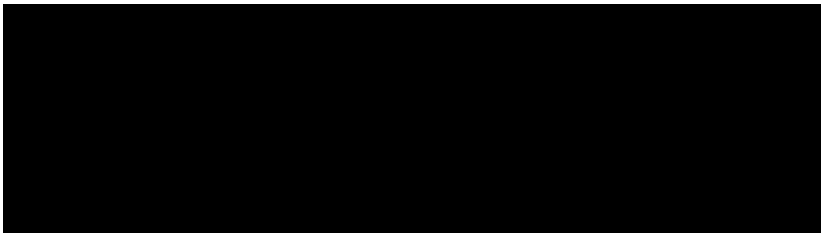
STATISTICAL ANALYSIS PLAN

Study Title:	A Phase 2, Multi-center, Open-label Study to Evaluate the Safety and Efficacy of VGT-309, a Tumor-Targeted, Activatable Fluorescent Imaging Agent, in Subjects Undergoing Surgery for Cancer in the Lung
Name of Test Drug:	VGT-309
Study Number:	VGT-309-2B-2023
Protocol Version:	3.0
Protocol Date:	29 February, 2024
Analysis Type:	Final
Analysis Plan Version:	1.0
Analysis Plan Date:	03 May, 2024
Analysis Plan Author:	[redacted]

AUTHOR(S):



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SPONSOR APPROVAL:

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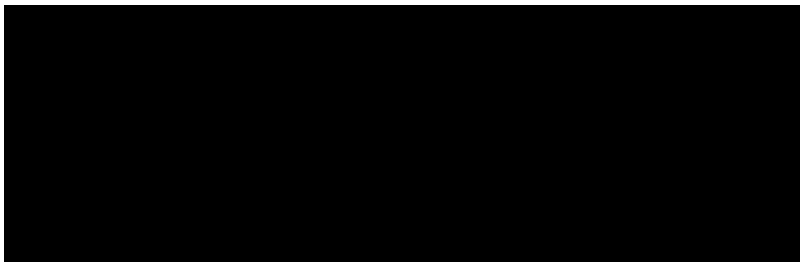

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CONFIDENTIAL AND PROPRIETARY INFORMATION

LIST OF ABBREVIATIONS

AE	Adverse Event
AR	Adverse Reaction
AUC	Area under the curve
BMI	Body mass index
BPM	Beats per minute
CDER	Center for Drug Evaluation and Research
CHMP	Committee for Medicinal Products for Human Use
CPMP	Committee for Proprietary Medicinal Products
CRF	Case report form
CRO	Contract research organization
CSE	Clinically Significant Event
CSR	Clinical study report
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
DMC	Data monitoring committee
ECG	Electrocardiogram
FN	False Negative
FP	False Positive
FR	Federal Register
GEE	Generalized Estimating Equation
HLT	High level term
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ITT	Intent to treat
IVRS/IWRS	Interactive voice/web response system
LLT	Lower level term
MedDRA	Medical dictionary for regulatory activities
NIR	Near-Infrared
NPV	Negative Predictive Value
PPV	Positive Predictive Value
PT	Preferred term
ROC	Receiver Operating Characteristic
SAP	Statistical analysis plan
SOC	System organ class
TFLs	Tables, figures, and listings
TN	True Negative
TP	True Positive
WHO	World Health Organization

1. INTRODUCTION

This statistical analysis plan (SAP) describes the statistical analysis methods and data presentations to be used in tables, figures, and listings (TFLs) to be implemented during the analyses of data collected within the scope of Protocol VGT-309-2B-2023 [A Phase 2, Multi-center, Open-label Study to Evaluate the Safety and Efficacy of VGT-309, a Tumor-Targeted, Activatable Fluorescent Imaging Agent, in Subjects Undergoing Surgery for Cancer in the Lung] sponsored by Vergent Bioscience, Inc. This SAP will be finalized before database lock. Any deviations from this plan after SAP finalization will be documented in the clinical study report (CSR).

2. STUDY OBJECTIVES

2.1. Primary Efficacy Objective

Assess the efficacy of VGT-309 with near-infrared (NIR) imaging for lesion visualization when compared to standard surgical techniques in subjects undergoing surgery for proven or suspected cancer in the lung.

2.2. Secondary Efficacy Objective

Assess the utility of VGT-309 with NIR imaging to identify cancerous tissue as confirmed by histopathology.

2.3. Safety Objective

Determine the safety and tolerability of VGT-309 in subjects with proven or suspected cancer in the lung.

3. STUDY ENDPOINTS

3.1. Primary Efficacy Endpoint

Identify the proportion of subjects with at least one Clinically Significant Event (CSE) as defined by:

1. Intraoperative localization of one or more preoperatively identified lung lesions using VGT-309 with NIR imaging when standard surgical techniques using white light and/or palpation failed to identify the lesion(s), and lesion(s) are confirmed by histologic examination to be other than normal lung tissue.
2. Identification of one or more synchronous or occult lung lesions using VGT-309 with NIR imaging when standard surgical techniques using white light and/or palpation and preoperative imaging failed to identify the lesion(s), and lesion(s) are confirmed by histologic examination to be cancerous or precancerous.
3. Identification of fluorescence within ≤ 10 mm from the inside edge of the closest staple line as measured by the investigator *ex vivo* in the operating room using NIR imaging, with pathologic margin confirmed by histologic examination to be ≤ 10 mm.

NOTE: Margins from excisions of known or suspected metastases at the time of the surgery, OR diagnostic wedge resections where a completion lobectomy or segmentectomy was subsequently performed, will not be considered for Margin CSE inclusion.

4. Identification of lymph nodes by VGT-309 with NIR imaging confirmed by histologic examination to be cancerous.

3.2. Secondary Efficacy Endpoints

To evaluate the sensitivity, positive predictive value (PPV), and 1-PPV of VGT-309 with NIR imaging for lesion(s) *in vivo*:

1. Sensitivity is defined as the probability that the tissue fluoresces when it is cancer, as confirmed by histology (TP/(TP+FN)) *
2. Positive predictive value (PPV) is defined as the probability that a tissue sample contains cancer on histologic exam if it fluoresces ((TP/(TP+FP)) *
3. 1-Positive predictive value (1-PPV) is defined as the probability that a tissue sample does not contain cancer when it fluoresces ((FP/(TP+FP))*

* TP = true positive, FP = false positive, FN = false negative

[redacted]

3.4. Safety Endpoint

Assessment of clinical laboratory tests, physical exam results, ECGs and vital sign measurements at various time points during the study, and documentation of treatmentemergent adverse events.

4. STUDY DESIGN

This is a Phase 2, multi-center, open-label study to evaluate the safety and efficacy of VGT-309, a tumor-targeted, activatable fluorescent imaging agent, in subjects undergoing surgery for proven or suspected cancer in the lung. Approximately 100 subjects will be enrolled to ensure at least 86 subjects are evaluable with the option to expand enrollment by protocol amendment if deemed necessary by the DSC (Data Safety Committee) to meet primary and/or secondary objectives.

Following agreement with and signing of the informed consent, subjects will undergo screening measurements for the study within 4 weeks prior to the anticipated dosing:

1. Medical, surgical and medication history.
2. Complete physical exam, including vital signs and height
3. Weight (needed for dose calculation)
4. Chemistry, hematology, coagulation and urinalysis with microscopy clinical laboratory studies.
5. 12-lead ECG.
6. Serum pregnancy test for females of child-bearing potential.

After meeting all enrollment criteria, each subject will receive 0.32 mg/kg VGT-309 by IV administration 12-36 hours prior to the induction of general anesthesia for surgery (refer to section 4 of protocol v3.0). Subjects will be observed for 1 hour after dosing is completed and asked about possible treatment emergent adverse events.

Subjects will undergo surgical resection within 12-36 hours after completion of VGT-309 dosing. Measurements of efficacy will be taken during surgery and during the pathological examination of all surgical specimens.

Following surgery, subjects will be monitored for safety during their hospitalization. Between 7 to 14 and 25 to 35 days after surgery, the subjects will return to the clinic or have a telehealth visit for final safety assessments. At the last visit, if there are no adverse events requiring further follow up, subjects will then be released from the study.

If a subject chooses to withdraw from the study prior to the final visit, they will be asked to undergo a final safety assessment before their departure.

5. DETERMINATION OF SAMPLE SIZE

[redacted]

6. TYPE OF PLANNED ANALYSIS

This analysis plan outlines planned analysis procedures for the Final Analysis, as described below. Data outliers and other raw data issues are planned to be identified and logged on a rolling basis throughout the study, with appropriate resolution decided upon and documented prior to the planned analyses outlined below. The Final Analysis will take place after the study is completed and the database has been locked. All tables, figures, and listings described in this SAP are planned to be included in the final analysis.

7. GENERAL CONSIDERATIONS FOR DATA ANALYSES

7.1. Data Presentation and Summarization

The statistical analyses will be reported using summary tables, listings, and figures (TLFs). The International Conference on Harmonisation (ICH) numbering convention will be used for all TLFs. Continuous variables will be summarized with means, standard deviations, medians, minimums, and maximums. Other summaries (e.g., quartiles, 5%, 95% intervals, CV or %CV) may be used as appropriate. Categorical variables will be summarized by counts and by percentage of patients in corresponding categories. Summaries will generally be presented by treatment cohort and for all participants overall. P-values will be presented for hypothesis testing, with a value of 0.05 being the threshold of significance under which the null hypothesis can be rejected. Descriptive statistics will be rounded to two decimal places with the exception of standard deviation which will be rounded to three decimal places.

All analyses and tabulations will be performed using SAS® Version 9.4 or higher. Tables, listings, and figures will be provided in PDF format.

7.2. Missing or incomplete dates

The most conservative approach will be systematically considered. [redacted]

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[redacted]

- [redacted]
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- [redacted]

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7.3. Analysis Populations

Analysis populations define the participants to be included in an analysis. Analysis populations and their definitions are provided in this section. The analysis population will be identified and included as a subtitle of each table.

Inclusion in the analysis population will be determined prior to database lock. A summary of the number and percent of participants in each analysis population will be provided. A by-participant listing of each analysis population will also be provided.

7.3.1. Enrolled Population

This population consists of all subjects who are enrolled in the study (signed informed consent).

7.3.2. Safety Population

This population consists of all subjects who received any amount of VGT-309.

7.3.3. Intent to Treat (ITT) Population

This population will consist of all subjects who received any amount of VGT-309 and had surgery with NIR imaging after the surgeon first attempts to locate the tumor/nodule by white light and palpation.

7.3.4. Evaluable Population

The Evaluable Population (EP) will consist of the subset of ITT subjects who were eligible, who had no major protocol deviations, who received at least 90% of the prescribed amount of

VGT-309, and the surgeon first attempted to locate the tumor/nodule by adequate white light and palpation followed by adequate NIR imaging.

8. STUDY POPULATION

In general, subjects who signed informed consent and were enrolled for the study will be used for data summaries and listings for subject disposition. Safety population will be used for safety summaries and listings, while the ITT and Evaluable Populations will be used for efficacy analyses, unless otherwise specified.

8.1. Subject Disposition

8.1.1. Subject Enrollment and Disposition

A summary of subject disposition will be provided and will present the number of subjects enrolled, and the number and percent of subjects:

- Included in the Safety population;
- Included in the ITT population;
- Included in the Evaluable population;
- Completing the study (includes the study treatment period, day 3 in-hospital visit, and at least one of the post-treatment safety follow-up visits); and
- Not completing the study, (with summary of reasons for not completing the study).

[redacted]

[redacted]

8.1.2. Exposure to Study Drug

Extent of exposure to study drug will be summarized for the safety population. [redacted]

8.2. Protocol Deviations

All protocol violations and deviations will be summarized by classification category (major/minor) and provided in a data listing. Subjects with major protocol deviations will be excluded from the Evaluable analysis population. Before database lock a Data Review Committee will document decisions around major/minor protocol deviations.

8.3. Inclusion/Exclusion Criteria

A listing will be provided for screen failures, defined as subjects in the Enrolled population who do not meet all inclusion criteria or who meet at least one exclusion criterion, along with the criterion number and description not met.

8.4. Demographics and Baseline Characteristics

Subject demographic data (e.g., age, sex, race, and ethnicity) and baseline characteristics (e.g., body weight, height, BMI) will be summarized using descriptive statistics (sample size, mean, standard deviation, median, Q1, Q3, minimum and maximum) for continuous data and by the number and percent of subjects for categorical data. The summaries of demographic data and baseline characteristics will be provided for the safety analysis set.

8.5. Medical History

Medical history will be summarized by Medical Dictionary for Regulatory Activities (MedDRA) system organ class (SOC) and preferred term (PT) (using minimum version 26.1). Participants will only be counted once at each level of summation. Summaries will be sorted by decreasing total incidence by SOC (overall) and then by PT (within each SOC); for SOCs or PTs (within SOC) with the same frequency, sorting will be performed alphabetically. By-participant listings of this information will also be provided along with onset and resolution dates and grades (collected in the CRF if the condition is ongoing).

8.6. Prior and Concomitant Medications

Concomitant medications are defined as medications that started on or after the treatment start date or were ongoing at the date of treatment start. Prior medications are defined as medications that started and stopped prior to the date of treatment start.

Medications will be coded using the World Health Organization (WHO) Drug Dictionary B3 Global (updated September 2023). Anatomical Therapeutic Chemical (ATC) Level 4 drug classes and preferred drug names (PN) will be attached to the clinical database. The number and percentage of subjects with at least one instance of medication use will be summarized by ATC class and PN (within ATC class) ordered by decreasing total incidence of ATC and PN (within ATC); ties in frequency will be broken alphabetically. Patients will only be counted once at each level of summation. By-participant listings will also be provided.

9. EFFICACY ANALYSES

9.1. Definition of the Primary Efficacy Endpoint

The proportion of subjects with at least one Clinically Significant Event (CSE) as defined by:

1. Intraoperative localization of one or more preoperatively identified lung lesions using VGT-309 with NIR imaging when standard surgical techniques using white light and/or palpation failed to identify the lesion(s), and lesion(s) are confirmed by histologic examination to be other than normal lung tissue.

2. Identification of one or more synchronous or occult lung lesions using VGT-309 with NIR imaging when standard surgical techniques using white light and/or palpation and preoperative imaging failed to identify the lesion(s), and lesion(s) are confirmed by histologic examination to be cancerous or precancerous.
3. Identification of fluorescence within ≤ 10 mm from the inside edge of the closest staple line as measured by the investigator *ex vivo* in the operating room using NIR imaging, with pathologic margin confirmed by histologic examination to be ≤ 10 mm. NOTE: Margins from excisions of known or suspected metastases at the time of the surgery, OR diagnostic wedge resections where a completion lobectomy or segmentectomy was subsequently performed, will not be considered for Margin CSE inclusion.
4. Identification of lymph nodes by VGT-309 with NIR imaging confirmed by histologic examination to be cancerous.

9.2. Statistical Hypothesis for the Primary Efficacy Endpoint

The primary study hypothesis is that VGT-309 with NIR imaging is superior to white light and palpation for the intraoperative localization of preoperatively identified lung lesions. The null hypothesis for the primary endpoint is that <= 10% of subjects will have at least one CSE, while the alternative hypothesis is that proportion of subjects with at least one CSE will exceed 10%.
For the primary endpoint analysis, the proportion of subjects with at least 1 CSE will be tested under the null hypothesis that this proportion is <=10%.

$$H_0: p \leq 0.10$$

The alternative hypothesis states that the proportion of evaluable subjects with at least 1 CSE will be greater than 10%:

$$H_a: p > 0.10$$

9.3. Analysis of the Primary Efficacy Endpoint

Efficacy analyses will be performed on the Evaluable population. This analysis will be repeated using the ITT population. Quantitative data will be presented as a standard set of summary statistics (n, mean, standard deviation, median, minimum, and maximum values). Categorical data will be expressed as frequency (%).

A one-sided exact binomial test will be used to test the null hypothesis using a one-sided alpha of .025, and Clopper-Pearson exact two-sided 95% confidence intervals will be reported. A right-sided p value will be provided, which is the probability of finding the observed number of successes (subjects with at least 1 CSE) or a larger number, given that the null hypothesis is true.

Frequency of CSEs will also be summarized by CSE type across subject characteristics including the following categories:

- Age
- Smoking history
- Overall cancer stage – pre-operative

- Overall cancer stage – post-operative
- Surgery type
- Primary lung cancer type
- Prior cancer treatment
- Imaging system
- Resection type
- Lesion type by histopathology

9.4. Secondary Efficacy Endpoints

9.4.1. Definition of Secondary Efficacy Endpoints

To evaluate the sensitivity, positive predictive value (PPV), and 1-PPV of VGT-309 with NIR imaging for lesion(s) *in vivo*:

4. Sensitivity is defined as the probability that the tissue fluoresces when it is cancer, as confirmed by histology (TP/(TP+FN)) *
5. Positive predictive value (PPV) is defined as the probability that a tissue sample contains cancer on histologic exam if it fluoresces ((TP/(TP+FP)) *
6. One minus Positive predictive value (1-PPV) is defined as the probability that a tissue sample does not contain cancer when it fluoresces ((FP/(TP+FP))*

* TP = true positive, FP = false positive, FN = false negative

9.4.2. Analysis Methods for Secondary Efficacy Endpoints

For the secondary endpoint analyses, sensitivity, PPV, and 1-PPV will be calculated and presented along with 95% confidence intervals for lesions and lymph nodes using in vivo assessments. Modeled point estimates and confidence intervals will be reported to account for the multiple dependent observations within subjects using Generalized Estimating Equations (GEE) since only between-subject observations can be modelled as independent. To account for the multiple dependent observations within a subject, the GEEs will be fit in SAS with an independent covariance matrix and binary (logit) outcomes. See Appendix 2.

Raw counts (%) will also be provided.

9.5. Exploratory Endpoints

9.5.1. Definition of Exploratory Endpoints

To evaluate the sensitivity, positive predictive value (PPV), and 1-PPV of VGT-309 with NIR imaging for lesion(s) *ex vivo*:

1. Sensitivity is defined as the probability that the tissue fluoresces when it is cancer, as confirmed by histology (TP/(TP+FN)) *
2. Positive predictive value (PPV) is defined as the probability that a tissue sample contains cancer on histologic exam if it fluoresces ((TP/(TP+FP)) *

3. One minus Positive predictive value (1-PPV) is defined as the probability that a tissue sample does not contain cancer when it fluoresces ((FP/(TP+FP))*

* TP = true positive, FP = false positive, FN = false negative

[redacted]

10. SAFETY ANALYSES

10.1. Adverse Events and Deaths

Adverse events will be summarized by the Medical Dictionary for Regulatory Activities (MedDRA) system organ class and preferred term. Events will be summarized on the basis of the date of onset for the event. Only treatment-emergent adverse events (TEAEs), defined as any adverse event that begins on or after the date of administration of VGT-309, will be collected and analyzed in this study. For purposes of this document, AEs refers to TEAEs.

An overall summary of TEAEs will be provided. The following summaries (number and percentage of subjects) of TEAEs (by System Organ Class and Preferred Term) will be provided:

- TEAEs
- Treatment-related TEAEs
- TEAEs leading to study drug discontinuation
- Serious TEAEs
- Treatment-related Serious TEAEs

All AEs collected during the study will be presented in a listing. Additionally, a listing will be provided for any AEs leading to death.

10.1.1. Adverse Event Dictionary

Clinical and laboratory adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA minimum version 26.1). System Organ Class (SOC), High Level Group Term (HLGT), High Level Term (HLT), Preferred Term (PT), and Lower-Level Term (LLT) will be attached to the clinical database.

10.1.2. Adverse Event Severity

Each AE will be assigned a “Grade” based on the Common Terminology Criteria for Adverse Events (CTCAE). Version 5.0 of the CTCAE will be used for this study. The severity grade of events for which the investigator did not record severity will be categorized

as "missing" for tabular summaries and data listings and will be considered the least severe for the purposes of sorting for data presentation.

10.1.3. Relationship of Adverse Events to Study Drug

The relationship of the Adverse Event to the study drug will be determined by the Principal Investigator, and assessed using the following definitions:

- **Related:** There is a distinct temporal relationship between the event onset and administration of the study drug. There is a known reaction to agent or chemical group or predicted by known pharmacology. The event cannot be explained by subject's clinical state or other factors.

- **Unrelated:** Evidence exists that the AE has an etiology other than the study drug (e.g., pre-existing condition, underlying disease, intercurrent illness, or concomitant medication).

Events for which the investigator did not record relationship to study drug will be considered related to study drug for summaries, but data listings will show relationship as missing.

10.1.4. Serious Adverse Events

An SAE is an AE from this study that results in any of the following outcomes:

- Death

- Life-threatening situation (subject is at immediate risk of death).

- Inpatient hospitalization or prolongation of existing hospitalization.

- Persistent or significant disability/incapacity.

- Congenital anomaly/birth defect in the offspring of a subject who received study drug.

Important medical events that may not result in death, be immediately life-threatening, or require hospitalization, may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject *and* may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

10.1.5. Summaries of Adverse Events and Deaths

Summaries (number and percent of subjects) of adverse events (by SOC, and PT) will be provided using the safety analysis set as follows:

- All treatment-emergent adverse events,

- All treatment-emergent treatment-related adverse events,

- All treatment-emergent treatment-related serious adverse events,
- All treatment-emergent adverse events that caused permanent discontinuation from study drug,
- All treatment-emergent adverse events leading to death.

A brief overall summary of TEAEs will show the number and percentage of subjects who had at least one of each of the above categories.

Multiple events will be counted once only per subject in each summary. For data presentation, SOC will be ordered alphabetically, with PT sorted by decreasing total frequency. For summaries by severity grade, the most severe event will be selected. In addition to the presentation by SOC, each summary will also be presented by preferred term only, ordered by decreasing frequency.

In addition to the summaries, data listings will be provided for the following:

- All treatment-related adverse events
- Serious adverse events
- Deaths
- Adverse events leading to discontinuation of study drug

10.2. Clinical Laboratory Evaluation

Laboratory parameters (including clinical chemistry, hematology, urinalysis, coagulation) will be summarized using descriptive statistics at baseline and at each protocol-specified visit time points. Unscheduled visits will be listed. Changes from baseline will also be summarized. Clinically significant abnormal laboratory values will be reported as AEs.

All laboratory parameters and pregnancy test results will be provided in listings.

10.2.1. Summaries of Numeric Laboratory Results

[redacted]

10.2.2. Shifts Relative to the Normal Range

The following summaries (number and percentage of subjects) relative to the respective laboratory test normal ranges will be provided:

- Shift from baseline NCI-CTCAE grade to post-baseline grade at each post-baseline analysis window.

10.3. Vital Signs

Vital signs will be summarized using descriptive statistics at baseline and at each protocol-specified visit time point. Unscheduled visits will be included in data listings. Changes from baseline will also be summarized.

All vital signs data will be provided in listings.

10.4. Physical Examination

Physical Examination results will be listed.

10.5. Electrocardiogram

All ECG results will be listed. Overall interpretation and percent change for each parameter from baseline will be summarized by time point.

[redacted]

10.6. Other Safety Measures

The following safety measures collected will also be provided in listings:

- Pregnancy test results

11. REFERENCES

Karjalainen J, Viitasalo M, Manttari M, Manninen V. 1994. Relation between QT intervals and heart rates from 40 to 120 beats/min in rest electrocardiograms of men and a simple method to adjust QT interval values. *J Am Coll Cardiol* **23**:1547-1553.

Ying GS, Maguire MG, Glynn RJ, Rosner B. Calculating Sensitivity, Specificity, and Predictive Values for Correlated Eye Data. Invest Ophthalmol Vis Sci. 2020 Sep 1;61(11):29. doi: 10.1167/iovs.61.11.29. PMID: 32936302; PMCID: PMC7500131.

12. SOFTWARE

SAS Software Version 9.4. SAS Institute Inc., Cary, NC, USA.

13. APPENDICES

Appendix 1. Table of Contents for Statistical Tables, Figures, and Listings

The following TFL numbering is completed according to ICH guidelines. The ICH heading number and description are in **bold**. Minor changes from this planned index do not need to be amended in the SAP. Formal organization of tabulations may be changed during programming if appropriate, e.g., tables for the different variables may be combined into a single table, or tables with more than one variable may be split into several tables.

List of Tables

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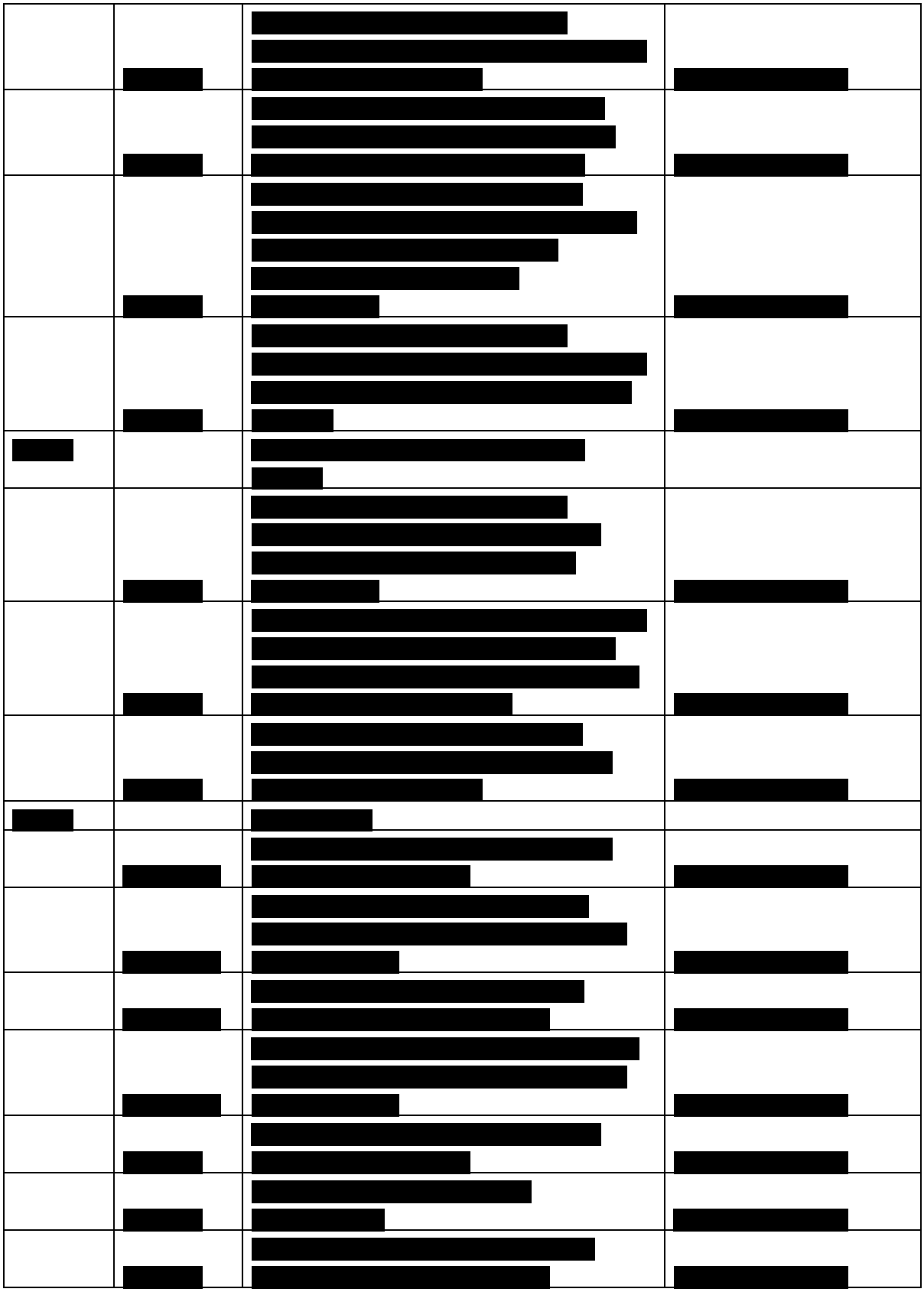

List of Data Listings

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