	X	X		X		X		X		X	X
Physical examination	X											
12-lead ECG	X	X	X[8]		X[8]		X[8]		X[8]		X[8]	
Holter monitor for continuous ECG readings[9]			X								X	
Pregnancy test for all females	X Serum	X Urine									X Serum	
FSH (females of non-childbearing potential only)	X											
Haematology[10]	X		X		X		X		X		X	
Biochemistry[10]	X		X		X		X		X		X	
Virology (HIV, Hep-B, Hep-C)	X											
Urinalysis	X										X	

	Screening	Study Day										Post-Study
Study Day	**-28 to -2**	**-1[1]**	**1-4[1]**	**4-11**	**12-13[2]**	**13-15**	**16-17[2]**	**17-19**	**20-21[2]**	**21-23**	**24-26[3]**	**6-8 days after last dose**
Drugs-of-abuse tests in urine	X	X										
Fagerström test for nicotine dependence		X									X	
Urine cotinine[11]	X		X		X		X		X		X	
Expired air CO[11]	X		X		X		X		X		X	
Verification of eligibility criteria	X	X	X									
Tobacco craving questionnaire (short form)		X	X		X		X		X		X	
Risks of smoking and quit advice[12]		X	X		X		X		X		X	X
IMP (cytisine) administration[13]			X	X	X	X	X	X	X	X	X	
Plasma collection for PK analysis[14]			X		X		X		X		X	
Urine collection for PK analysis[15]			X								X	
Adverse events monitoring[16]			X		X		X		X		X	X

[1] 4 overnight stays in clinic, starting evening of Day -1.
[2] 1 overnight stay in clinic.
[3] 2 overnight stays in clinic.
[4] Subject to record number of cigarettes smoked in past 24 hours on a daily basis throughout study, actual time of each medication dose and record any adverse events. Information to be reviewed and documented during clinic days. Subject to also maintain record of number of cigarettes smoked in each 24 hour period from Day 26 until final Post-Study Visit (see protocol Section 13.9).
[5] Pre-First Dose on Day 1
[6] Vital signs (supine blood pressure, pulse rate and oral temperature) will be recorded at pre-dose and again prior to discharge from clinic. Vital Signs will also be recorded 3 hours post-last dose on Days 1, 2, 3, 12, 16, 20 and 24.
[7] Clinically relevant changes will be reported as adverse events.
[8] Obtain 12-lead ECG (triplicate) at any time if clinically indicated during clinic visits. Repeat 12-lead ECG and assess prior to discharge.
[9] Attach ECG Holter monitor and begin recording prior to first dose on Day 1 and continue for 24 hours (discontinue just prior to first dose on Day 2. Repeat again by starting Holter monitor prior to dose on Day 25 and record for 24 hours.
[10] Haematology and biochemistry testing to be completed prior to discharge on Days 4, 13, 17, 21, 26
[11] Urine cotinine and expired CO testing to be obtained at screen and prior to discharge on Days 4, 13, 17, 21, 26.
[12] Each subject given advice about the risks of smoking and quitting. This will take the form of a brief interview following WHO evidence-based recommendations on the treatment of tobacco dependence on Day -1 and reminders prior to discharge on Days 4, 13, 17, 21, 26 and on the final post-study visit.
[13] IMP administration to follow 25-day dosing schedule (see Table 1).
[14] Plasma collection for PK analysis is outlined in protocol Section 12.1.
[15] Urine to be collected for PK on Day 1 and Day 25 as follows: pre-dose and 0-24 hours.
[16] Adverse event recording to begin upon admissions on Day -1 to post-study.

3.5 SAMPLE SIZE CONSIDERATIONS

A total of approximately 36 subjects will be randomised to the study, with approximately 18 subjects per treatment arm (12 aged 18-65 and 6 aged >65).

No previous pharmacokinetic studies with cytisine have reported the effects of repeated administration, so no estimates of intra-subject variability of C_{max}, C_{min} or AUC are available. No specific hypothesis testing is planned. Post-hoc analyses may be performed for PK or PD effects that may appear significantly different between the dosage groups or age groups. In general, the sample size of 36 should be sufficient to meet the exploratory objectives of the study.

Subjects who withdraw from study at any time after randomisation will not be automatically replaced. Replacement based on the number and reason of withdrawals will be at discretion of the Sponsor, following discussion with the Principal Investigator.

3.6 RANDOMISATION

Subjects will be allocated to Group A (1.5 mg) or Group B (3.0 mg) in accordance with a randomisation code produced by [redacted] using the PROC PLAN procedure of SAS® version 9.3. Subjects will be numbered sequentially from 001 (i.e. 001, 002 etc.). Any replacement subjects will be assigned the same randomisation as the subjects they are replacing with 100 added to the subject number (i.e. 101 would replace 001 etc.). Subjects will be stratified for age (elderly > 65 years: yes vs no) to ensure that each dose group has 6 elderly subjects and 12 non-elderly subjects.

4 STUDY VARIABLES AND COVARIATES

4.1 PRIMARY VARIABLES

The plasma cytisine pharmacokinetic endpoints for this study are as follows:

- C_{max} — Maximum observed plasma concentration post dose, directly obtained from the observed concentration versus time profile.
- T_{max} — Time of occurrence of C_{max}.
- C_{min} — Minimum observed plasma concentration prior to the last daily dose or prior to each scheduled dose change.
- AUC_{0-t} — Area under the plasma concentration versus time curve (AUC) from time zero to the last sampling time at which concentrations were at or above the lower limit of quantification (LLOQ), calculated by the linear-up/log-down trapezoidal rule.
- AUC_{0-inf} — Total AUC from zero to infinity, calculated as $AUC_{0-inf} = AUC_{0-t} + (C_{last}/\lambda_z)$, where C_{last} is the last measurable plasma concentration and λ_z the apparent terminal elimination rate constant.
- %AUC — Residual area or percentage of extrapolated part for the calculation of $AUC_{0-\infty}$, calculated as $100*[1-(AUC_{0-t}/ AUC_{0-\infty})]$.
- λ_z — Apparent terminal elimination rate constant.
- $t_{1/2}$ — Apparent terminal elimination half-life, calculated from $ln\ 2/\lambda_z$.

The pharmacodynamic endpoints for this study are as follows:

- Number of cigarettes smoked daily during treatment and at Day 26
- Reduction in number of cigarettes smoked from baseline, daily during study treatment and at Day 26
- Reduction in expired air carbon monoxide (CO) from baseline, during study treatment and at Day 26;
- Number of subjects at Day 26 that did not smoke any cigarettes for the past 24 hrs and had biochemical confirmation of a CO level less than 10ppm on Day 26 (smoking cessation)
- Change from baseline urine cotinine results;
- Change from baseline Tobacco Craving Questionnaire – Short Form (TCQ-SF) scores: total score and component scores for emotionality, expectancy, compulsivity, purposefulness.

4.2 SECONDARY VARIABLES

The urine cytisine pharmacokinetic endpoints for this study are as follows:

- Ae Amount excreted in urine over time.
- Ae% Percentage of drug excreted in urine.

The safety endpoints for this study are as follows:

- Adverse events (AEs).
- Laboratory safety data (biochemistry, haematology, urinalysis and microscopy).
- Vital signs (supine systolic/diastolic blood pressure, pulse rate and oral temperature).
- 12-lead ECG (heart rate, PR interval, QRS duration, QT interval and QT interval corrected using Fridericia's formula (QTcF interval).
- ECG Holter Monitoring (heart rate, PR interval, QRS duration, QT interval and QTcF interval).

5 DEFINITIONS AND DERIVED VARIABLES

Study drug/IMP: 1.5 mg or 3.0 mg cytisine.

Treatment/dose: 1.5 mg or 3.0 mg cytisine as per commercialised 25-day dosing schedule.

Dose group: 1.5 mg Cytisine, 3.0 mg Cytisine.

Age group: 18-65 yrs, >65 yrs, all ages.

Baseline: In general, baseline is defined by subject and by variable as the last non-missing value (including repeats) before the first dose of study drug. This is normally the pre-dose assessment on Day 1 but if this assessment is missing (or not planned) then the assessment at the Screening/Day -1 visit will be used instead, if available.

For Holter ECG endpoints, a baseline value will be derived for each subject and ECG parameter (heart rate, PR interval, QRS duration, QT interval and QTcF interval) by calculating the mean of all individual results recorded prior to the administration of study drug on Day 1 (i.e., at the -30 minute and -15 minute time points).

Study Day: Study day is the number of days since start of treatment where the date of first dose is counted as Day 1.

Bleed Time Deviation: Actual time of blood sample – theoretical time of blood sample.

Protocol Deviation: a deviation related to study inclusion or exclusion criteria, conduct of the trial, subject management or subject assessment. This refers to any change, divergence, or departure from the study design or procedures defined in the protocol. Deviations recorded by the Project Manager or detected by Data Management or by statistical programming checks will be identified and discussed at the Data Review Meeting (DRM) before Database Lock (DBL). All protocol deviations within the study database will be classified as either 'Major' or 'Minor' prior to DBL, details of which will be included within the Protocol Deviations listing.

Duration of Exposure: (Date of last dose of cytisine – date of first dose of cytisine) + 1.

6 ANALYSIS SETS

Membership of the analysis sets will be reviewed and agreed prior to DBL. These will be reviewed by the Sponsor, Study Statistician, PK analyst and Project Manager and signed off. If PK data is not available at the time of DBL, then subjects will be assumed to be included in the analysis set unless the PK data provide reason to exclude a subject, in which case this will be discussed with the Sponsor and documented within the Analysis Sets listing (Listing 16.2.3.1).

6.1 SAFETY SET

All randomised subjects who receive at least one dose of cytisine will constitute the Safety Set.

This analysis set will be used for baseline and safety summaries as well as for all study listings.

6.2 PK SET

The PK Set will include all subjects who have completed all cytisine dosing on Days 1-3, completed >90% cytisine dosing on Days 4-25 and comply with the following criteria:

- Do not have an occurrence of vomiting or severe diarrhoea which renders the concentration profile unreliable (i.e. vomiting occurring at or before 2 times median T_{max});
- Do not use a concomitant medication which renders the concentration profile unreliable;
- Have at least one evaluable concentration that is preceded by a lower evaluable concentration and followed by a lower evaluable concentration for the calculation of C_{max}, T_{max} and AUCs (i.e. at least 3 evaluable concentrations in total);
- Do not violate the protocol (major protocol deviation) in a way that may invalidate or bias the PK results.

6.3 PD SET

The PD Set will include all subjects in the PK Set who have an available baseline result and at least one on-treatment result with regards to urine cotinine, expired air CO or daily cigarette consumption and do not incur a major protocol deviation in a way that may invalidate or bias the PD results.

6.4 ECG SET

The ECG Set will include all subjects who receive at least one dose of cytisine, with at least one available baseline ECG and at least one on-treatment ECG.

6.5 ECG-PK SET

The ECG-PK Set will include all subjects who receive at least one dose of cytisine, with a time-matched ECG-PK pair at baseline and at least one time-matched pair on-treatment.

7 SAFETY MONITORING

Not applicable.

8 INTERIM ANALYSES

Not applicable.

9 DATA

9.1 CRF DATA

CRF data will be provided by [redacted] Data Management to the Statistics department as SAS datasets in [redacted] standard format. SDTM datasets will be derived from the raw database and ADaM from SDTM. Both SDTM and ADaM domains will be used for programming the outputs to be included in the Clinical Study Report (CSR). SDTM/ADaM programming will begin when populated [redacted] standard SAS datasets are available.

9.1.1 Laboratory Data

Transfers of safety laboratory data will be available from the Pathology laboratory and delivered to Data Management via electronic transfer and stored within the study database. Details of laboratory data are documented in the Data Management Plan (DMP). Populated test transfers will be received before programming can start. The following results will be included:

- **Haematology**: Haemoglobin, haematocrit, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, red blood cells, white blood cells, neutrophils, lymphocytes, monocytes, eosinophils, basophils and platelets.

- **Biochemistry**: Total protein, albumin, total bilirubin, alanine transaminase, aspartate transaminase, gamma glutamyl transferase, alkaline phosphatase, glucose, sodium, potassium, calcium, bicarbonate, creatinine and urea.
- **Urinalysis:** Glucose, specific gravity, protein, leukocytes, ketones, urobilinogen, bilirubin, nitrites, pH and blood.
- **Microscopy:** In the event that the 'dipstick' is positive for leukocytes, nitrites, protein and blood, the parameters red blood cells, white blood cells, epithelial cells, crystals, bacteria and casts will be examined microscopically.
- **Virology:** Hep B, Hep C and HIV.
- **Drugs of Abuse and Alcohol:** Urine alcohol and drugs of abuse screen: cannabinoids, amphetamines, cocaine, benzodiazepines, opiates and cotinine.
- Urine cotinine**:** Urine cotinine will be collected throughout the study as a PD parameter.
- **Pregnancy/post-menopausal assessment:** serum/urine pregnancy test and follicle stimulating hormone.
- **Other parameters:** Any further parameters that are taken that are not included as part of the above categories will be included in an 'Other Laboratory Data' listing.

9.1.2 Pharmacokinetic Data

Plasma and urine concentration data will be received as an Excel file from the Bioanalytical department via an electronic transfer and stored as a SAS dataset. This data will then be stored in the appropriate SDTM domain and subsequent ADaM domain which will be used to produce the file provided to the pharmacokinetic team in order to derive the PK parameters using Phoenix WinNonlin 6.4. Derived PK parameters will be received by the Statistics department from the Project Manager/PK analyst in a SAS.xpt file in an agreed format and then stored as a SAS dataset and subsequently within SDTM/ADaM domains.

9.1.3 ECG Holter Monitoring Data

ECG Holter data will be received as an Excel file from CardioAnalytics (3rd party cardiac monitoring service managed by [redacted] via an electronic transfer and stored as a SAS dataset. This data will then be stored in the appropriate SDTM domain and subsequent ADaM domain.

9.2 RANDOMISATION LIST

The randomisation list will be uploaded to a SAS dataset and incorporated into the relevant SDTM/ADaM domains.

9.3 PROGRAMMING AND DATA REVIEW

Programming of datasets, tables, figures and listings may be ongoing while study data management activities are in progress.

Prior to DBL, a review of the clinical database (i.e. CRF data, laboratory data) in the form of Excel data listings will be conducted. A Data Review Meeting (DRM) will be held to discuss the outcome of this review, any potential impact on the analyses, analysis sets and protocol deviations. Once all data issues have been resolved and the analysis sets approved, the database will be locked. The SDTM/ADaM datasets will be finalised and the final run of outputs and quality control (QC) will take place.

Due to issues with the recruitment of the elderly cohort, two database locks are required. The database for non-elderly subjects (<=65 yrs) will be locked while recruitment for the elderly cohort (>65 yrs) is ongoing. The database for the elderly subjects will be locked at a later date. Therefore, two sets of TFLs will be produced – an 'interim' set presenting data for the non-elderly subjects only followed by a second set which will include data from both the non-elderly and elderly cohorts. The second set consisting of data from all subjects will be included in the clinical study report (CSR).

10 STATISTICAL METHODS

10.1 GENERAL PRINCIPLES

- All statistical methods will be based on the International Conference on Harmonisation (ICH) E9 document "Statistical Principles for Clinical Trials".
- All data collected will be presented within data listings and any unscheduled visits will be listed.
- Data will be summarised by dose group (1.5 mg Cytisine, 3.0 mg Cytisine), age group (18-65 yrs, >65 yrs, all ages) and overall (both dose groups pooled) where appropriate. The format of the summaries is defined in the shells at the end of this document.
- In summary and analysis tables of continuous variables, standard descriptive statistics (N [number within analysis set, or cohort, or subgroup], n [number of observations included in analysis], mean, standard deviation [SD], median, minimum and maximum) will be presented. Least squares mean (LS mean) and 90/95% confidence interval (CI) will be presented in the statistical analysis outputs as appropriate. For PK summaries, geometric mean, and coefficient of variation (%CV) will also be used to summarise the data.
- Unless otherwise specified, the minimum and maximum statistics will be presented in summary tables to the same number of decimal places as the original data. The mean, median, LS mean, geometric mean and CI will be presented to one more decimal place than the original data. SD will be presented to two more decimal places than the original data. %CV will be presented to one decimal place.
- In summary tables of categorical variables, the number of non-missing observations by category will be presented along with percentages. Unless otherwise specified, the denominator for each percentage will be the number of non-missing observations. All percentages will be presented to one decimal place.
- All plots will use a linear time scale for the nominal times of the visits and will be labelled by time point.
- Original values will be used in summary tables, unscheduled measurements will be listed only. However, where repeats of baseline values occur, unless specified otherwise (i.e. 12-lead ECG), the last assessment will be used within any summary tables and used to calculate change from baseline. In case an unscheduled measurement is performed immediately after the scheduled

measurement due to an error in the original measurement, unless specified otherwise (i.e. 12-lead ECG) the unscheduled measurement will be included in the analysis and the original erroneous measurement will be excluded.

- The date format for all output presentations will be as captured in the database.
- All statistical analysis will be performed using SAS 9.3 or higher.
- All hypothesis testing will be carried out at the 5% (2-sided) significance level unless stated otherwise.
- P-values will be rounded to four decimal places. P-values less than 0.0001 will be reported as <0.0001 in tables.
- Generally, character values will be left aligned and numeric values will be decimally aligned.
- If any of the assumptions underlying the formal statistical methods proposed are violated during the analysis of the final data, alternative statistical methods will be used and any changes documented in the statistical methods section of the clinical study report (CSR), including the rationale for use.
- For numeric data which includes non-numeric values (e.g. PK data reported as BLQ or laboratory results reported as < 10 or >100) the following principles will be applied when summarising the data:
  - BLQ will be replaced with a zero.
  - Results reported as <*x* or >*x* will be treated as *x*.

10.2 STRATIFICATION AND COVARIATE ADJUSTMENT

In order to investigate any possible effects of age, analyses will be presented by non-elderly subjects (18-65 years) and elderly subjects (>65 years).

10.3 MISSING DATA

Generally, no methods to impute missing data will be used. However, for the purpose of calculating change from baseline, in the instance of a missing baseline result (generally the assessment at Day 1, Pre-Dose) the results obtained at the Screening/Day -1 visit will be used instead, if available.

In the instance of missing pharmacokinetic blood samples, the linear-up/log-down trapezoidal rule will be employed between the samples immediately before and after the missing sample for the AUC calculations.

10.4 POOLING OF SITES

Not applicable.

10.5 MULTIPLE COMPARISONS

Not applicable.

10.6 SUBGROUP ANALYSES

Analyses will be performed by age group (18-35 yrs, >65 yrs, all ages). Due to the small sample sizes, noteably for subjects > 65 years, these analyses are exploratory in nature. The primary comparison of interest is between dose groups.

10.7 STATISTICAL ISSUES

None.

11 STATISTICAL OUTPUT

General principles for layout of the statistical output are described in Section 10.1. Layout and specifications are illustrated for each unique table and listing within the shells presented in Section 14.

11.1 SUBJECT DISPOSITION

The subject disposition table will summarise the following data for all randomised subjects:

- The number (%) of subjects within each analysis set;
- The number (%) of subjects dosed;
- The number (%) of subjects who completed the study/withdrew from the study and the associated reasons for early study termination.

The disposition summary will be presented by dose group and overall.

Screening and study completion/termination data will also be listed. A listing of all subjects with protocol deviations will be presented including major/minor classification. A data listing presenting subject eligibility for each analysis set and the reason for exclusion from an analysis set will also be presented.

11.2 SUBJECT CHARACTERISTICS AT BASELINE

11.2.1 Demographic and Baseline Characteristics

Demographic data will be listed (including informed consent information) and descriptive statistics for the continuous variables age, height, weight and BMI and frequencies for the categorical variables race and gender will be tabulated. These descriptive statistics will be presented by dose group, age group and overall.

Alcohol and cotinine results collected at Screening and Day -1 will be listed and summarised using frequencies (n, %).

Demographic and baseline data will be listed and summarised using the Safety Set. Demographic data may also be summarised using the PK Set, if appropriate.

Smoking history variables reported at baseline will be listed and summarized by dose group, age group and overall. The total number of cigarettes smoked in the past 24h will be summarized as a continuous

variable, and also using frequencies (n, %). Descriptive statistics will be reported for baseline expired CO (ppm), urine cotinine, FTND total score, and TCQ-SF total score. Baseline is defined as results obtained at Screening for expired CO and urine cotinine and as Day -1 for the daily cigarette consumption, FTND and TCQ-SF.

11.3 EFFICACY ANALYSES

Not applicable.

11.4 PK ANALYSES

The primary analysis set for summaries of PK data will be the PK Set. If the PK Set and the Safety Set are not equivalent, then all PK summaries described below will also be produced for the Safety Set.

11.4.1 Plasma Concentration Data

Plasma PK samples will be collected for measurement of cytisine at the following time points:

- Day 1, first dose: Pre-first dose, 0.5h, 0.75h, 1h, 1.25h, 1.5h, 2h;
- Day 1, last dose: Pre-last dose, 0.5h, 1h, 1.5h, 2h, 2.5h, 3h, 3.5h, 4h, 5h;
- Days 2 and 3: Pre-last dose, 0.5h, 1h, 1.5h, 2h, 2.5h, 3h, 3.5h, 4h, 5h;
- Days 4, 13, 17, 21: Pre-first dose;
- Days 12, 16, 20, 24: Pre-last dose, 1h, 1.5h, 2h, 2.5h, 3h, 3.5h, 4h;
- Day 25: Pre-dose, 0.5h, 0.75h, 1h, 1.25h, 1.5h, 1.75h, 2h, 2.25h, 2.5h, 2.75h, 3h, 3.25h, 3.5h, 3.75h, 4h, 5h, 6h, 8h, 10h, 12h, 24h.

Cytisine concentrations in plasma will be listed and summarised by dose group and age group using the descriptive statistics N, n, arithmetic mean, arithmetic standard deviation (SD), coefficient of variation (CV%), minimum, median and maximum.

The individual subject plasma cytisine concentration profiles over time will be presented graphically using actual blood sampling times on a linear and semi-logarithmic scale. Arithmetic mean plasma cytisine concentration profiles over time will be presented on linear and semi-logarithmic scales with profiles presented for each study day as appropriate and a separate plot for each dose group and age group.

For inclusion within the summary tables and linear scale plots, concentrations below the limit of quantification (BLQ) will be assigned a value of zero. For inclusion within the semi-logarithmic scale plots, concentrations below the limit of quantification (BLQ) will be set to missing. Concentrations above the upper limit of quantification (ULOQ) will be obtained via dilution into the calibration range and are valid results.

Plasma concentration data listings and individual plots will be presented using the Safety Set. Plasma concentration data summaries and mean plots will be presented using the PK Set and the Safety Set. All plasma concentration data included in listings and summaries will be presented to three significant figures.

11.4.2 Urine Concentration Data

Urine PK samples will be collected for measurement of cytisine on Day 1 and Day 25 at the following time intervals: Pre-dose (taken upon waking), 0-24h post-dose.

Cytisine concentrations in urine will be listed and summarised for each dose group and age group using the descriptive statistics N, n, arithmetic mean, arithmetic standard deviation (SD), coefficient of variation (CV%), minimum, median and maximum.

Urine concentration data listings will be presented using the Safety Set. Urine concentration data summaries will be presented using the PK Set and the Safety Set.

11.4.3 Derived PK Parameters

The derived pharmacokinetic parameters of cytisine in plasma (listed in section 4) will be determined using WinNonlin Phoenix 6.4 from the individual concentration versus time data using standard non-compartmental methods. The derived urine pharmacokinetic parameters, Ae and Ae% (individual and cumulative), will be determined from the individual concentration versus time data using SAS version 9.3.

For the calculation of derived pharmacokinetic parameters, concentrations below the limit of quantification (BLQ) will be assigned a value of zero. In case of a deviation from the theoretical time, the actual time of blood sample will be used in the calculation of the derived pharmacokinetic parameters.

The terminal elimination rate constant (λ_z) will be determined by plotting the concentration data versus time on a semi-logarithmic scale. The parameter will be estimated by linear least square regression analysis, using the last three (or more) non-zero concentrations. The upper and the lower time points, as well as the number of time points, used for λ_z estimation will be reported.

AUC_{0-t} will be calculated using the linear-up/log-down trapezoidal method.

The derived urine PK parameter Ae will be calculated from as: urine volume * urine cytisine concentration. Ae% will be derived as: 100*Ae/Dose.

Values of λ_z, $AUC_{0-\infty}$, %AUC and $t_{½}$ will not be reported for cases where λ_z cannot be reliably determined.

C_{max} (and T_{max}) will be calculated after the first dose and the last dose on Day 1, after the last dose on days 2, 3, 12, 16, 20, 24 and after the morning dose on Day 25. C_{max} and T_{max} values will be calculated as follows:

- On Day 1, C_{max} after the first daily dose will be estimated from venous blood samples collected 0.5h, 0.75h, 1h, 1.25h, 1.5h, and 2h after the first dose.
- On Day 1, Day 2, and Day 3 estimates of C_{max} after the last daily dose will be made from venous blood samples collected 0.5 h, 1h, 1.5h, 2h, 2.5h, 3h, 3.5h, 4h, and 5h following the last dose.
- On Day 12, 16, 20, and 24, C_{max} estimates will be made from venous blood samples collected 1h, 1.5h, 2h, 2.5h, 3h, 3.5h, and 4h after the last daily dose.
- On Day 25 C_{max} will be estimated from venous blood samples collected 0.5h, 0.75h, 1h, 1.25h, 1.5h, 1.75h, 2h, 2.25h, 2.5h, 2.75h, 3h, 3.25h, 3.5h, 3.75h, 4h, 5h, 6h, 8h, 10h, 12h, and 24h post-dose.

C_{min} will be derived from the pre-first dose plasma values on days 4, 13, 17, 21 and 25.

The $t_{½}$, λ_z, AUC_{0-t}, AUC_{0-inf} and $\%AUC_{extrap}$ values will be determined after the administration of the final dose of cytisine on Day 25. On Day 25 $t_{1/2}$ and AUC will be determined after the administration

of the final dose of cytisine from venous blood samples collected 0.5h, 0.75h, 1h, 1.25h, 1.5h, 1.75h, 2h, 2.25h, 2.5h, 2.75h, 3h, 3.25h, 3.5h, 3.75h, 4h, 5h, 6h. 8h, 10h, 12h, and 24h post-dose.

The derived urine parameters Ae and Ae% will be calculated from 0-24h on Day 1 and Day 25.

Derived pharmacokinetic parameters will be listed and summarised for each dose group and age group. The descriptive statistics presented will be N, n, arithmetic mean, arithmetic standard deviation (SD), coefficient of variation (CV%), minimum, median, maximum and geometric mean (with the exception of T_{max}).

PK parameter data listings will be presented using the Safety Set. PK data summaries will be presented using the PK Set and the Safety Set. All PK parameter data included in listings and summaries will be presented to three significant figures with the exception of the CV% which will be presented to one decimal place.

11.4.3.1 Statistical Analysis of PK Parameters

Dosing Schedule Comparison

The dosing schedule comparison analysis will be performed separately within each of the dose groups.

In order to perform comparisons between schedule changes within each dose group, following logarithmic transformation, C_{max} values (post-last dose on days 3, 12, 16, 20, 24 and 25), and C_{min} values (pre-first dose on days 4, 13, 17, 21 and 25) will be subjected to an analysis of variance (ANOVA) including a fixed effect for study day and a random effect of subject. Point estimates and 90% confidence intervals (CI) will be constructed for the contrasts between consecutive study days using the residual mean square error obtained from the ANOVA. The point and interval estimates will be back-transformed to give estimates of the ratios of the geometric least squares means (LSmean) and corresponding 90% CI. In addition, estimated geometric means will be presented. The analysis will be performed by age group (18-65 yrs, >65 yrs, all ages).

Dose Group Comparison

The dose group comparison will be a between-group analysis.

Following logarithmic transformation, C_{max}, AUC_{0-t} and AUC_{0-inf} values on Day 25 will be subjected to an analysis of variance (ANOVA) including a fixed effect for dose group. Point estimates and 90% confidence intervals (CI) will be constructed for the contrast between the 1.5 mg and 3.0 mg dose groups using the residual mean square error obtained from the ANOVA. The point and interval estimates will be back-transformed to give estimates of the ratios of the geometric least squares means (LSmean) and corresponding 90% CI. In addition, estimated geometric means will be presented. The analysis will be peformed by age group (18-65 yrs, >65 yrs, all ages).

11.5 PD ANALYSES

11.5.1 Number of Cigarettes Smoked and Expired CO

The number of cigarettes smoked in the past 24 hours will be recorded at Screening and daily from Day -1 to Post-Study. Expired air carbon monoxide (CO) data will be obtained at Screening and prior to discharge on days 4, 13, 17, 21 and 26.

The number of cigarettes smoked during the past 24 hours will be taken from the values recorded in the CRF (at CPU) for Screening and Day -1 to Day 3 and from the subject diaries thereafter. In order to evaluate any reduction in smoking, the number of cigarettes smoked daily will be assessed as well as smoking cessation status at Day 26. For each individual subject a smoking cessation status of 'Ceased smoking' will be defined as not having smoked any cigarettes for the past 24 hours on Day 26 and confirmed by an expired CO level <10ppm on Day 26. If these criteria are not satisfied, a smoking cessation status of 'Continued smoking' will be assigned to a subject, including subjects who have missing data at Day 26 for either the number of cigarettes smoked or expired CO level, or both.

The number of cigarettes smoked daily and smoking cessation status will be listed. Frequencies (n, %) of smoking cessation status at Day 26 will be calculated by dose group and age group. For those subjects who had ceased smoking by Day 26, frequencies of the day of first smoking cessation (defined as smoking 0 cigarettes and where cessation is sustained without relapse) will be presented by dose group and age group. Descriptive statistics (N, n, mean, SD, minimum, median and maximum) of absolute and change from baseline (Day -1) number of cigarettes smoked will be tabulated by dose group and age group. The mean absolute values and mean change from baseline will also be presented graphically. Individual subject plots of the number of cigarettes smoked daily will also be produced.

Expired CO levels will be included in the listing that presents the smoking cessation status data. Descriptive statistics (N, n, mean, SD, minimum, median and maximum) of absolute and change from baseline (Screening) expired CO results will be tabulated by dose group and age group. The mean absolute values and mean change from baseline values will also be presented graphically. Individual subject plots of expired CO levels will also be produced.

The summary tables described above for number of cigarettes smoked daily and expired CO levels will also be produced for two subgroups of subjects, defined by smoking cessation status ('Ceased smoking', 'Continued smoking'). The graphs described above of mean absolute values and mean change from baseline will also be presented by smoking cessation subgroup.

11.5.2 Urine Cotinine

Urine cotinine data will be obtained at Screening and prior to discharge on days 4, 13, 17, 21 and 26.

Urine cotinine levels will be included in the listing that presents the smoking cessation status data. Descriptive statistics (N, n, mean, SD, minimum, median and maximum) of absolute and change from baseline (Screening) urine cotinine levels will be tabulated by dose group and age group. The mean absolute values and mean change from baseline values will also be presented graphically. Individual subject plots of urine cotinine levels will also be produced.

11.5.3 Tobacco Craving Questionnaire

The Tobacco Craving Questionnaire – Short Form (TCQ-SF) will be completed on days -1, 4, 13, 17, 21 and 26.

The TCQ-SF is a 12-item questionnaire that assesses 4 components of tobacco craving: emotionality (3 items), expectancy (3 items), compulsivity (3 items) and purposefulness (3 items). Responses to each item are scored from 1 (strongly disagree) through 7 (strongly disagree). Component scores are defined

as the sum of the scores within each component. The total score is defined as the sum of the 4 component scores. If any item responses are missing then the relevant component score(s), as well as the total score, will not be calculated.

The TCQ-SF total score and component scores for emotionality, expectancy, compulsivity and purposefulness will be listed and descriptive statistics (N, n, mean, SD, minimum, median and maximum) of absolute and change from baseline (Day -1) results will be tabulated by dose group and age group. For the total score, the mean absolute values and mean change from baseline values will also be presented graphically.

11.6 SAFETY ANALYSES

11.6.1 Adverse Events

All AEs will be coded using the MedDRA dictionary using the version specified in the Data Management Plan (DMP).

All AEs, including those which occurred prior to the first dose of study drug, will be listed. Only treatment emergent adverse events (TEAEs), i.e., existing conditions that worsen or events that occur during the course of the study after administration of IMP, will be included within the summary tables. TEAEs will be summarised by dose group, age group and overall.

An overall summary of AEs will be produced including the number of TEAEs; the number and % of subjects reporting at least 1 TEAE, serious TEAE (where SAE is reported as 'Yes'), TEAE leading to withdrawal from study drug (Action recorded as 'Study Drug Discontinued'); the number and % of subjects reporting TEAEs by severity and relationship to study drug. A subject with multiple occurrences of any AE is counted only once at the maximum level of severity or the highest association to study drug.

The number of TEAEs and the number and % of subjects reporting at least one TEAE will be tabulated by system organ class (SOC) and preferred term (PT). A subject reporting multiple episodes of a particular AE will only contribute one count towards the corresponding SOC and preferred term. The number of TEAEs and the number and % of subjects reporting at least one TEAE will also be tabulated by preferred term (PT), with the PTs sorted in descending order of frequency. A subject reporting multiple episodes of a particular AE will only contribute one count towards the corresponding PT.

In addition, the number and % of subjects reporting TEAEs will be tabulated by maximum severity and strongest relationship to study drug. For the summary of TEAEs by severity, if a subject has multiple events occurring within the same SOC or preferred term the event with the highest severity will be counted. Similarly, for TEAEs by relationship to study drug, if a subject has multiple events occurring within the same SOC or PT, the event with the highest association to study drug will be counted.

Data will be listed by dose group and age group. For any adverse event taken prior to first administration of study drug, treatment will be described as 'Prior to Dosing'.

Where there are only partial dates/times recorded for adverse events, adverse events will be assigned to treatment unless it can be ruled out based on the partial information.

The derived variables, 'Time from Dose' and 'Duration' will be presented where full date and time are present. If partial dates are present for any parameter required in the calculation, then the variable will not be populated. The following will be used to calculate the variables:

Duration (dd:hh:mm): (Date/Time of Resolution-Date/Time of Onset) + 1 minute;

Time from Dose (dd:hh:mm): (Date/Time of Onset-Date/Time of Start of Dose).

The following will be presented in listing format within the data summaries:

Serious Adverse Events – If there are none present, the listing will be produced stating: 'No subjects experienced any serious adverse events'.

Adverse Events which Led to Withdrawal of Study Drug – If there are none present, the listing will be produced stating: 'No subjects experienced any adverse events that led to withdrawal of study drug'.

Adverse event data will be summarised and listed using the Safety Set.

11.6.2 Laboratory Data

Routine biochemistry and haematology tests will be carried out at Screening and prior to discharge on days 4, 13, 17, 21 and 26. Urinalysis will be performed at Screening and Day 26.

The laboratory parameters required for this study are listed in section 9.2.1.

Laboratory data listings will be presented in two ways:

- Out of range values - any values that fall outside of the normal/alert ranges (presented in listing format within the data summaries)
- All laboratory data (including physician's review (Normal, Abnormal-NCS, Abnormal-CS)) with any out of range values flagged (presented within the data listings).

Descriptive statistics (N, n, mean, SD, minimum, median and maximum) of absolute and change from baseline (Screening) biochemistry and haematology parameters at each time point up to and including Day 26 will be tabulated by dose group and age group.

Laboratory data will be listed and summarised using the Safety Set.

11.6.3 Vital Signs

Supine blood pressure, pulse rate and oral temperature will be taken as follows: Screening; Day -1; Days 1, 2 and 3 pre-first dose and 3h post-last dose; Days 4, 13, 17, 21, 25 pre-first dose; Day 12, 16, 20 and 24 3h post-last dose; Day 25 24h.

Vital signs parameters (supine systolic and diastolic blood pressure and pulse rate and oral temperature) will be listed with any out of normal range values (see Appendix 1) flagged (flag 'H' or 'L' appended to relevant result). Descriptive statistics (N, n, mean, SD, minimum, median and maximum) of absolute and change from baseline (Day 1, pre-first dose) at each time point up to and including Day 25, 24h will be tabulated by dose group and age group.

Vital signs data will be summarised and listed using the Safety Set.

11.6.4 Electrocardiogram

11.6.4.1 12-Lead ECG

12-lead ECGs will be performed in triplicate as follows: Screening; Day -1; Days 4, 13, 17 and 21 pre-first dose; Day 26.

12-lead ECG parameters (heart rate, PR interval, QRS duration, QT interval and QTcF interval) will be listed with any out of normal range values (see Appendix 16.1) flagged (flag 'H' or 'L' appended to relevant result). The ECG findings (i.e. parameter values as well as morphologic findings (rhythm, conduction, evidence of MI, ST segments, T waves etc.)) will also be assessed by a physician as either 'normal' or abnormal' with comments on abnormal results also presented.

Descriptive statistics (N, n, mean, SD, minimum, median and maximum) of absolute and change from baseline (Day -1) values at each time point up to and including Day 26, will be tabulated by dose group. The mean of the triplicate recordings at each time point will be calculated for use in the summary tables. Any ECG traces that are determined to contain a technical error/interference will not be included in the calculation of mean values. If an ECG recording is repeated, all 3 of the original triplicate recordings (or whatever number of ECGs are free of technical errors) and the repeat recording(s) will be included in the calculation of the mean, provided that the original and repeat recordings are within 5 minutes of each other. Otherwise, the mean will be derived separately for the original and repeat recordings and the last mean value prior to dosing will be used for the summary tables.

12-lead ECG data will be listed and summarised using the Safety Set.

11.6.4.2 Holter Monitoring

The ECG extraction time points will be from extraction windows which precede the PK blood draws. ECG parameters (heart rate, PR interval, QRS duration, QT interval and QTcF interval) will be extracted in triplicate from Holter recordings on Day 1 and Day 25 at 30 and 15 minutes prior to the first dose and at 0.5h, 1h, 2h, 3h, 4h, 6h, 8h, 12h and 24h post dose.

The extracted ECG parameters will be listed with any out of normal range values (see Appendix 1) flagged (flag 'H' or 'L' appended to relevant result). The ECG results will also be assessed as either 'normal' or abnormal' with comments on abnormal results also presented.

The mean of the triplicate recordings at each time point will be calculated for use in the summary tables and plots. Any ECG traces that are determined to contain a technical error will not be included in the calculation of the mean values. At each time point, the mean value will be derived from all ECGs (generally three) that are free of technical errors. Descriptive statistics (N, n, mean, SD, minimum, median and maximum) of absolute and change from baseline (see definition, Section 5) values on Day 1 and Day 25 at each time point up to 24h, will be tabulated for each dose group and age group. The change from baseline ECG results will also be plotted over time by dose group and age group.

In addition, frequencies of QTcF data will be calculated according to the following categories:

For absolute values:

- QTcF > 450 mSec
- QTcF > 480 mSec
- QTcF > 500 mSec

For change from baseline:

- QTcF increase > 30 mSec
- QTcF increase > 60 mSec.

The presence of outliers will be explored for heart rate, PR interval, QRS duration, QT interval and QTcF interval). The outlier analysis will use the time-averaged baseline (see definition, Section 5) and compare that baseline value to each of the individual on-treatment ECG time point values. Outliers are defined as follows for this analysis:

- Heart rate: A value for a subject will be considered to be an outlier at a pre-defined post dose time point if the heart rate measurement at that time point is <50 bpm and the measure is at least a 25% decrease from the subject's baseline mean heart rate (i.e., a bradycardic event) or if the heart rate measurement at the pre-defined post dose time point is >100 bpm and the measure is at least a 25% increase from the baseline mean heart rate (i.e., a tachycardic event).

- PR interval: A value for a subject will be considered to be an outlier at a pre-defined post dose time point if the PR interval at that pre-defined post dose time point is >200 mSec and it is at least a 25% increase from the subject's baseline mean PR interval.

- QRS duration: A value for a subject will be considered to be an outlier at a pre-defined post dose time point if the QRS duration at that pre-defined post dose time point is >100 mSec and it is at least a 25% increase from the subject's baseline mean QRS duration.

- QT interval: A value for a subject will be considered to be an outlier at a pre-defined post dose time point if the QT interval at that pre-defined post dose time point is >500 mSec and the subject's baseline mean QT interval is ≤500 mSec.

- QTcF interval: A value for a subject will be considered to be an outlier at a pre-defined post dose time point if the QTcF interval at that pre-defined post dose time point is >500 mSec and the subject's baseline mean QTcF interval is ≤500 mSec. Outlier values will also be presented if the QTcF interval at a pre-defined post dose time point is >480 mSec when the subject's baseline mean QTcF interval is ≤480 mSec and when a pre-defined post dose time point is >450 mSec when the subject's baseline mean QTcF interval is ≤450 mSec. Subjects will be included on a 'worst case' basis, i.e. if a subject has a baseline ≤450 mSec and has a post dose value of >500 mSec, the subject will be counted in the >500 mSec category only.

The number of events and number (%) of subjects with at least one outlier will be summarized on Day 1 and Day 25 by dose group and age group for each ECG parameter. For heart rate, separate summaries will be provided for bradycardic events (i.e., decreases) and tachycardic events (i.e. increases).

Holter ECG data will be listed for the Safety Set and summarised using the ECG Set.

11.7 PK/PD

In order to explore the effects of cytisine on QTc prolongation, individual cytisine concentrations in plasma and individual (mean of triplicate recordings) absolute and change from baseline Holter QTcF values will be presented together in a listing. A scatter plot will be produced with the individual change from baseline Holter QTcF values at the extraction time points 0.5h, 1h, 2h, 3h, 4h, 6h, 8h, 12h and 24h plotted against the corresponding cytisine concentrations. Both dose groups will be presented on the same plot along with their correlation coefficients with plots for each age group (18-65, >65, all ages) presented separately.

Holter ECG versus plasma concentration data will be listed for the Safety Set and plotted for subjects in the ECG-PK Set.

11.8 OTHER

11.8.1 Prior and Concomitant Medications

All medications will be coded using the WHO Drug Dictionary (version as specified in the DMP) and listed using the ATC Level 4 class, Preferred Term and verbatim text. A medication will be regarded as '*prior*' if it stops prior to dosing, '*prior and ongoing*' if it starts prior to dosing and continues after dosing and '*concomitant*' if it starts after dosing. Frequencies of prior medications and concomitant medications within an ATC Level 4 class and Preferred Term will be presented by dose group, age group and overall. Medications regarded as '*prior and ongoing*' will be assigned to treatment and included within the summary of concomitant medications.

11.8.2 Extent of Exposure

Exposure will be summarized by dose group and age group using counts and percentages for categorical variables and descriptive statistics (N, n, mean, SD, minimum, median and maximum) for continuous variables. The following will be calculated for each subject and summarized by dose group, age group and overall: duration of exposure to cytisine (days), number of cytisine doses administered, total dose of cytisine (mg) administered, percentage of planned dose of cytisine administered. One-hundred (100) doses of cytisine are to be administered over 25 days. The total planned dose of cytisine is 100 x 1.5 mg in the 1.5 mg dose group and 100 x 3.0 mg in the 3.0 mg dose group. Duration of exposure to cytisine will be summarized both as a categorical variable and as a continuous variable.

11.8.3 All Other Data

All data will be listed, including the following: Tobacco and Alcohol Use, Visit Dates, Medical History and Concurrent Conditions, Menstrual and Obstetric History, Pregnancy Test Results, Eligibility Check, Physical Examination, Inclusion/Exclusion Criteria Failures, Virology Results, Alcohol and Drugs of Abuse Results, Dose Administration, Urine Volumes, Additional Notes, Holter Monitoring Information and Daily Smoking and Study Medication Diary, Drug Accountability, Fagerström Test for Nicotine Dependence Questionnaire.

Derivations within listings:

PK Blood Sampling Time Deviations: Calculate sample time deviation as: actual time – theoretical time, display in minutes.

Analysis Sets: Detail whether subject should be included in each of the analysis sets along with corresponding comments if not included.

Inclusion/Exclusion Criteria: Only failures to be recorded. If no failures display '*All subjects passed inclusion/exclusion criteria*'.

Protocol Deviations: Major/minor classification to be assigned and confirmed by Sponsor.

Fagerström Test for Nicotine Dependence Questionnaire: The FTND responses will be assigned numerical values as follows:

- A question with a yes/no response: '*No*' = 0; '*Yes*' = 1.
- How soon after you wake up do you smoke your first cigarette: '*After 60 minutes*' = 0; '*31 – 60 minutes*' = 1; '*6 – 30 minutes*' = 2; '*Within 5 minutes*' = 3.
- Which cigarette would you hate most to give up: '*All others*' = 0; '*The first one in the morning*' = 1.
- How many cigarettes in a day do you smoke: '*10 or less*' = 0; '*11 – 20*' = 1; '*21 – 30* '= 2; '*31 or more*' = 3.

The FTND total score is defined as the sum of the scores from all 6 questions, provided all 6 questions have been completed. If any of the questions have a missing response, the total score will not be calculated.

12 VALIDATION

All tables, figures and listings will be subject to independent quality control and visual review. Unique tables will be independently programmed. Findings will be documented in an Output Summary file quality control form and actions taken will also be documented.

The study summary sheet of the Output Summary file will be completed and signed by all persons who performed QC. The final version of the Output Summary, including the signed summary sheet, will be printed and stored in the Data Management File. The study summary sheet of the Output Summary file will be completed and signed by all persons who performed QC. The final version of the Output Summary, including the signed summary sheet, will be printed and stored in the Data Management File (DMF).

13 LITERATURE CITATIONS/REFERENCES

None.

14 LIST OF TABLES, FIGURES AND LISTINGS

List of Tables and Figures Contained in Report Section 14

14.1 Disposition and Demographic Data

14.1.1 Disposition Data

14.1.2 Demographic Data

14.1.3 Smoking History

14.2 Efficacy Data

Not Applicable.

14.3 Safety Data

14.3.1 Adverse Events

14.3.2 Laboratory Safety

Table 14.3.2.1	Biochemistry Out of Range Data	Safety Set
Table 14.3.2.2	Haematology Out of Range Data	Safety Set
Table 14.3.2.3	Urinalysis Out of Range Data	Safety Set
Table 14.3.2.4	Summary of Absolute and Change from Baseline Biochemistry Data	Safety Set
Table 14.3.2.5	Summary of Absolute and Change from Baseline Haematology Data	Safety Set

14.3.3 Vital Signs

Table 14.3.3.1	Summary of Absolute and Change from Baseline Vital Signs Data	Safety Set

14.3.4 ECG

Table 14.3.4.1	Summary of Absolute and Change from Baseline 12-lead ECG Data	Safety Set
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Table 14.3.4.3	Summary of Holter ECG QTcF Categories	ECG Set
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Figure 14.3.4.1	Mean Change from Baseline Holter ECG Data	ECG Set
Figure 14.3.4.2	Individual Change from Baseline Holter ECG QTcF versus Plasma Cytisine Concentration Data	ECG-PK Set

14.4 Pharmacokinetics

14.4.1 Concentration-Time Data

Table 14.4.1.1	Summary of Plasma Cytisine Concentration Data	PK Set
Table 14.4.1.2	Summary of Plasma Cytisine Concentration Data	Safety Set
Table 14.4.1.3	Summary of Urine Cytisine Concentration Data	PK Set
Table 14.4.1.4	Summary of Urine Cytisine Concentration Data	Safety Set
Figure 14.4.1.1	Mean Plasma Cytisine Concentration-Time Curves - Linear Scale	PK Set
Figure 14.4.1.2	Mean Plasma Cytisine Concentration-Time Curves - Linear Scale	Safety Set
Figure 14.4.1.3	Mean Plasma Cytisine Concentration-Time Curves - Semi-Logarithmic Scale	PK Set

Figure 14.4.1.4	Mean Plasma Cytisine Concentration-Time Curves - Semi-Logarithmic Scale– Non-Elderly Subjects	Safety Set

14.4.2 Derived Pharmacokinetic Data

Table 14.4.2.1	Summary of Derived Cytisine Pharmacokinetic Parameters	PK Set
Table 14.4.2.2	Summary of Derived Cytisine Pharmacokinetic Parameters	Safety Set
Table 14.4.2.3	Summary of Statistical Analysis of Cytisine C_{max} and C_{min} – Dose Schedule Comparison	PK Set
Table 14.4.2.4	Summary of Statistical Analysis of Cytisine C_{max} and C_{min} – Dose Schedule Comparison	Safety Set
Table 14.4.2.5	Summary of Statistical Analysis of Day 25 Cytisine C_{max} and AUC – Dose Group Comparison	PK Set
Table 14.4.2.6	Summary of Statistical Analysis of Day 25 Cytisine C_{max} and AUC – Dose Group Comparison	Safety Set

14.5 Pharmacodynamics

14.5.1 Pharmacodynamic Data

Table 14.5.1.1	Summary of Absolute and Change from Baseline Daily Cigarette Consumption	PD Set
Table 14.5.1.2	Summary of Smoking Cessation Status	PD Set
Table 14.5.1.3	Summary of Absolute and Change from Baseline Daily Cigarette Consumption by Smoking Cessation Status	PD Set
Table 14.5.1.4	Summary of First Day of Smoking Cessation for Subjects Who Ceased Smoking by Day 26	PD Set
Table 14.5.1.5	Summary of Absolute and Change from Baseline Expired Air Carbon Monoxide (CO) Data	PD Set
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Subject Data: Listings Contained in Report Appendix 16.2

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Not applicable.

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Figure 16.2.13.3	Individual Urine Cotinine Data	Safety Set

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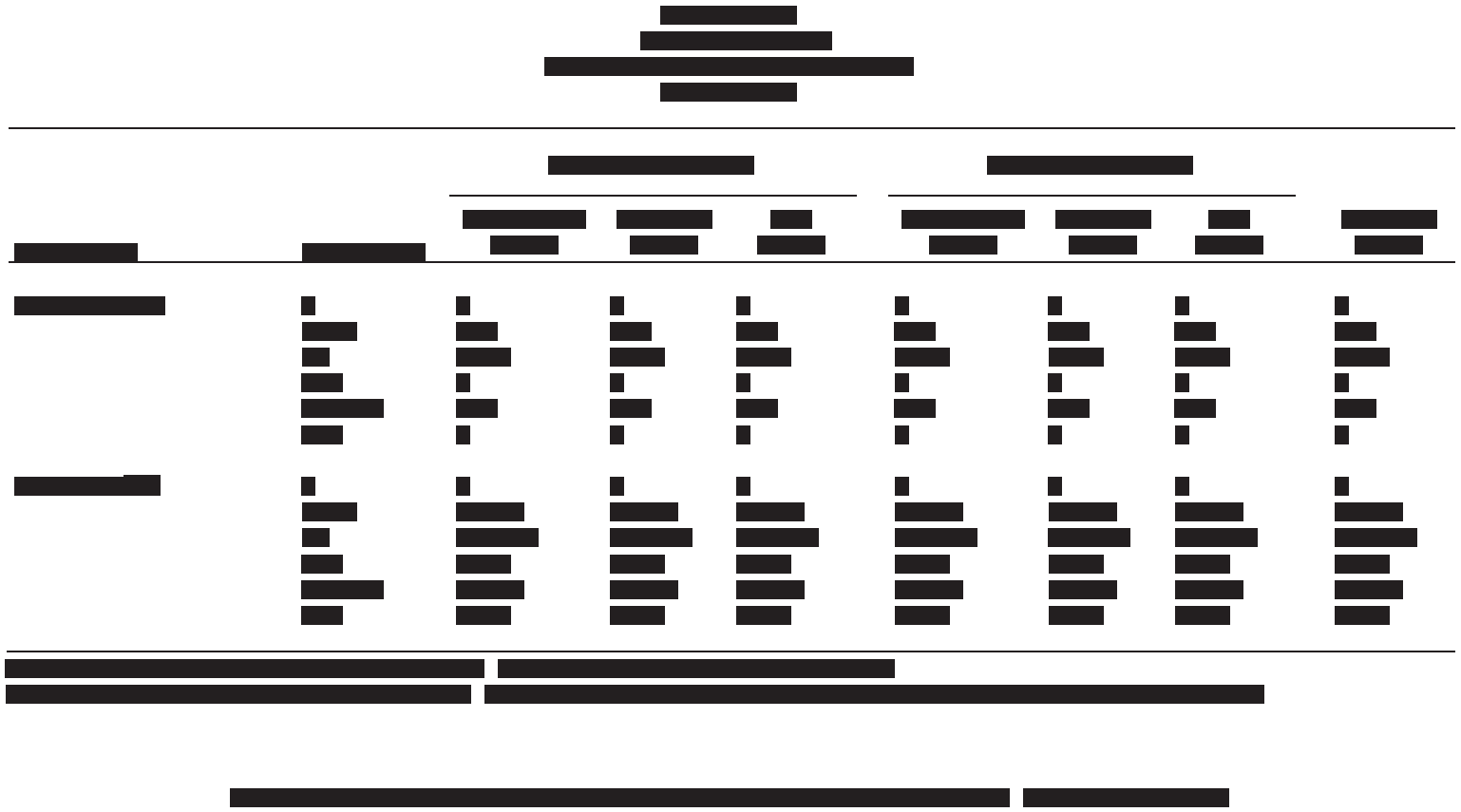

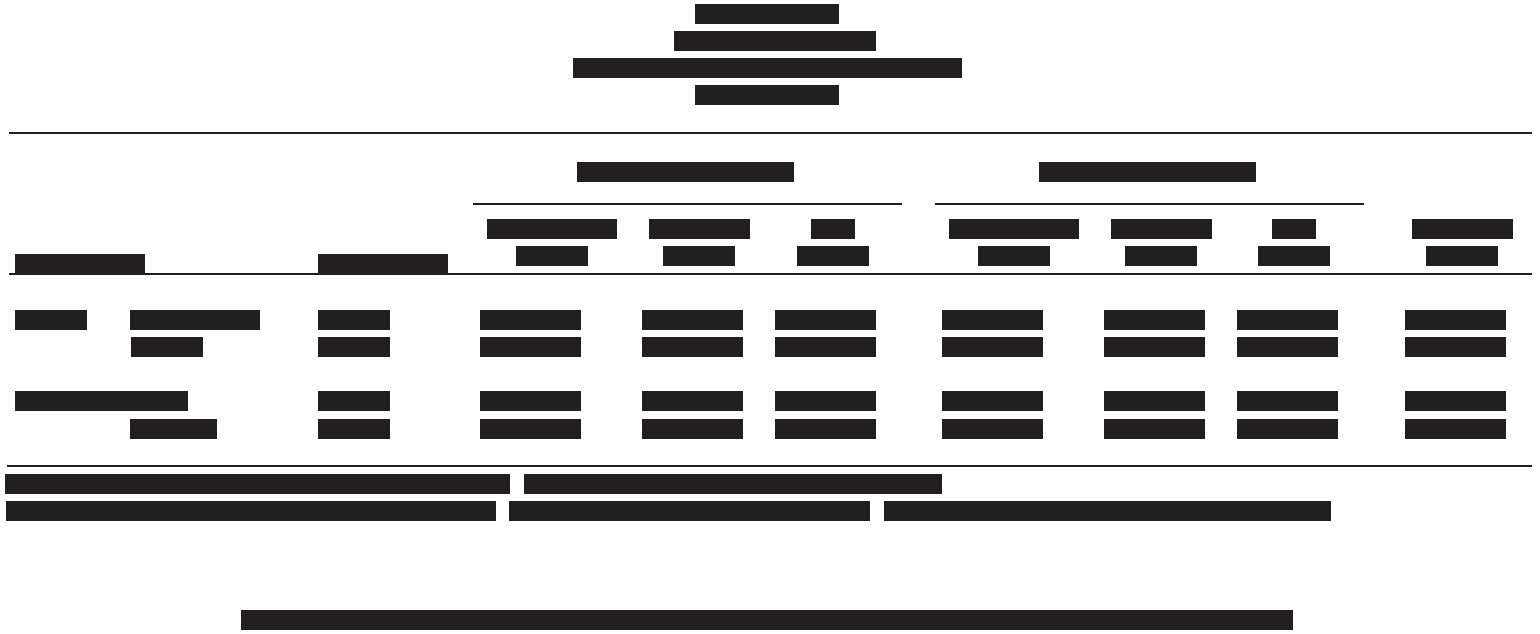

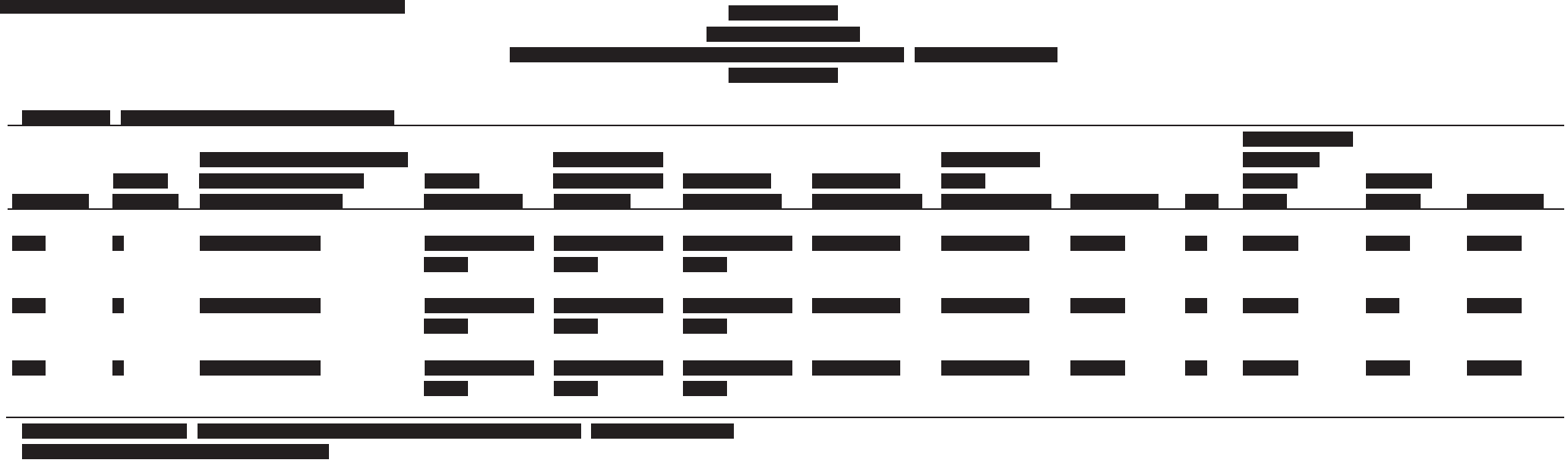

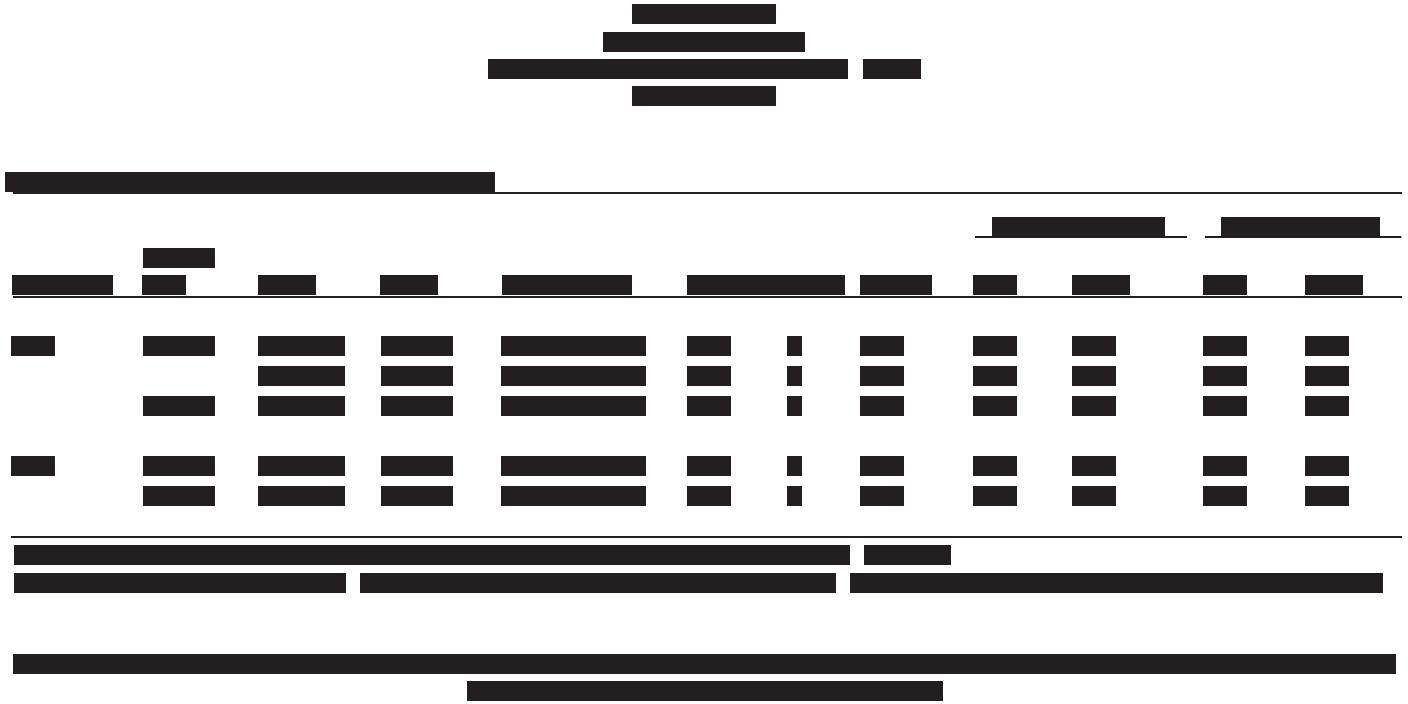

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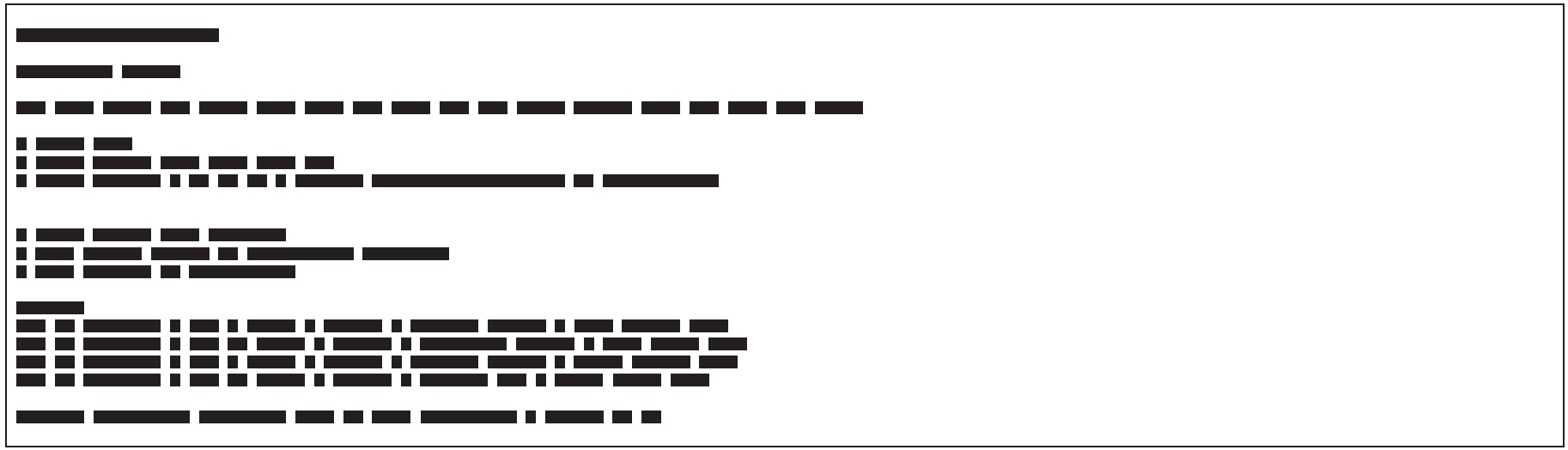

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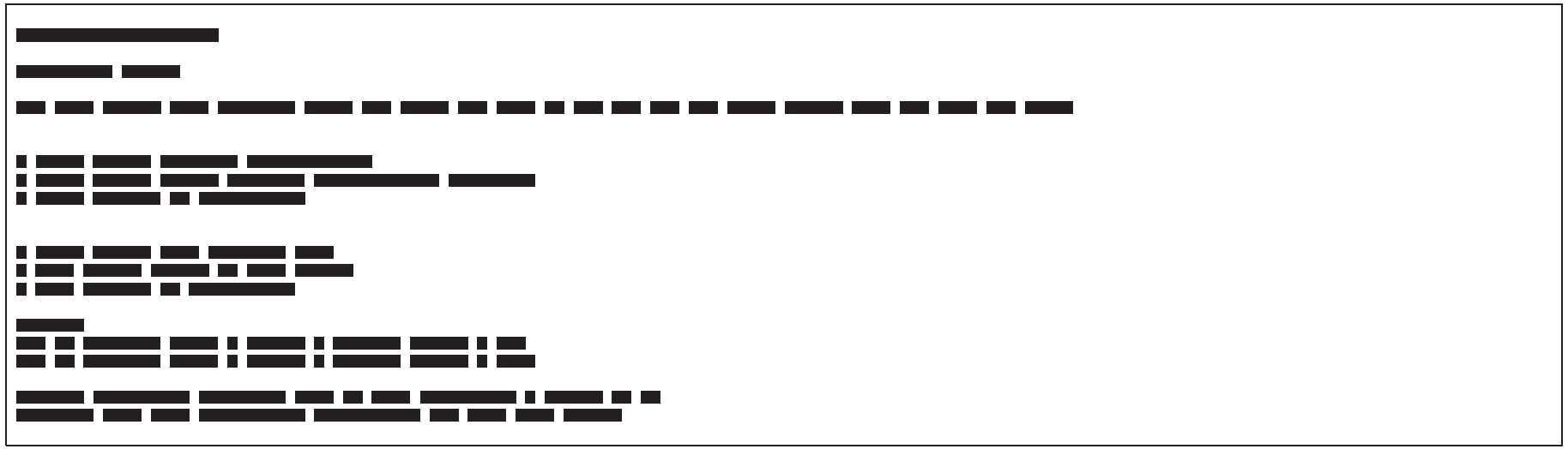

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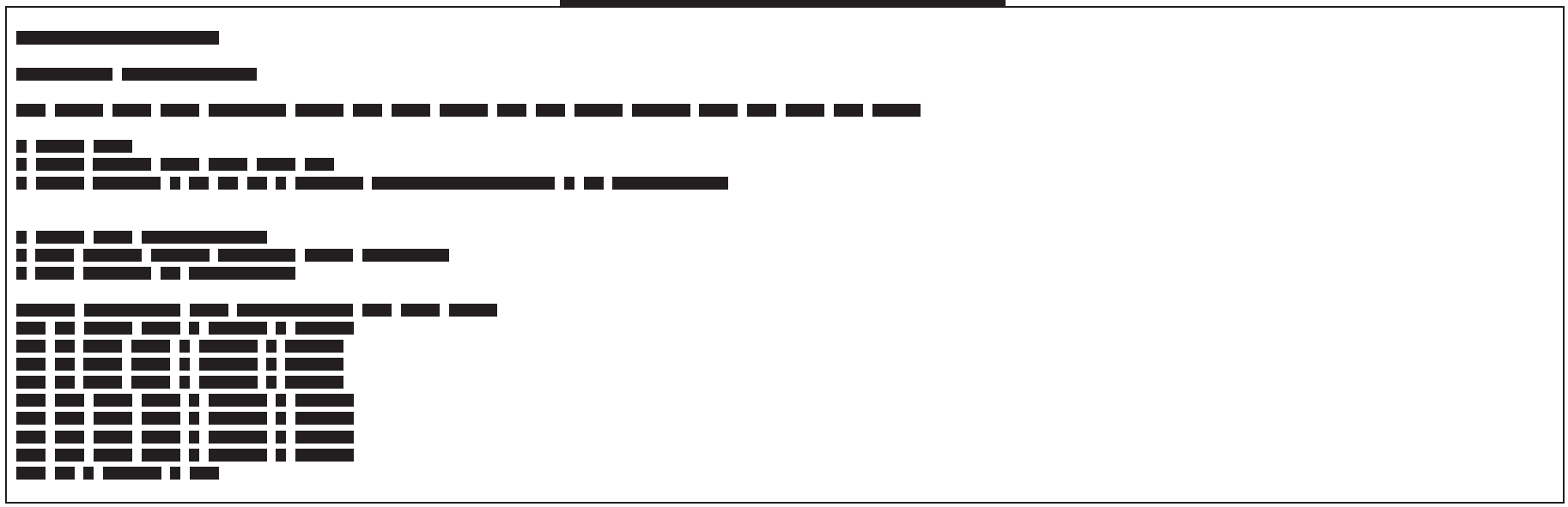

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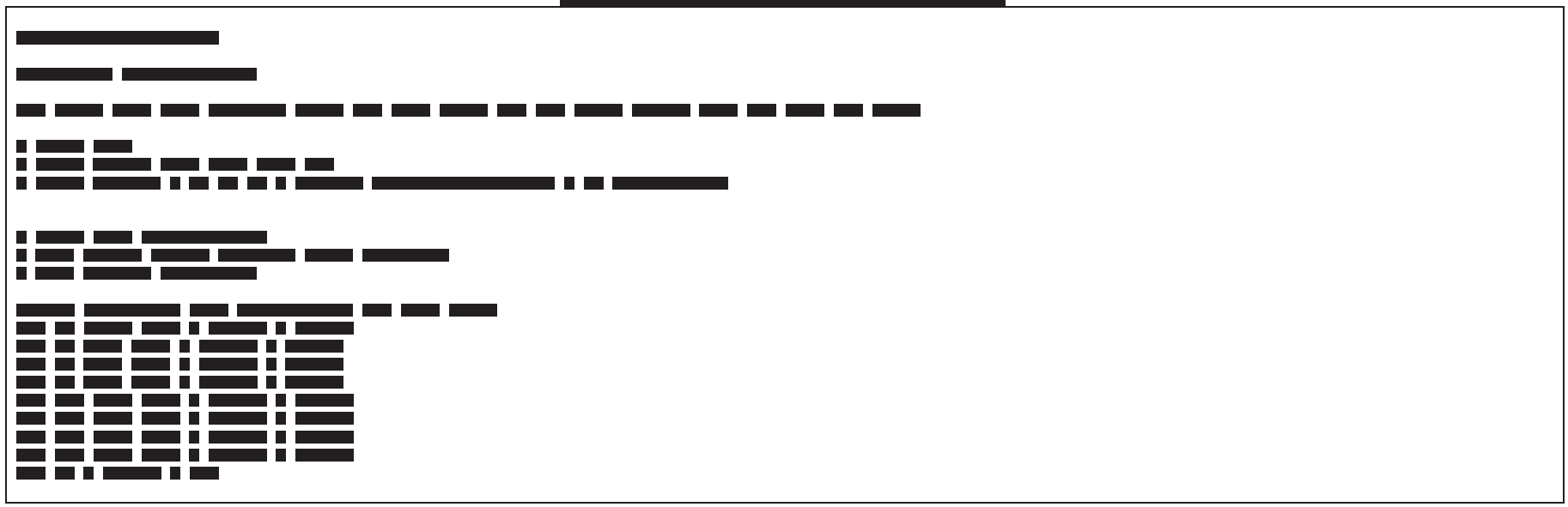

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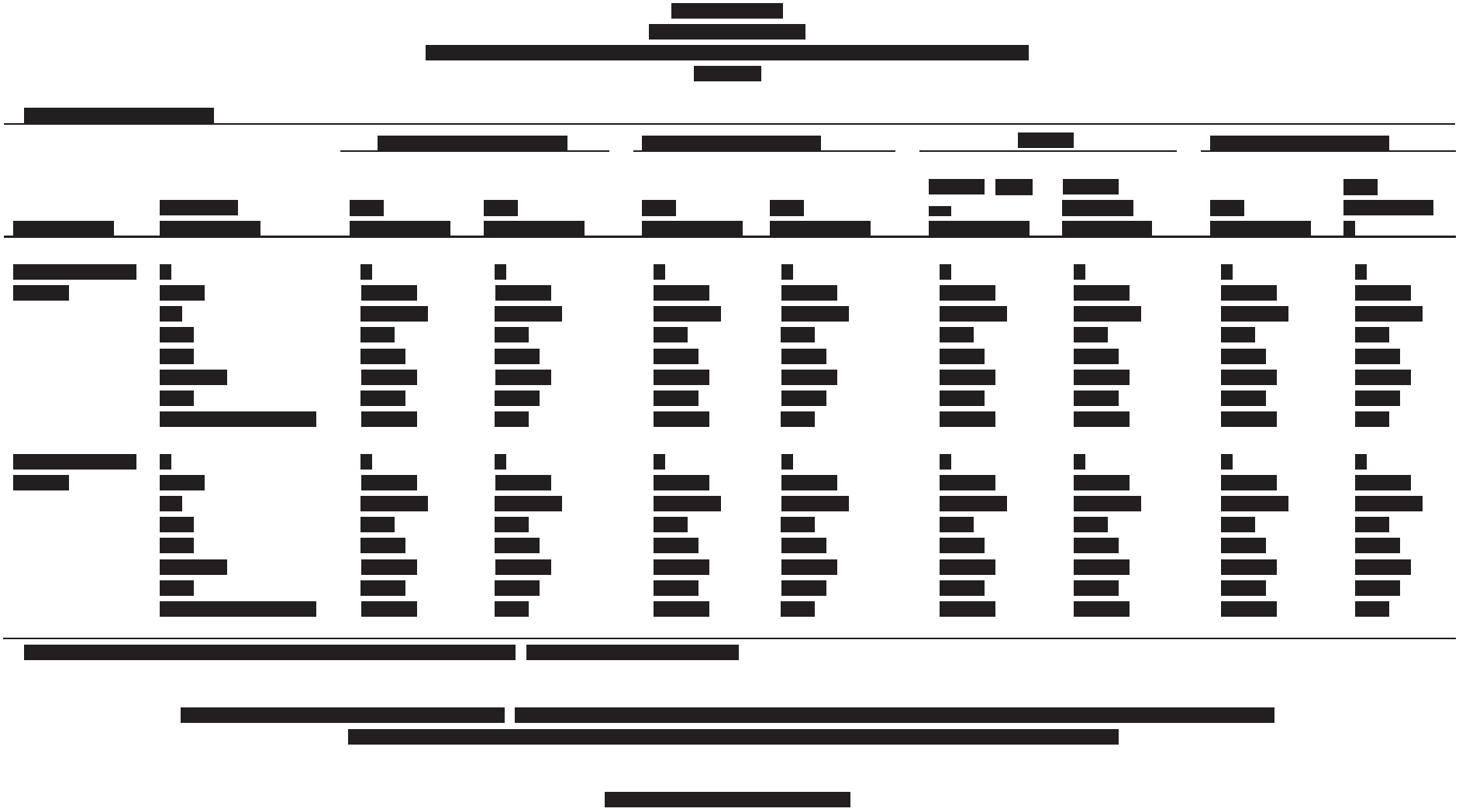

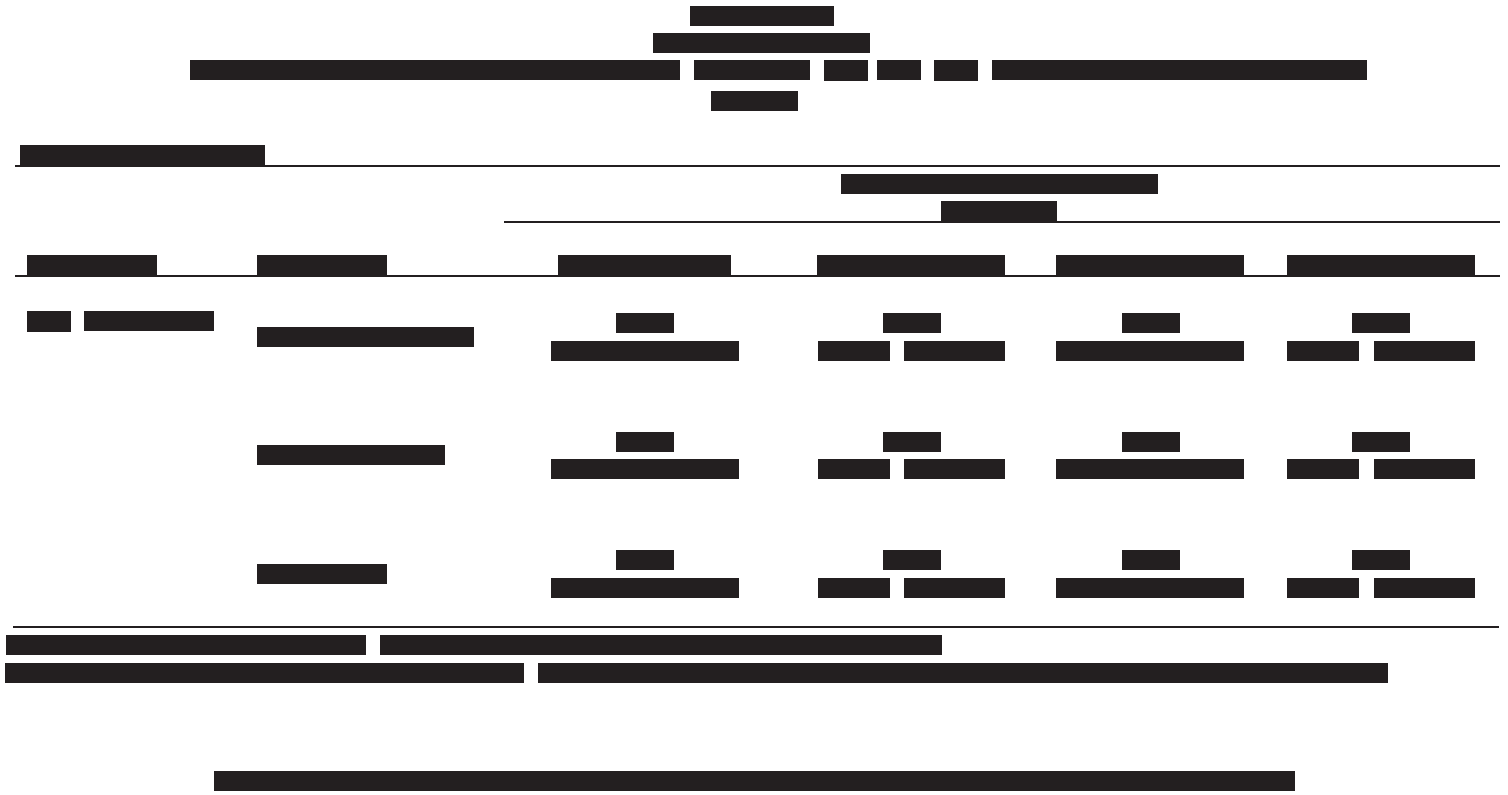

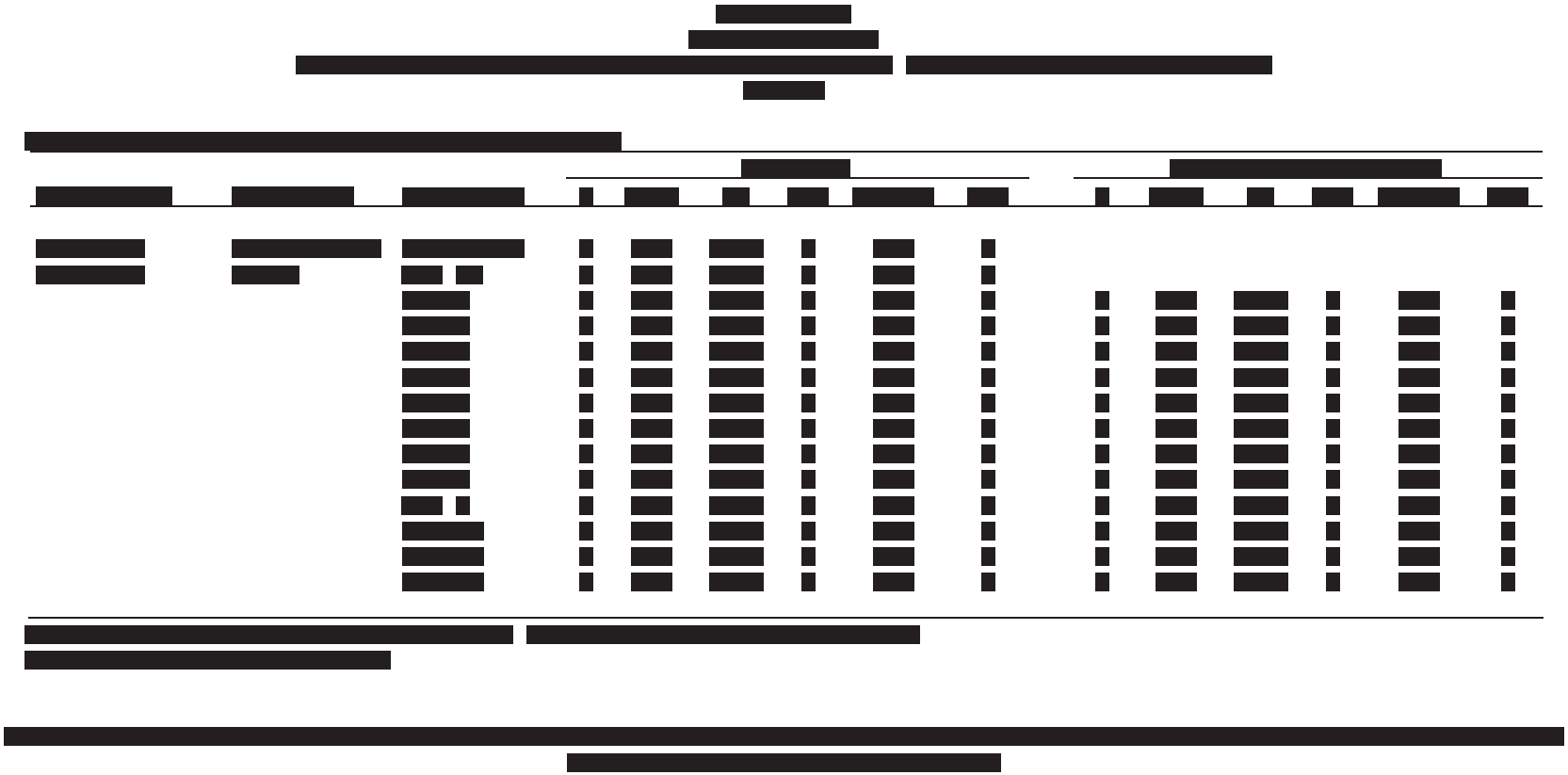

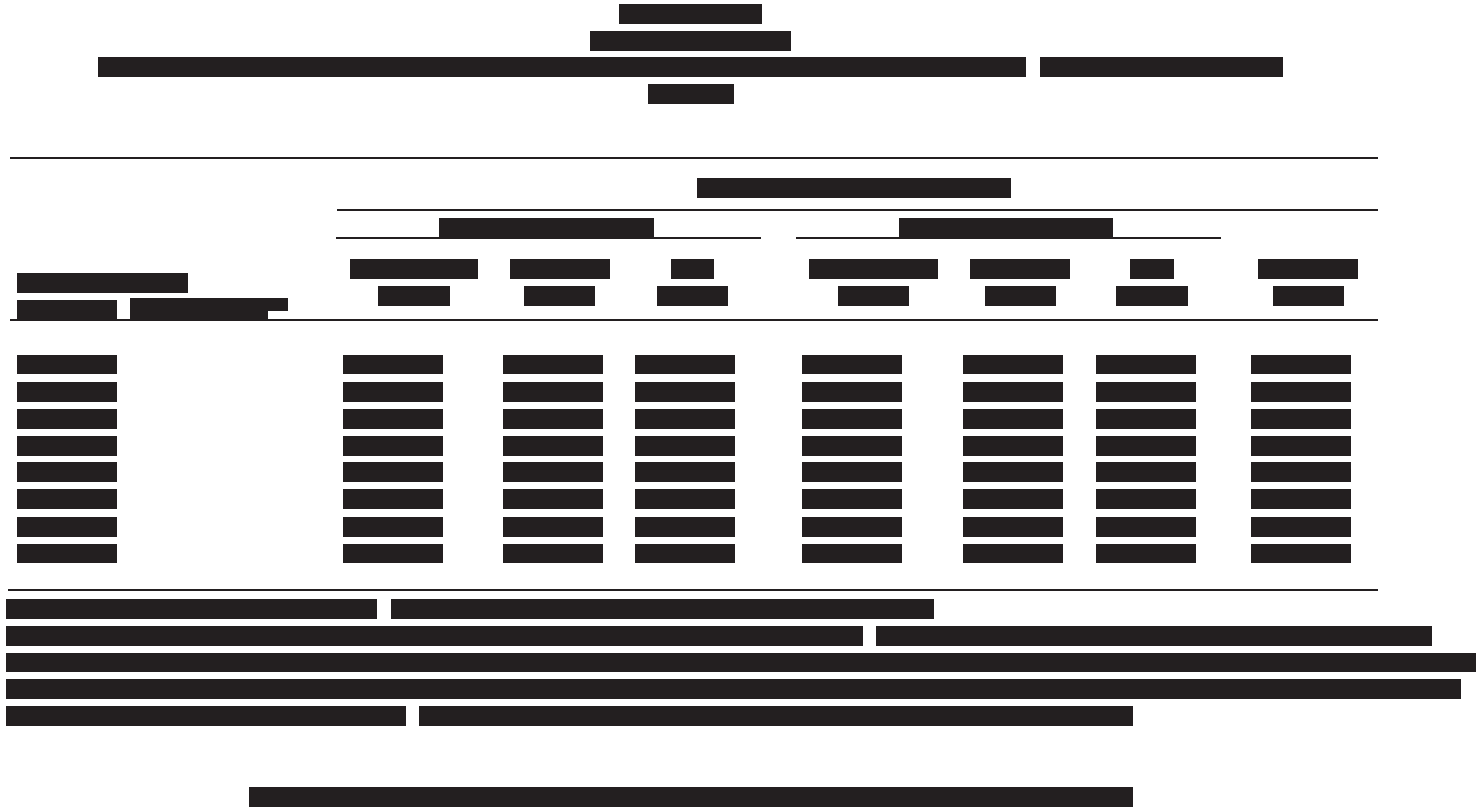

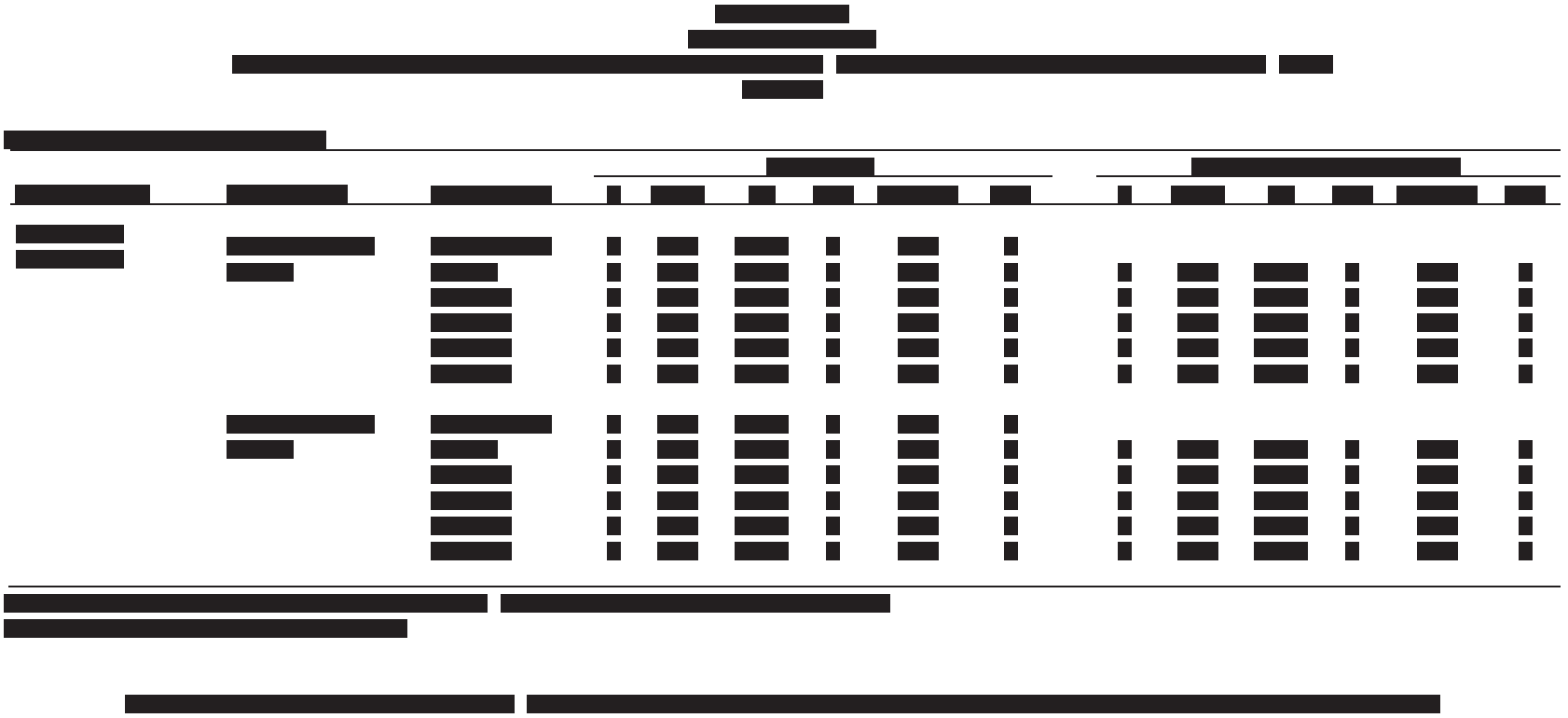

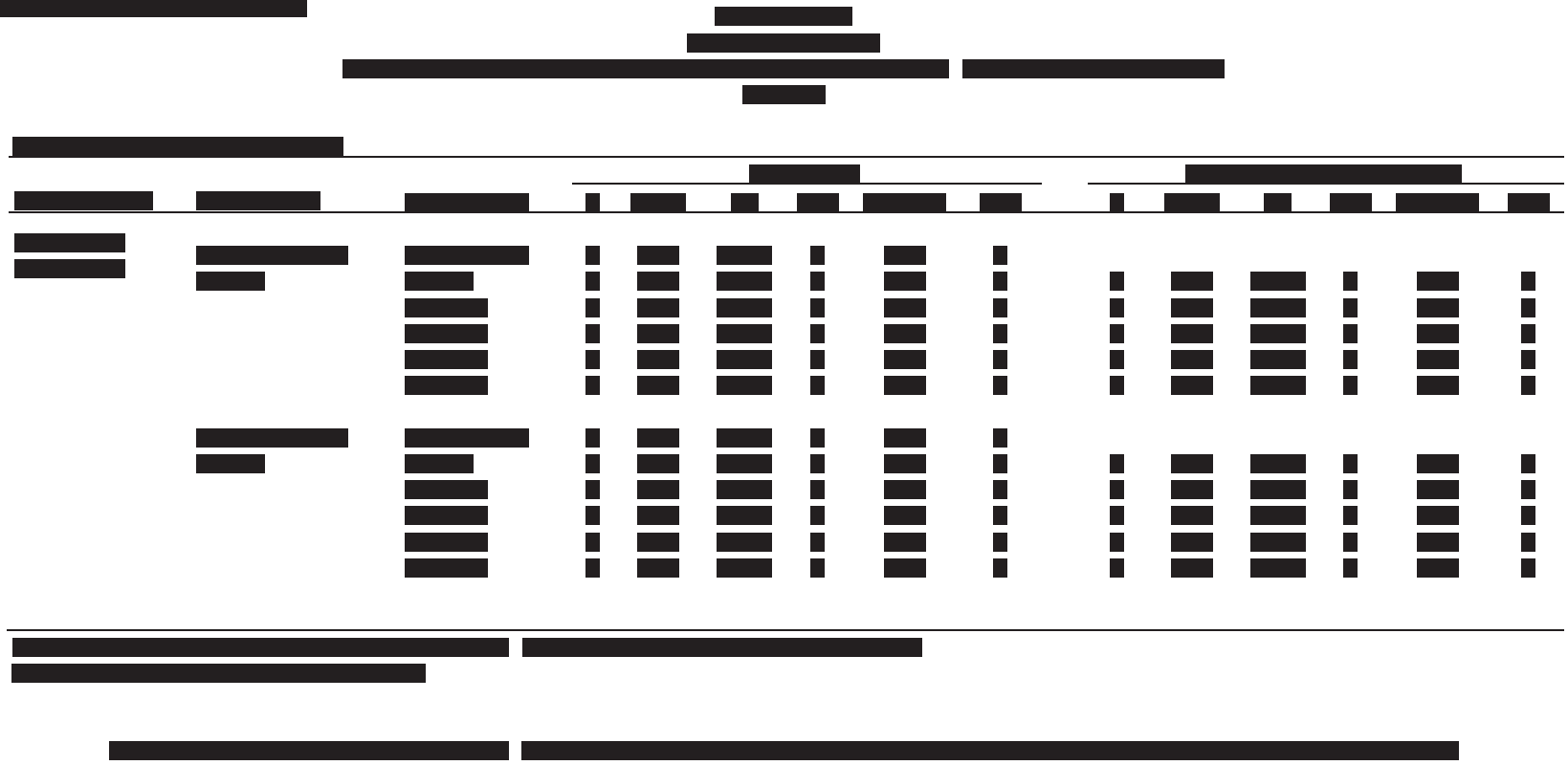

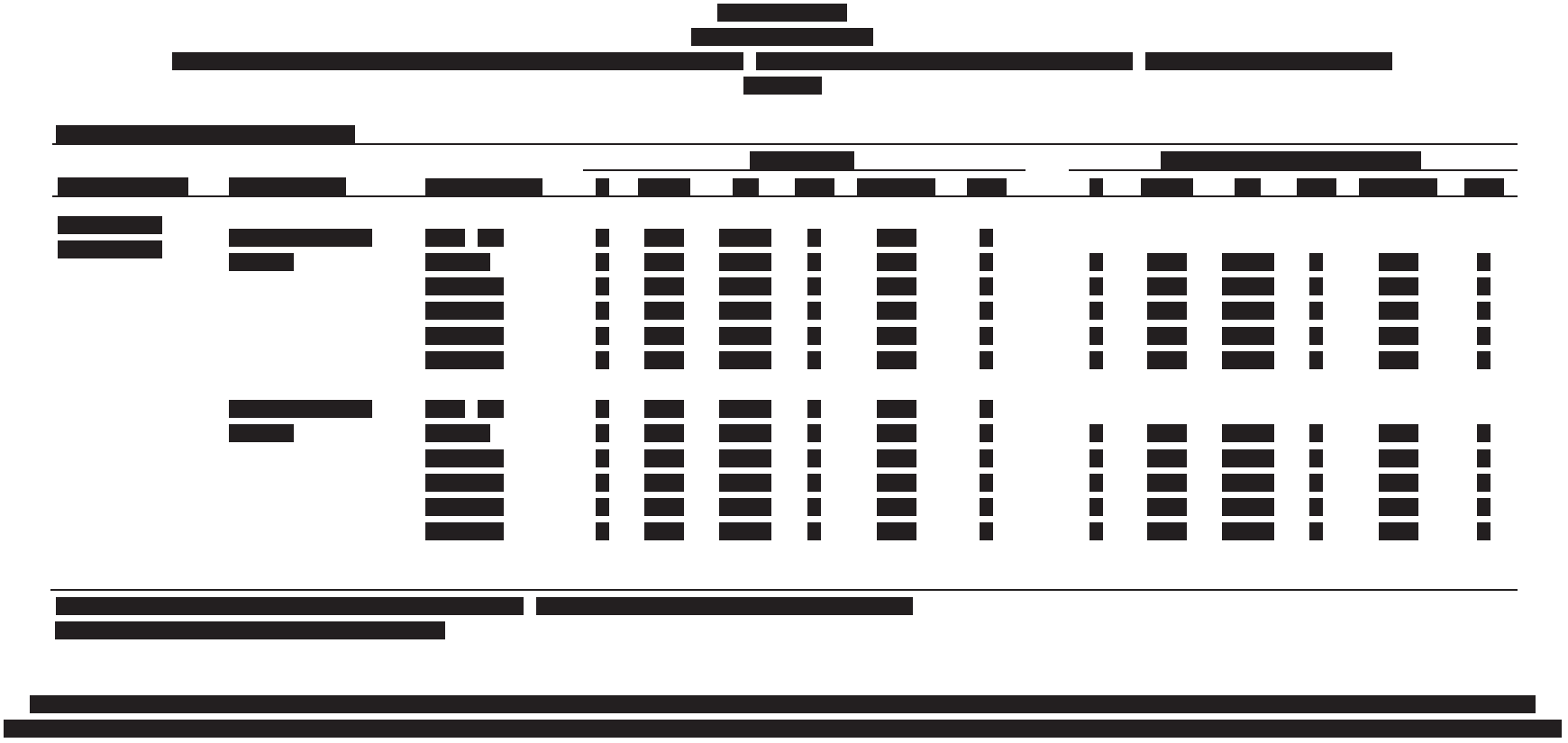

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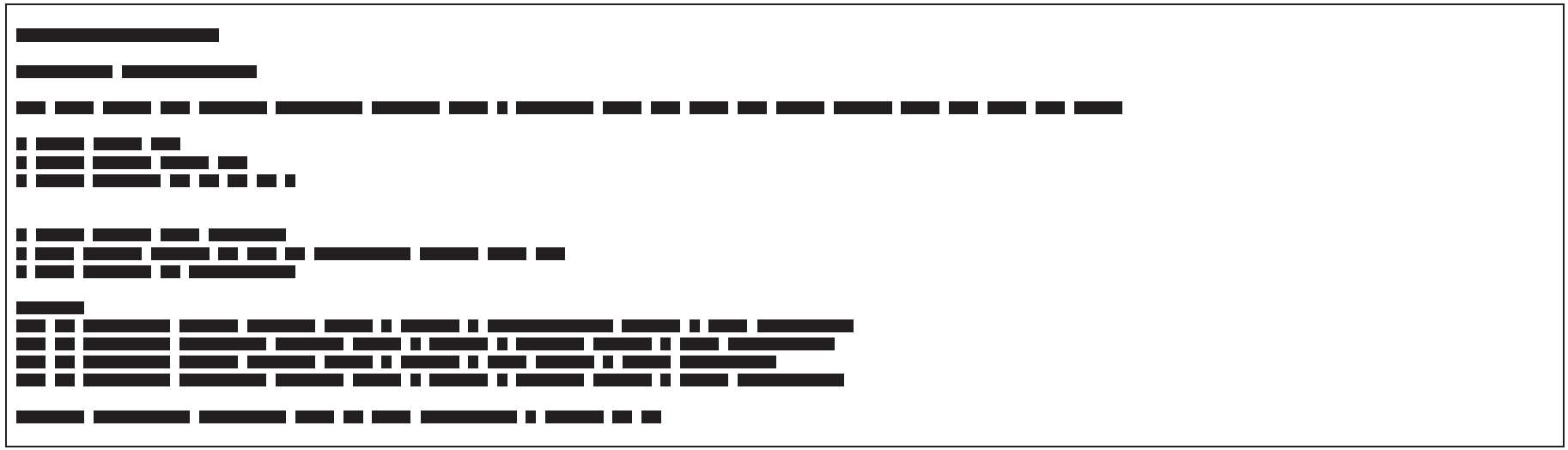

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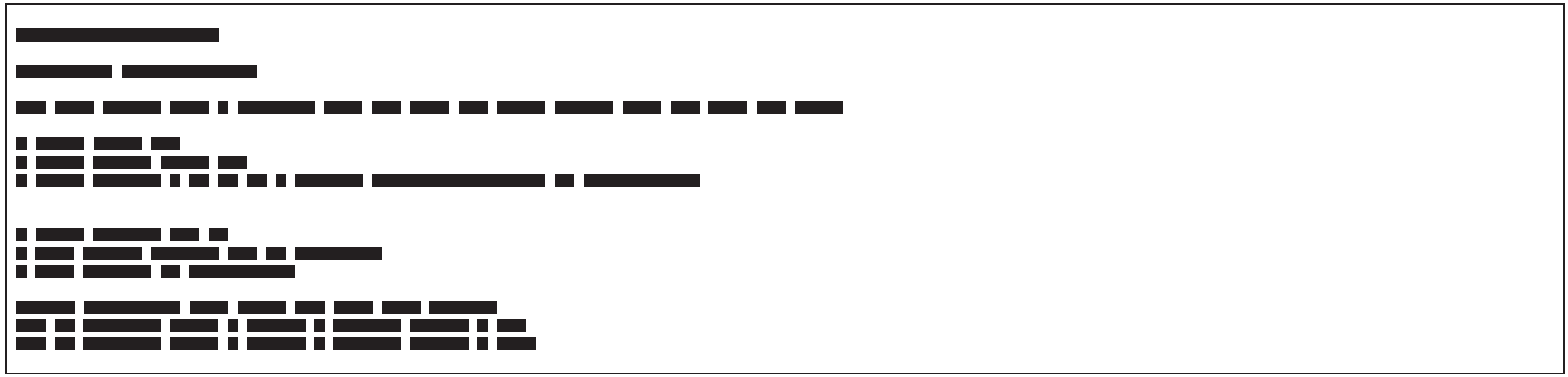

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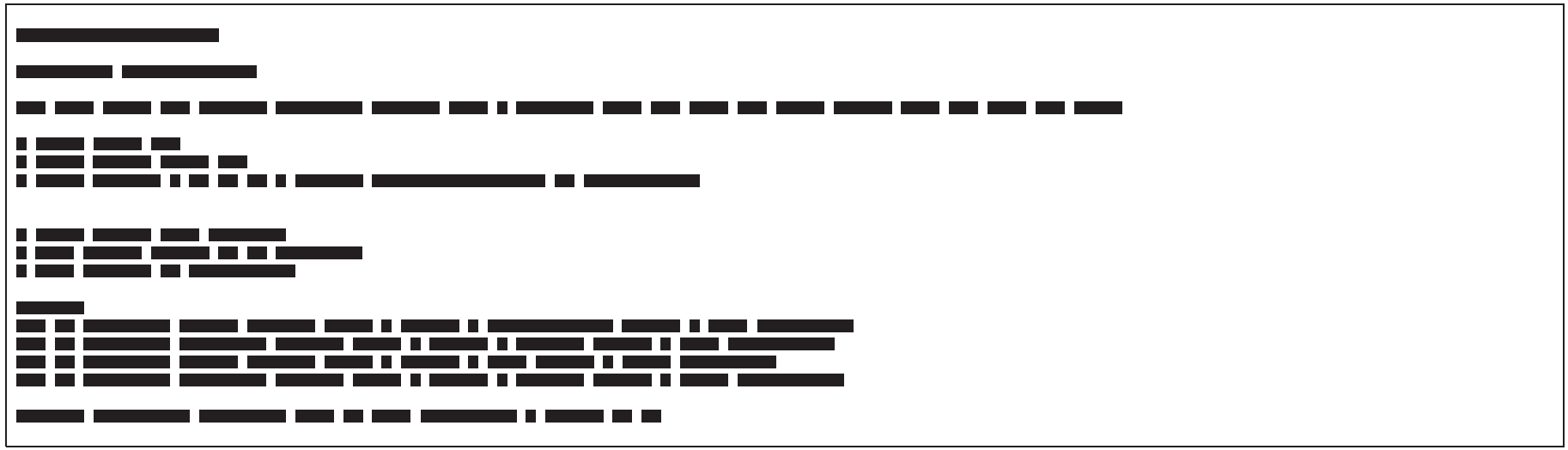

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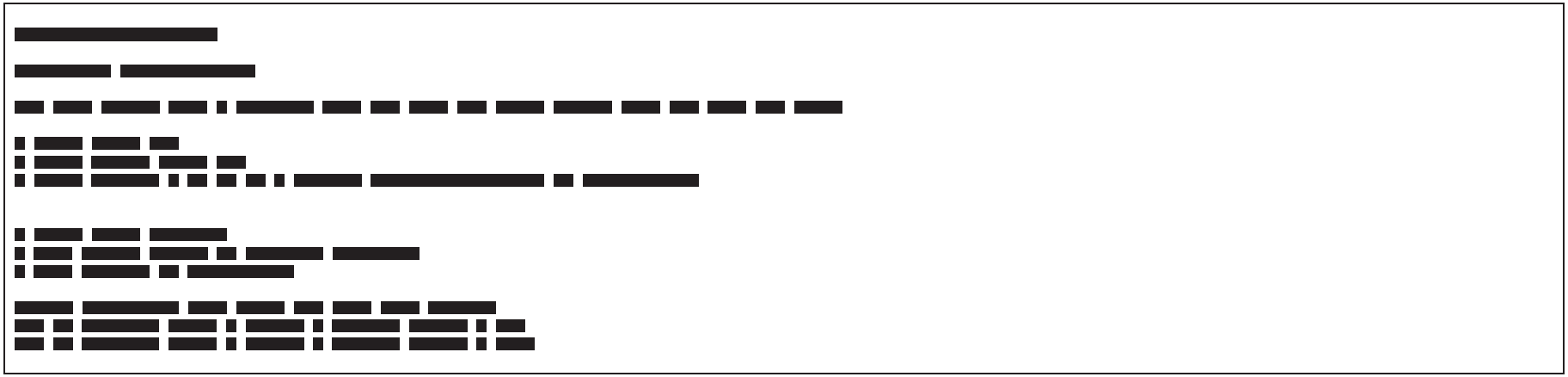

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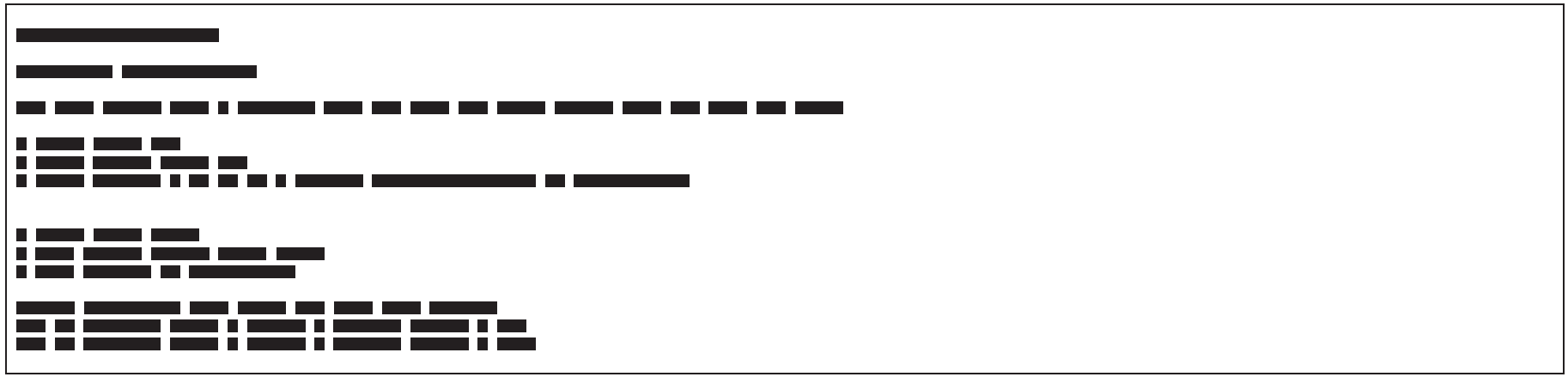

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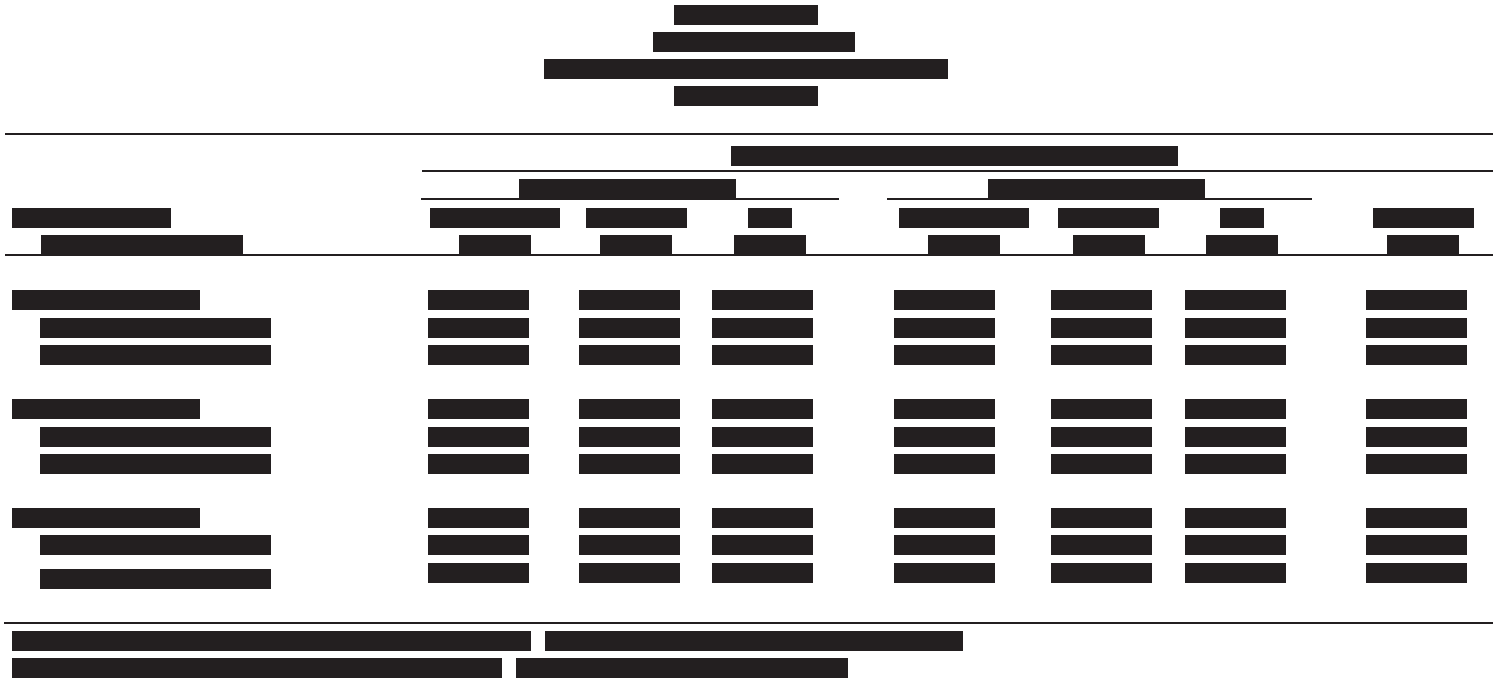

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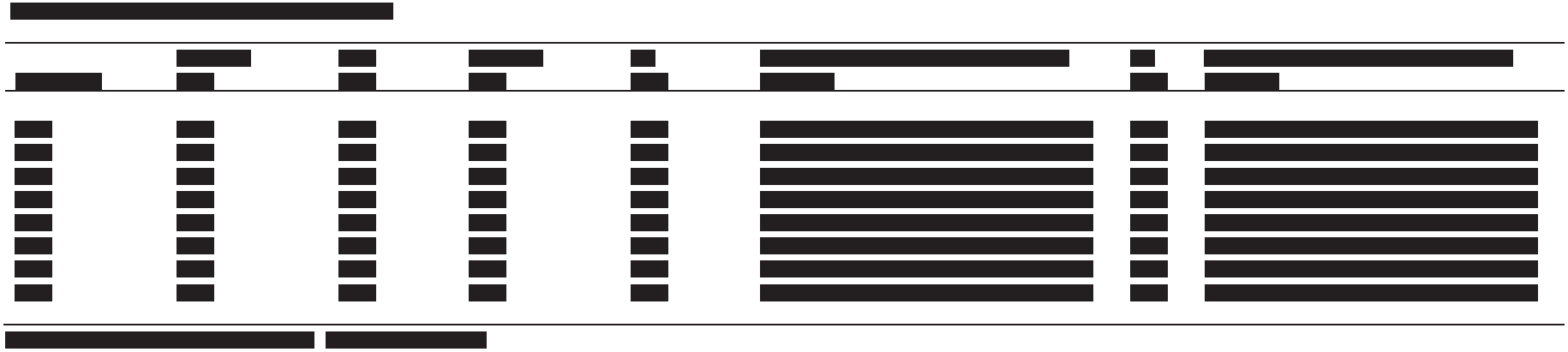

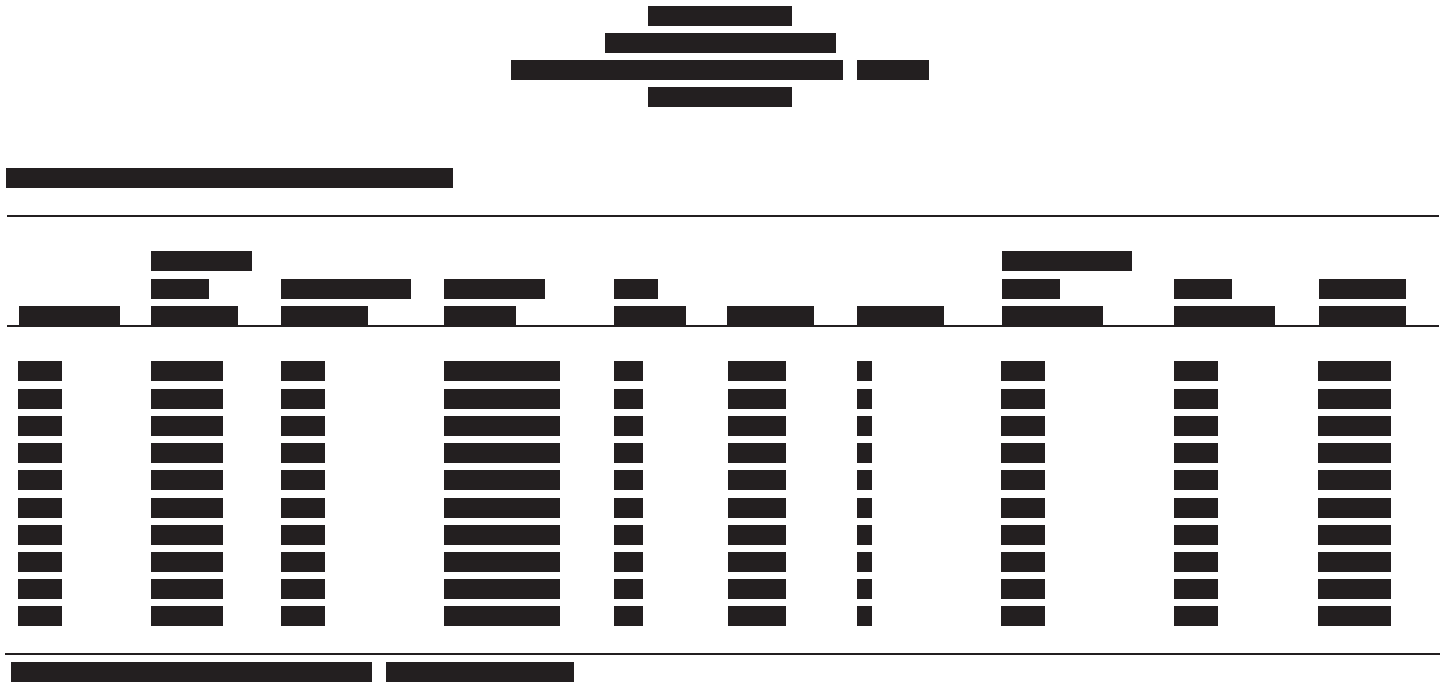

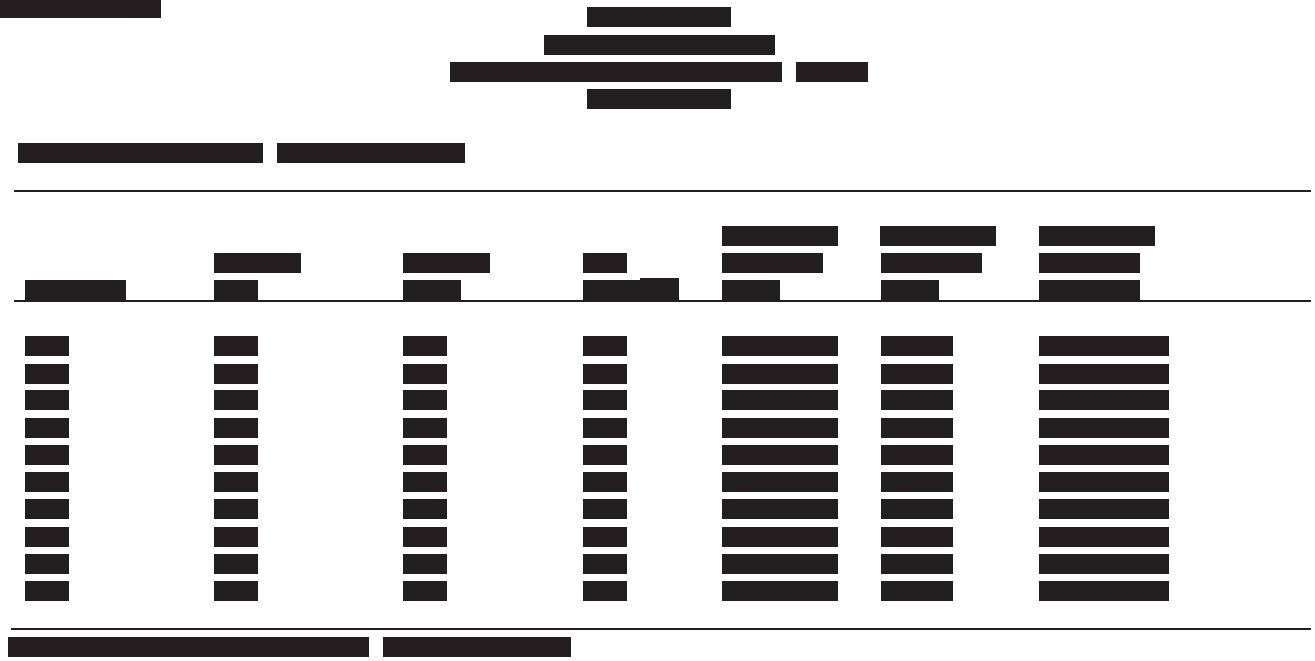

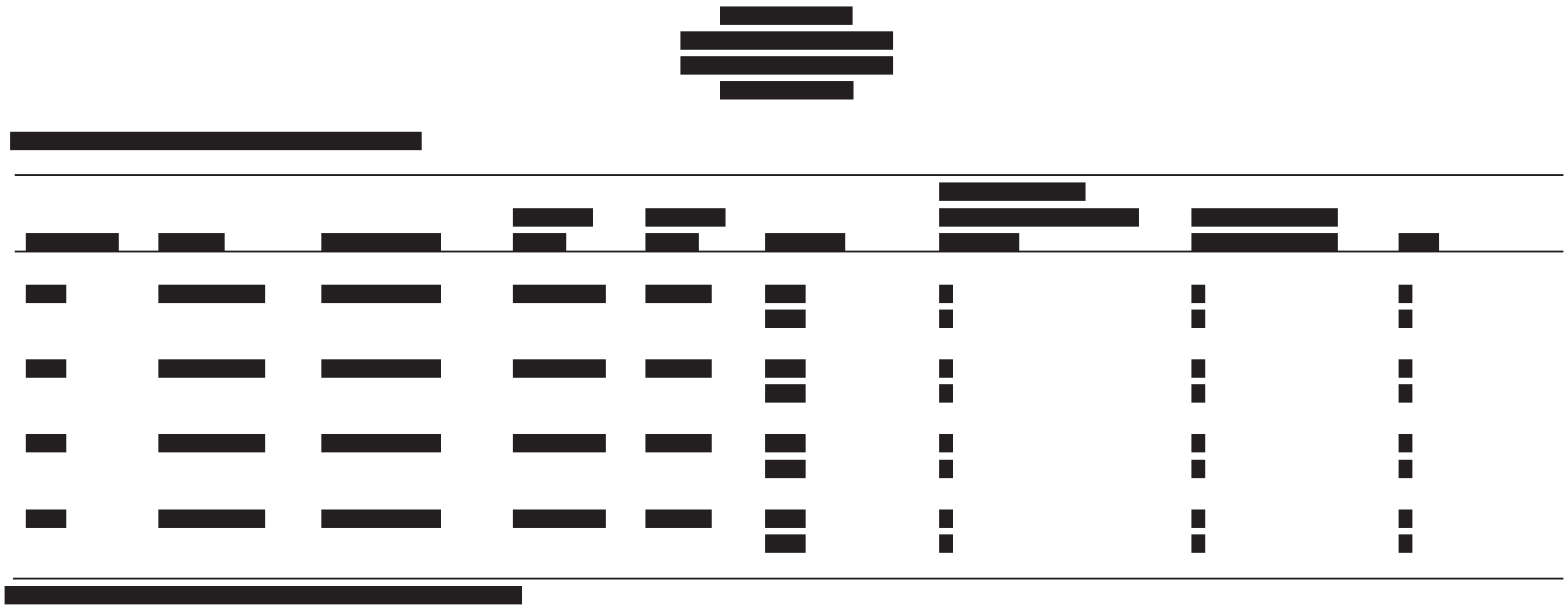

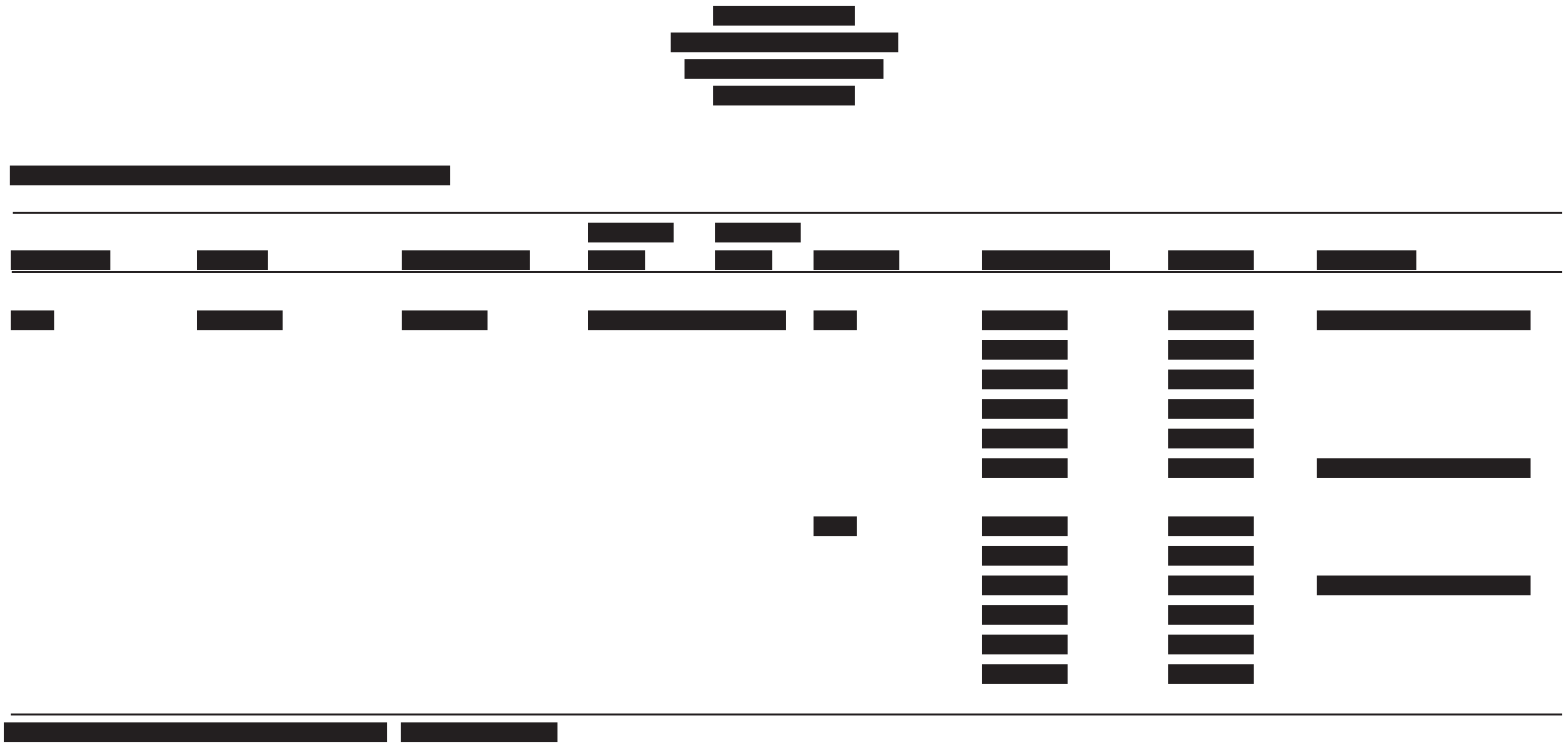

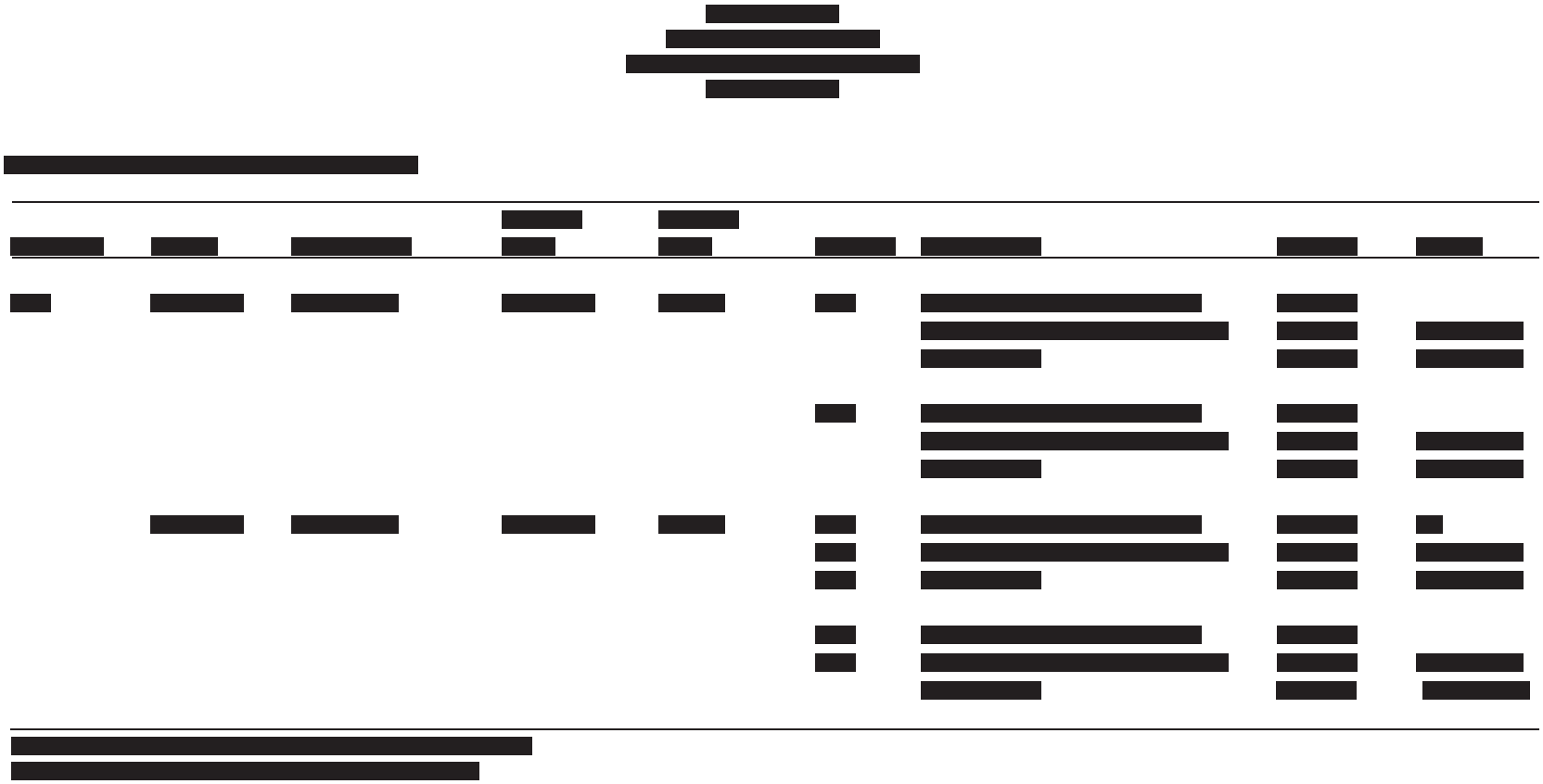

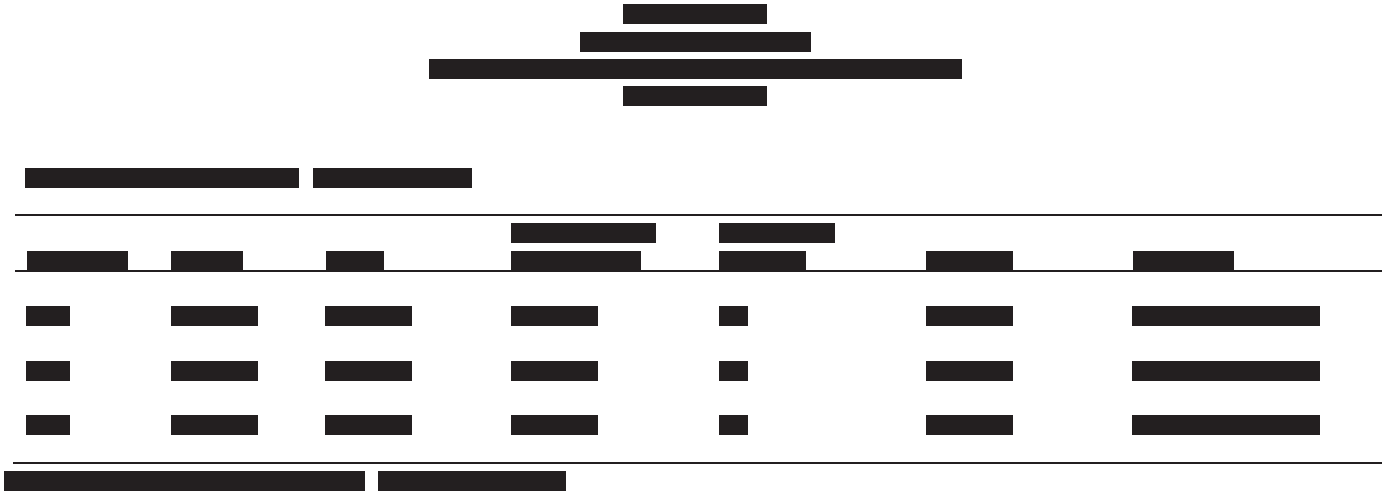

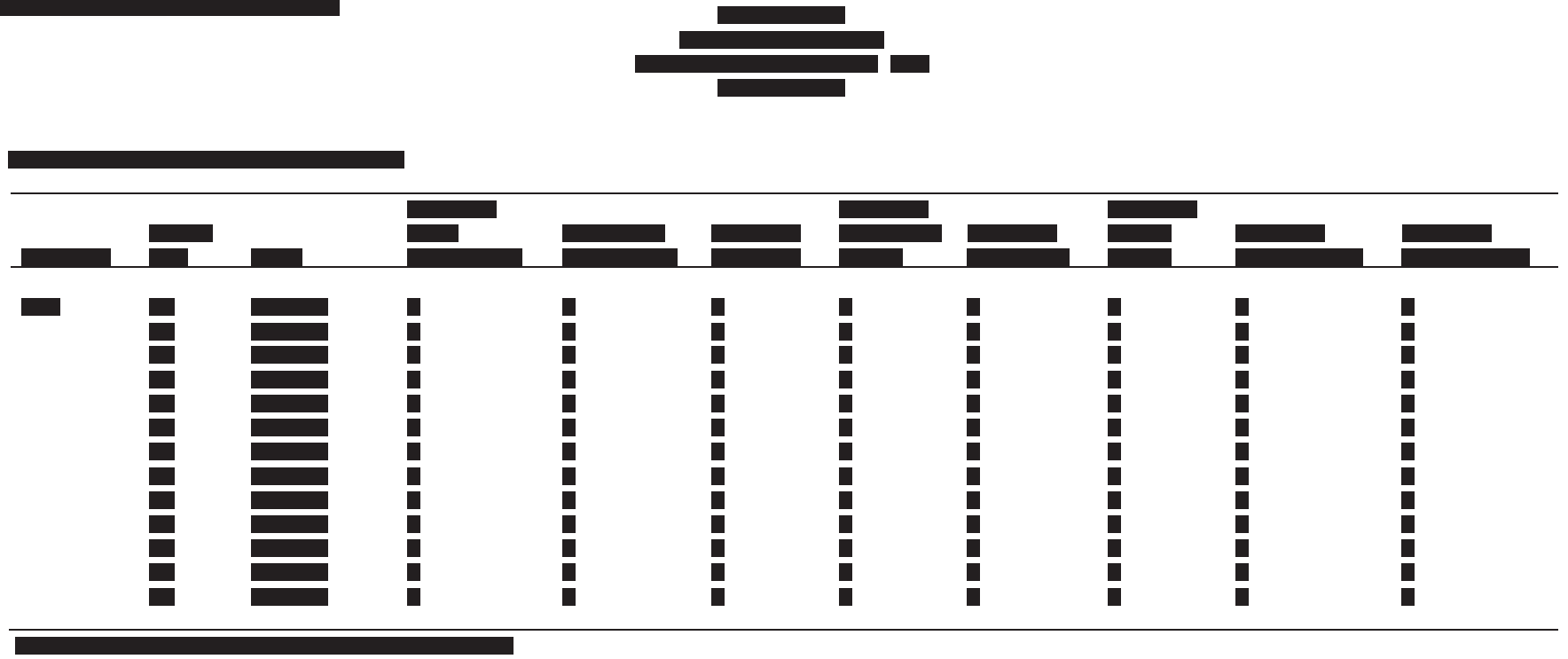

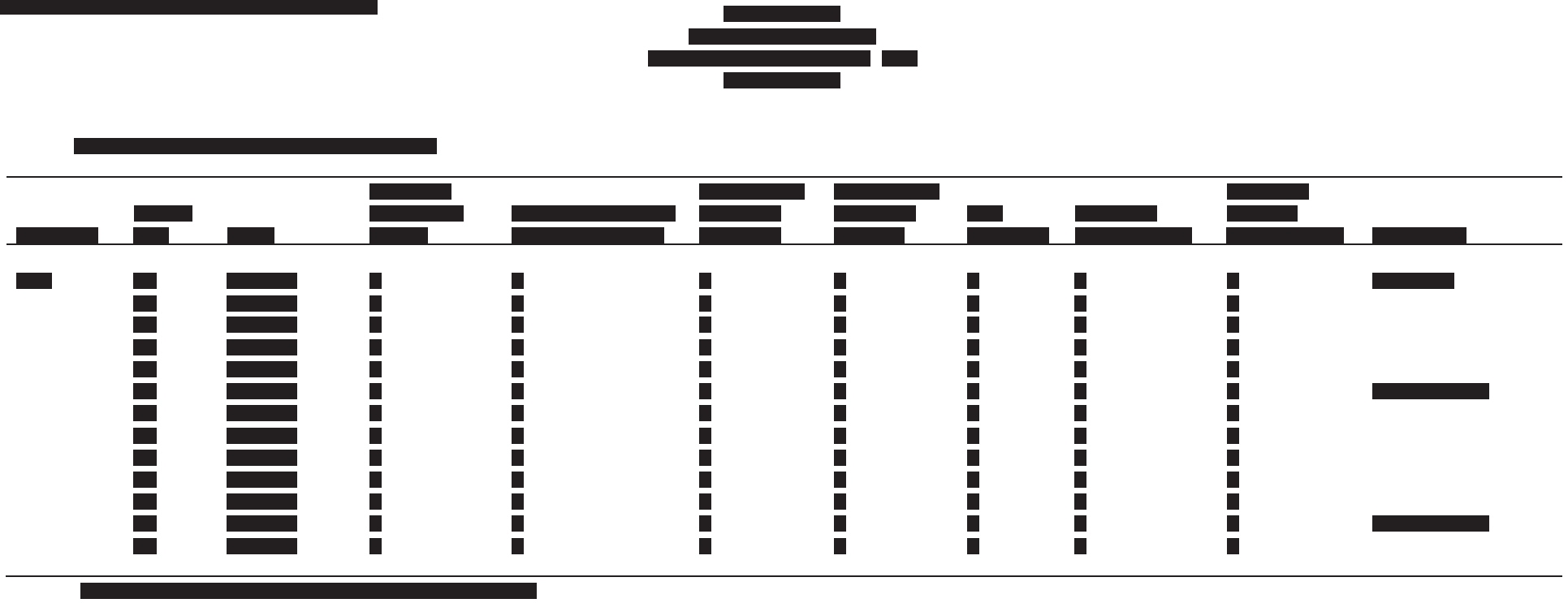

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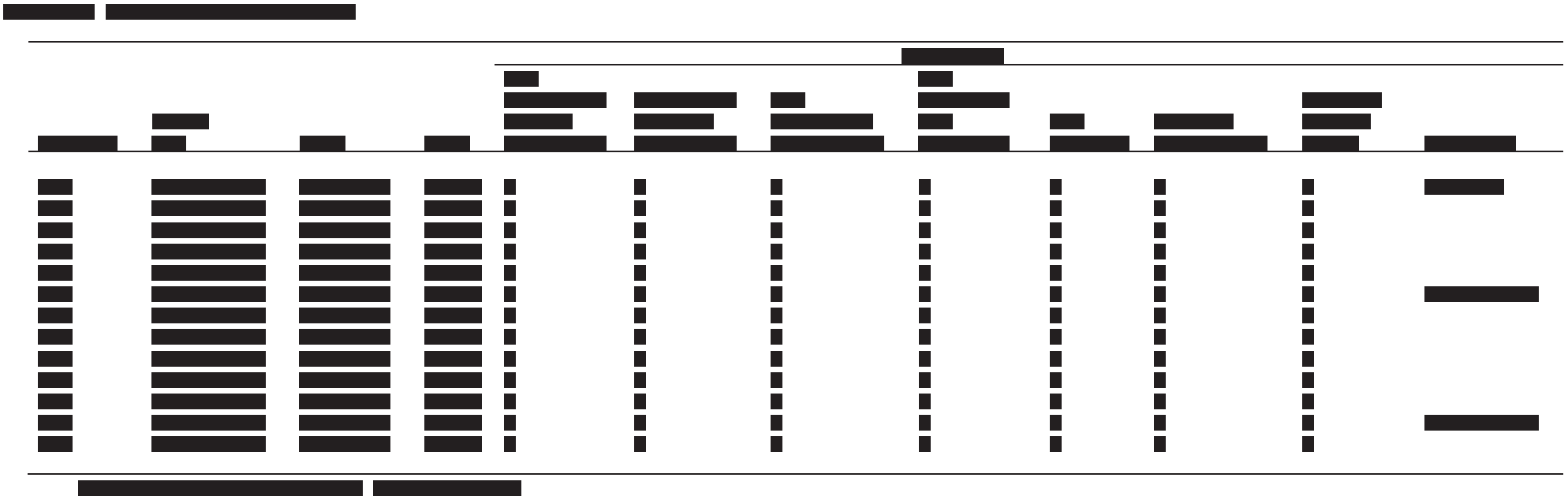

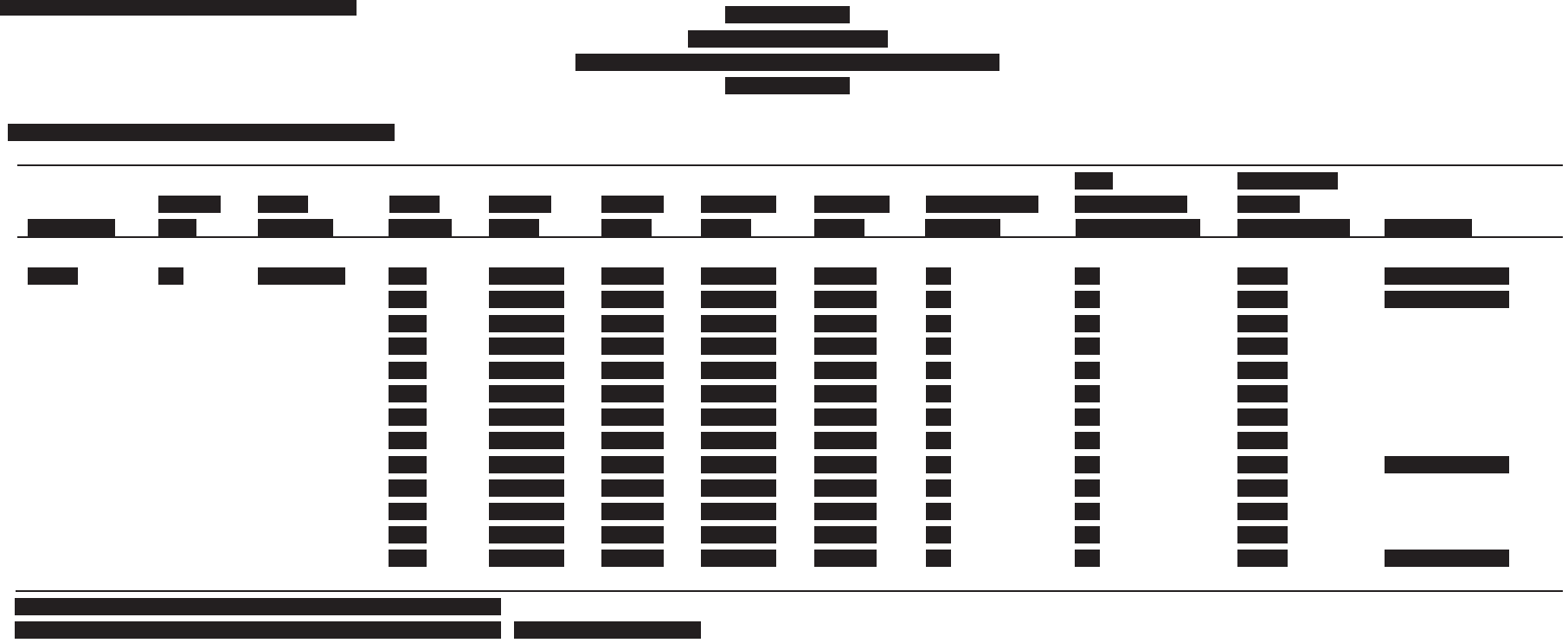

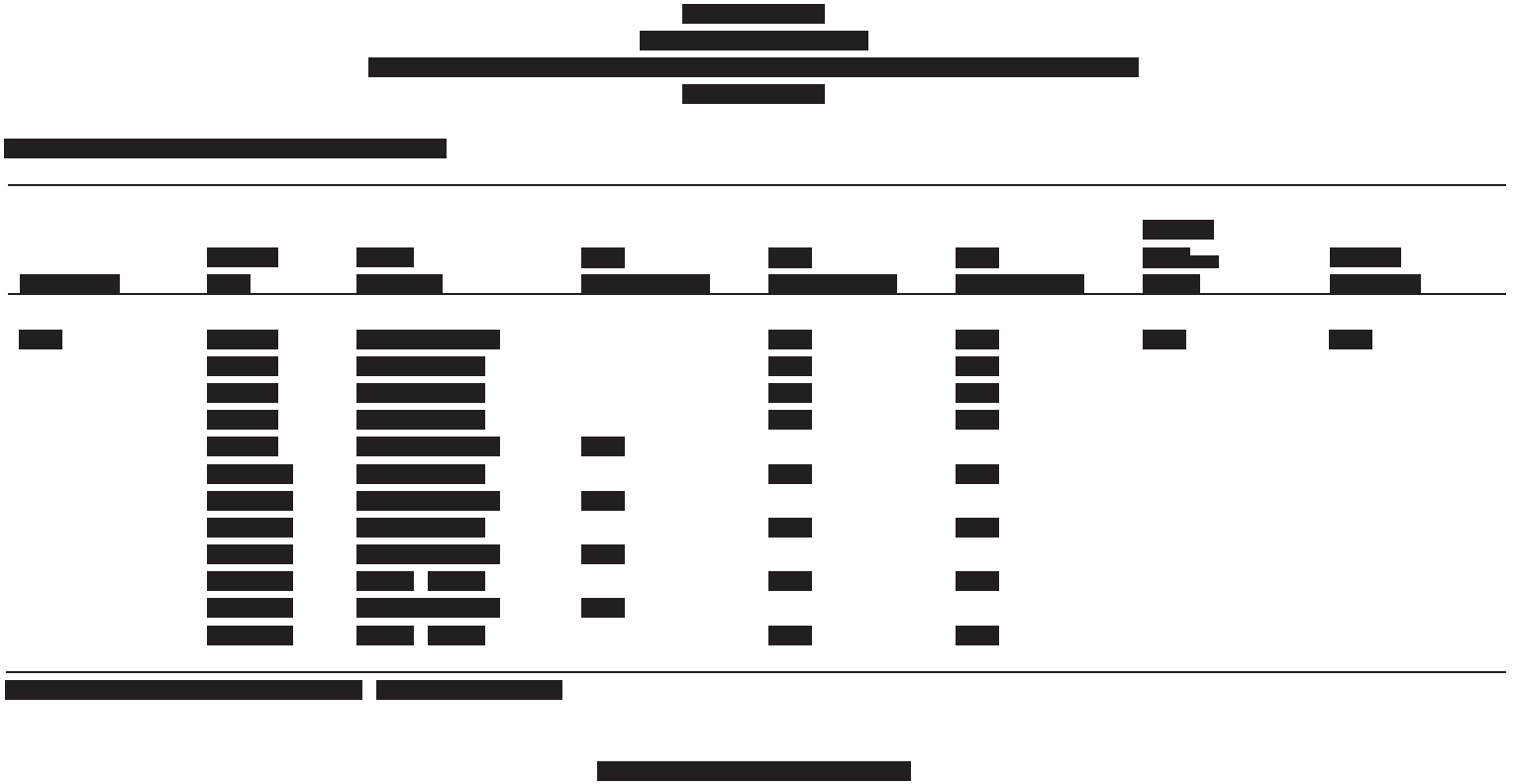

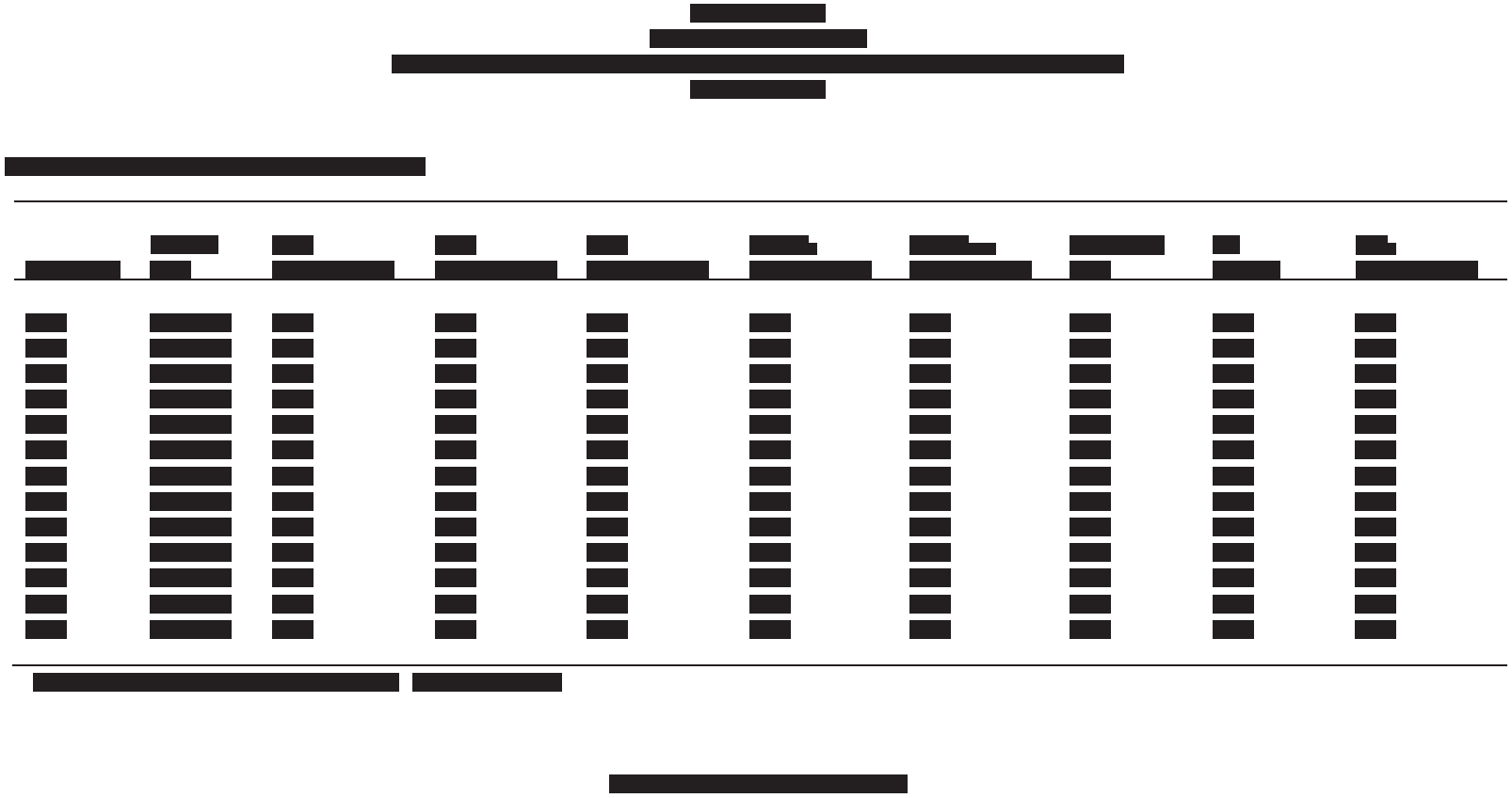

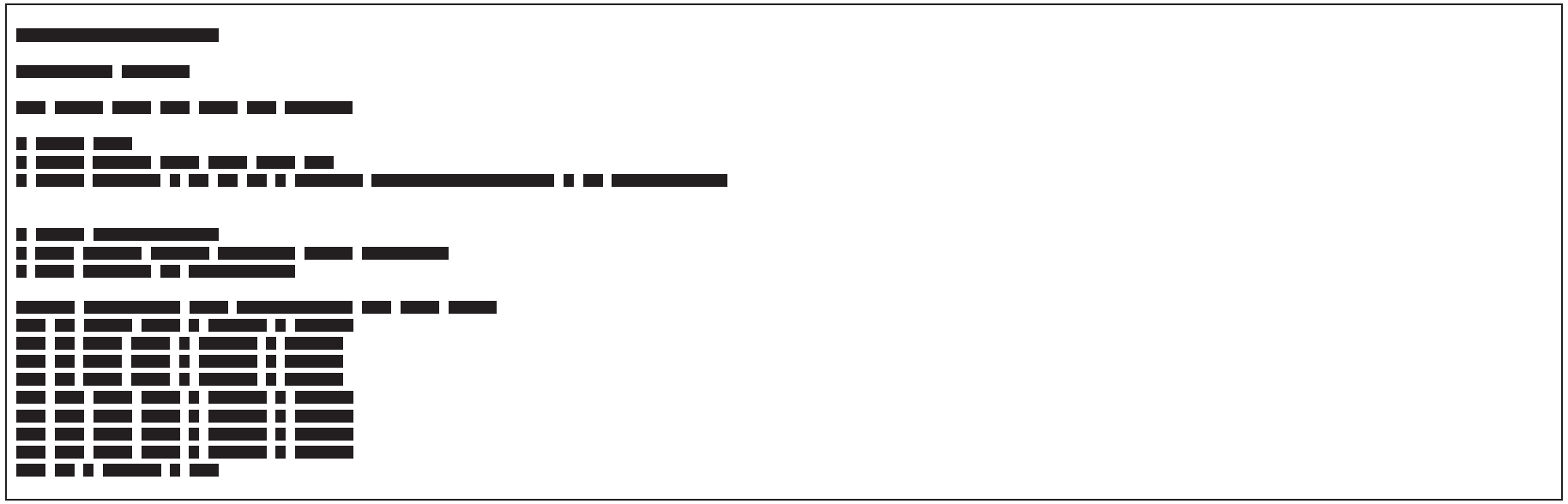

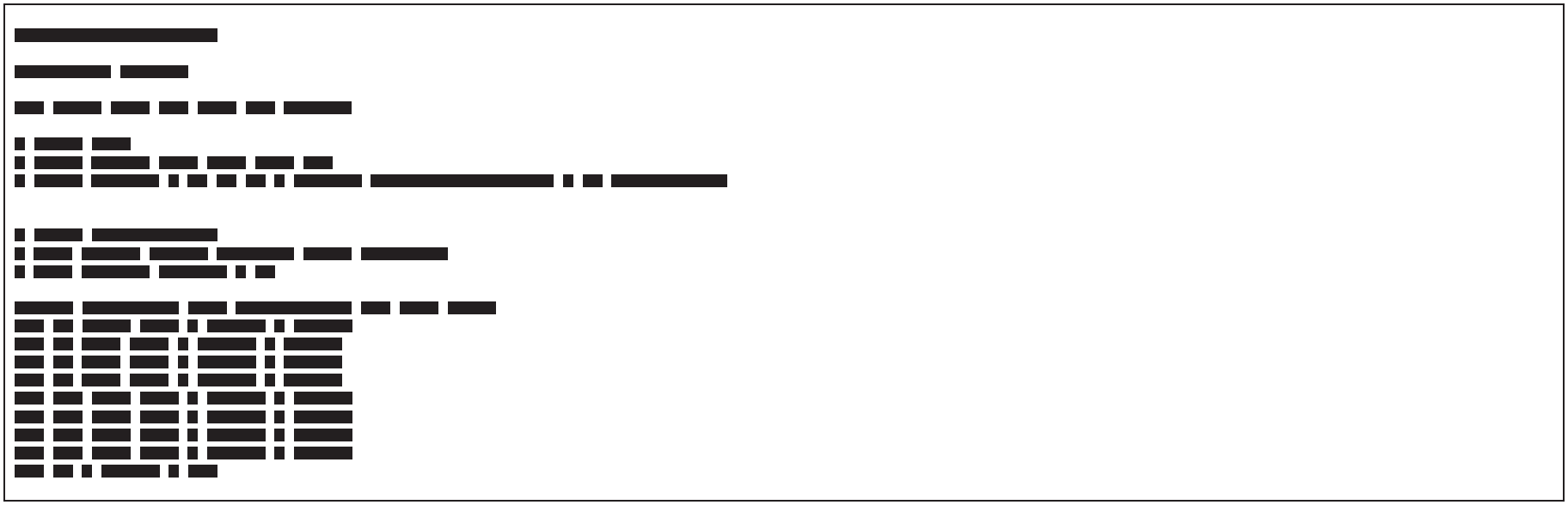

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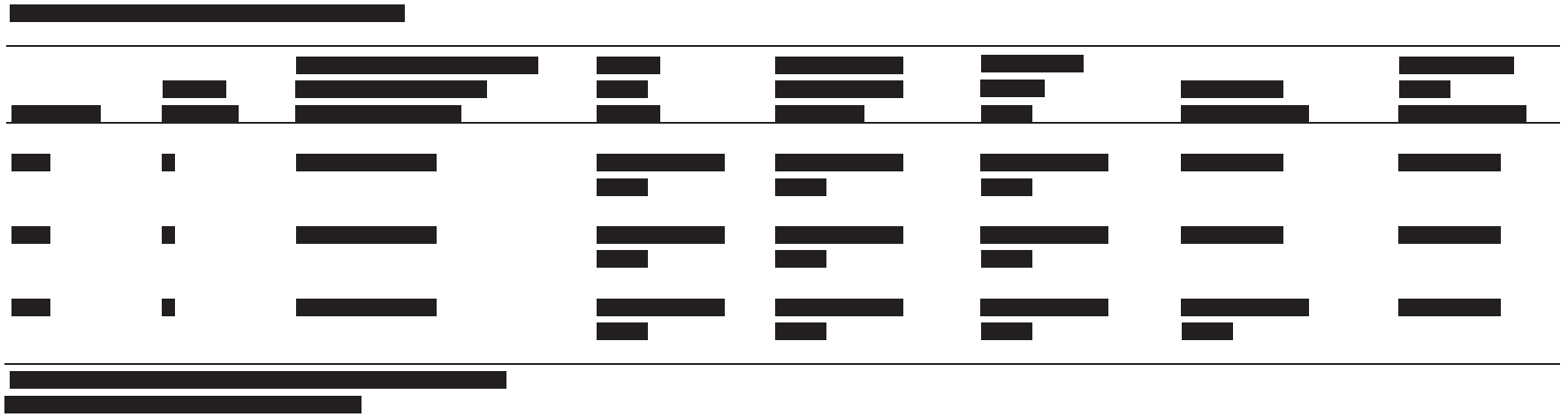

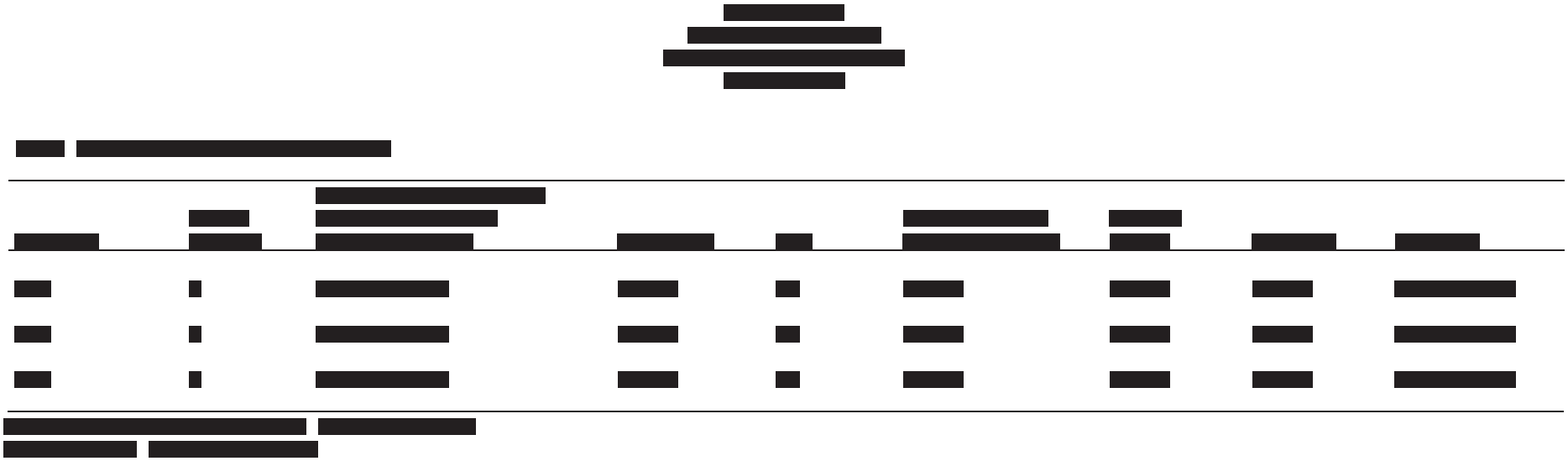

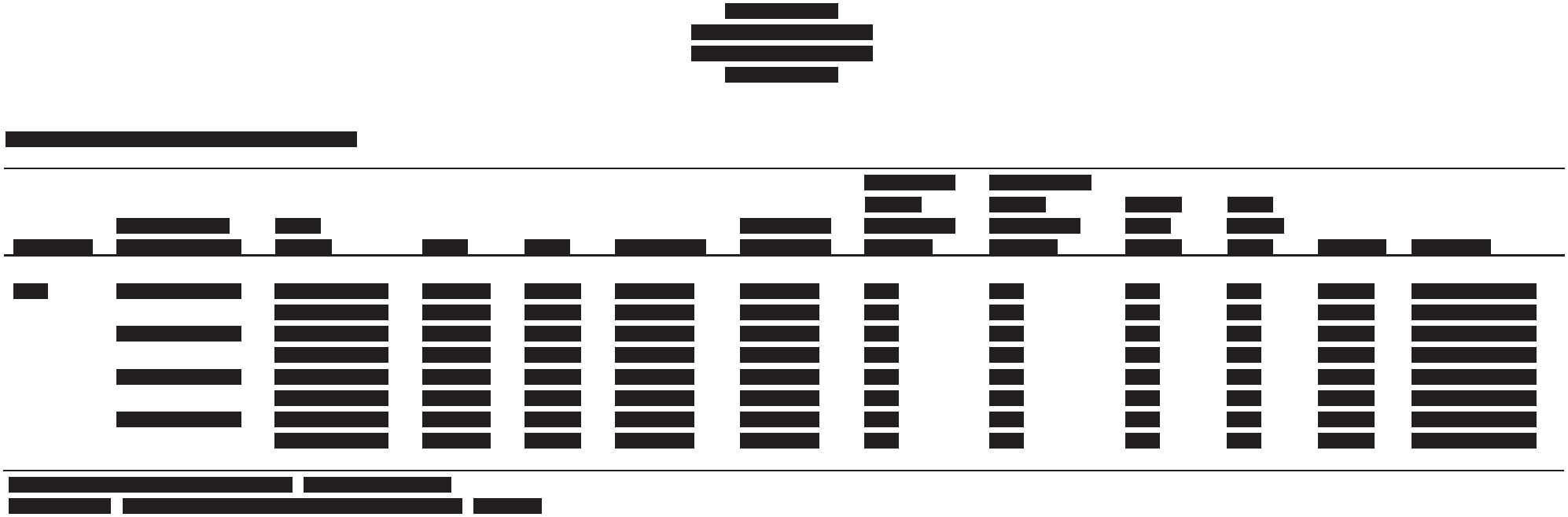

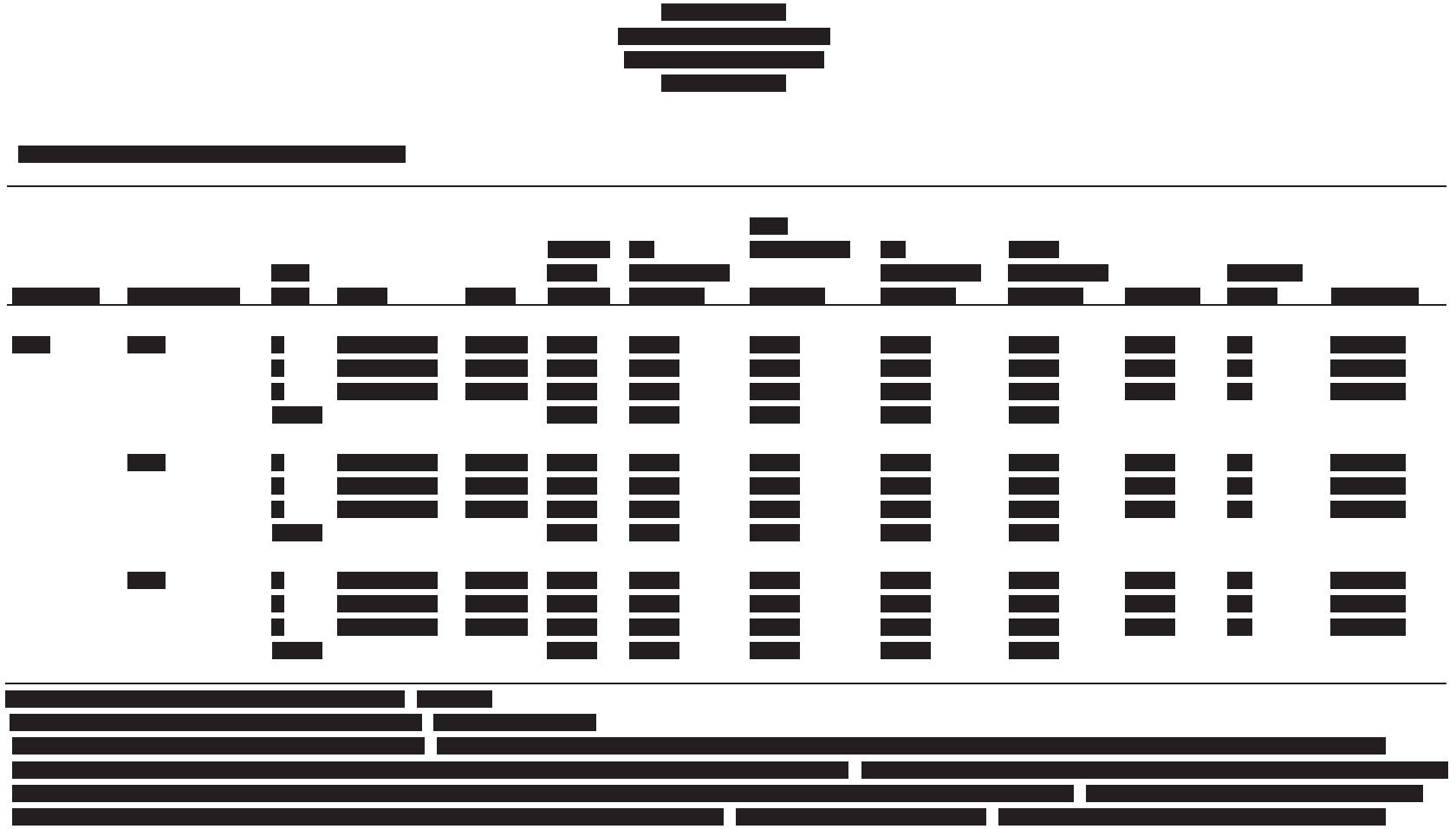

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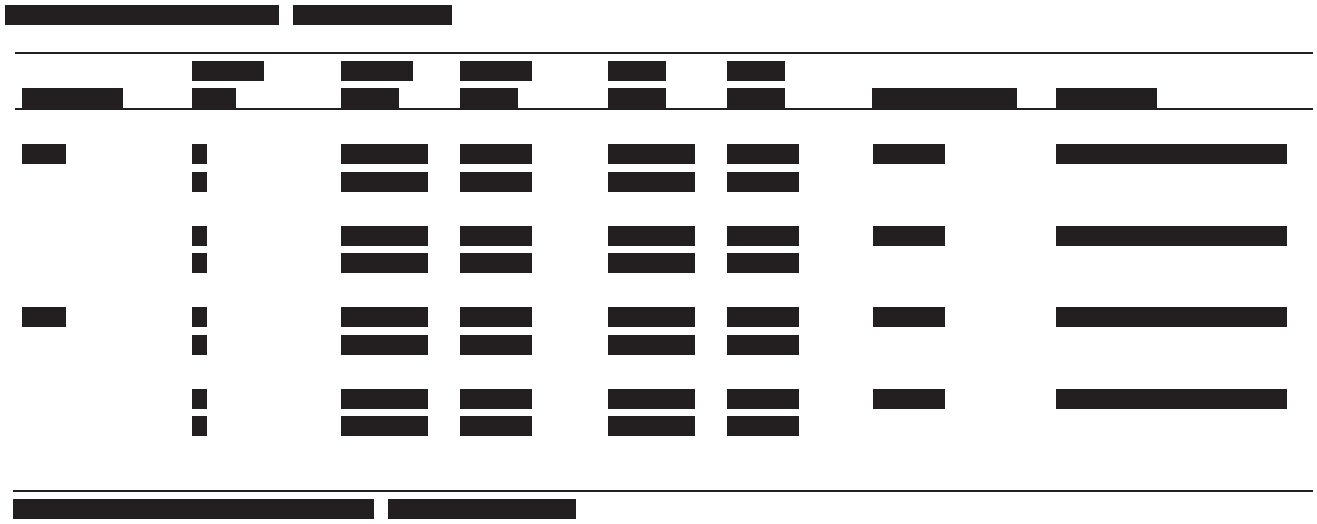

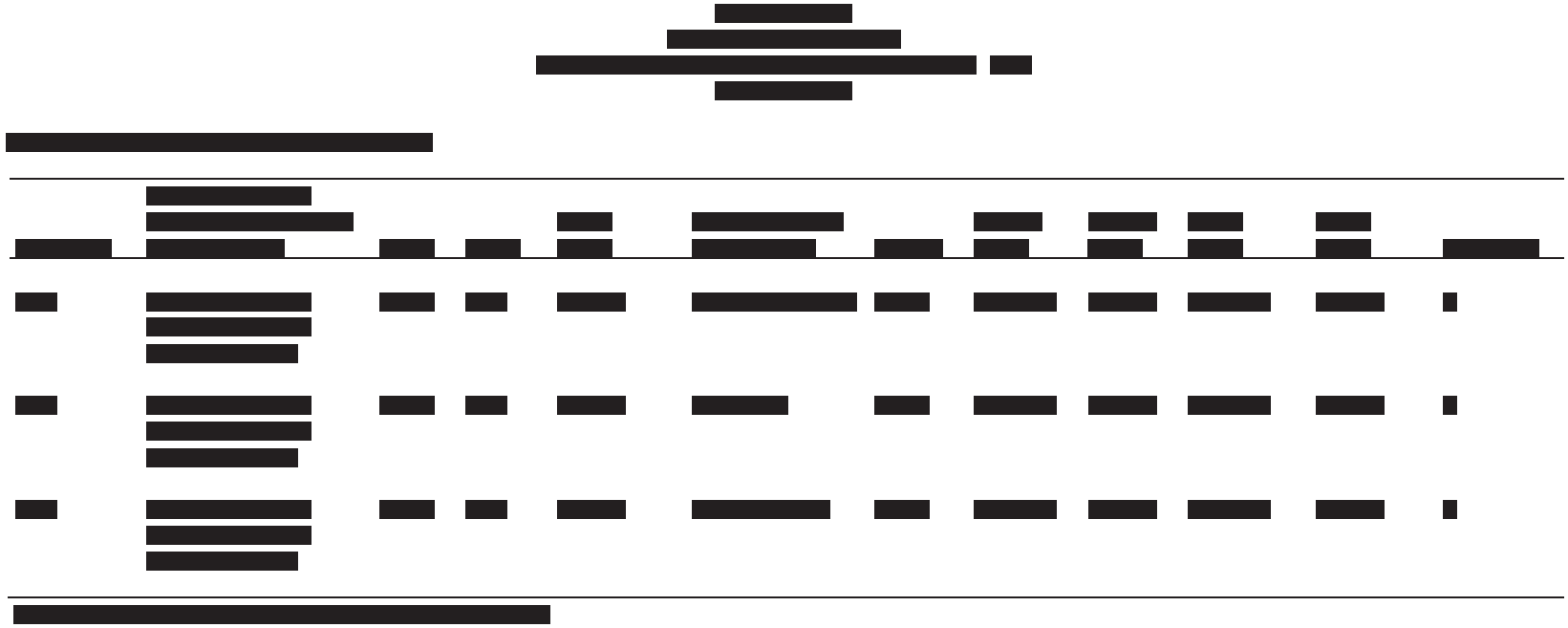

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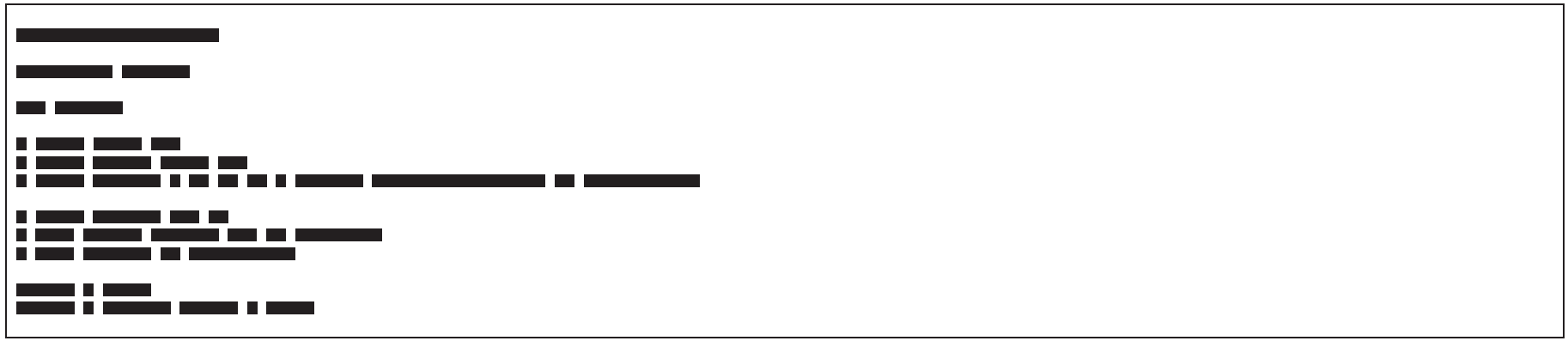

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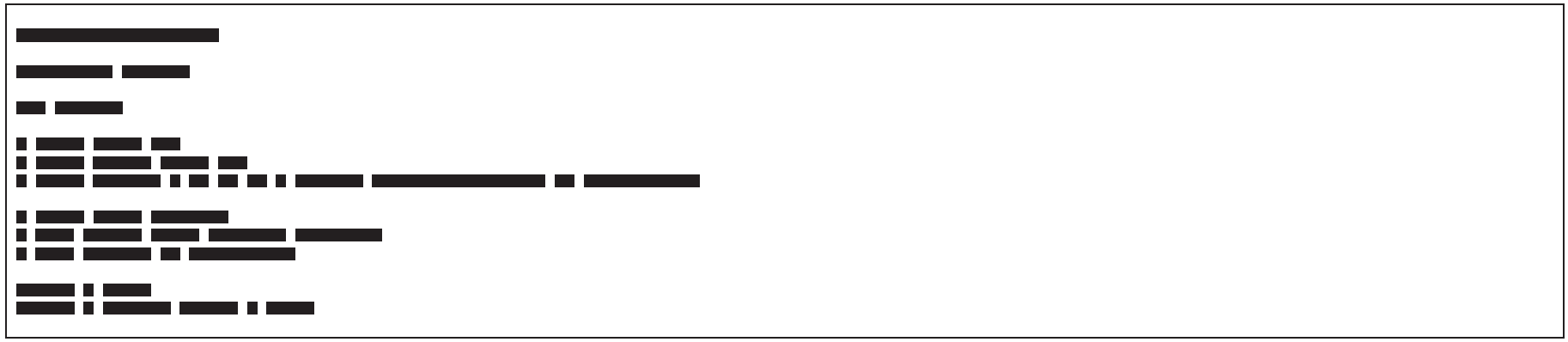

16 APPENDICES

16.1 NORMAL RANGES

Vital Signs Normal Ranges:

Parameter	Normal Range	Units
Pulse Rate	40-110	Beats per minute (bpm)
Systolic Blood Pressure	90-150	mmHg
Diastolic Blood Pressure	50-90	mmHg
Respiratory Rate	12-18	Breaths per minute
Oral Temperature	35.0-37.5	Degrees Celsius (°C)
Pulse Oximetry	94-100	%

12-Lead ECG Normal Ranges:

Parameter	Normal Range	Units
Heart Rate	40-110	Beats per minute (bpm)
PR Interval	120-220	mSec
QRS Duration	70-120	mSec
QT Interval	N/a	N/a
QTc Interval (=QTcF) (Fridericia's)	350-430 (males)	mSec
	350-450 (females)	mSec