Obtaining occurrence data of contaminants in foods

MAFF
Ministry of Agriculture Forestry and Fisheries
Food Safety and Consumer Affairs Bureau

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Contents

- Concept, Basic terms for sampling
- Sampling
  - for surveillance to obtaining occurrence data
  - for conformity assessment in regulation
- Validation of analytical method / use of validated analytical method
- Requirement for laboratories

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1. Concept, Basic terms for sampling

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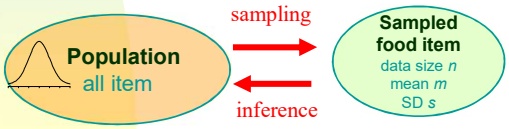
Purpose of analysis

Risk manager should decide

- To know the population nation-wide or regional (Surveillance)
  - To know average or high end
- Whether a certain sample meet the requirement (e.g. MRL) – conformity assessment



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Lot

- Commodities which are presumed uniform
  - Example: crops of the same variety harvested in the same year from the same field
- a quantity of a food material delivered at one time and known, or presumed, by the sampling officer to have uniform characteristics such as origin, producer, variety, packer, type of packing, markings, consignor, etc. (CXG 33)

Sublot

- Part of a large lot

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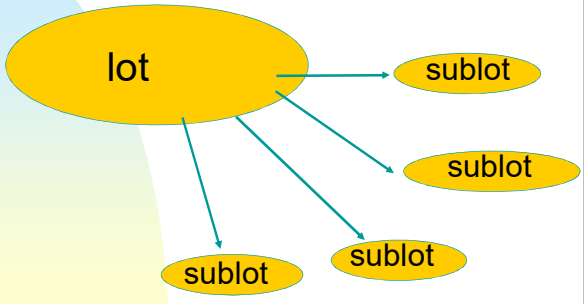
Sampling



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Consignment

Commodities delivered at one time.

- Consignment may consist of multiple lot. If possible, each lot in a consignment should be considered separately.
- Consignment may be a portion of a lot.
- Each one of a series of wagons, lorries, ship's bays, etc., may be considered a separate lot.

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Sample

Incremental sample (Increment)

A quantity of material taken from a single random place in the lot or sublot.

Aggregate sample

The combined total of all the incremental samples taken from the lot or sublot.

Laboratory sample

The sample finally submitted to the laboratory

(CXS 193)

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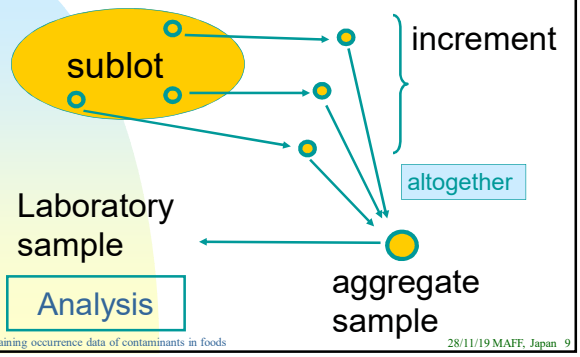
Sampling



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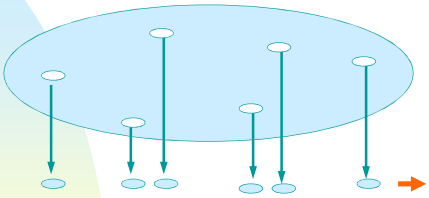
Sampling from homogeneous lot



- e.g. liquid food

small sampling uncertainty (small sampling error)

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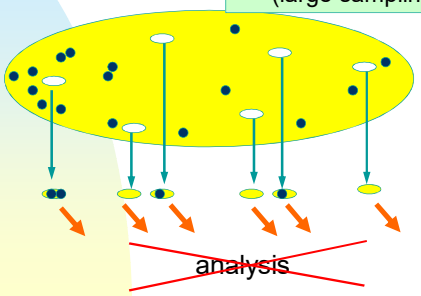
Sampling from heterogeneous lot

large sampling uncertainty (large sampling error)



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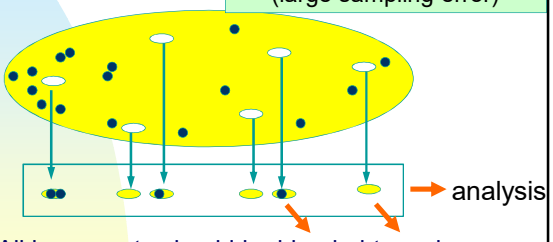
Sampling from heterogeneous lot

large sampling uncertainty (large sampling error)



- All increments should be blended to make homogeneous laboratory sample

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Sampling = Sampling plan + Sampling procedure

- Sampling plan

A scheme defining the number of items to collect and the decision criterion
1) the number of non-confirming items required in a sample to evaluate the compliance status of a lot; or
2) Calculation using data (analytical results)

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- Sampling procedure

Operational requirements and/or instructions relating to the use of a particular sampling plan; i.e. the planned method of selection, withdrawal and transport to the laboratory of sample(s) from a lot or consignment to yield knowledge of its characteristic(s).

- ✓ size and number of individual items forming the sample taken from the lot or consignment
- ✓ collecting, handling and recording the sample(s)

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Basic concepts of sampling

- **Random sampling** (not intentionally chosen)
  - Taking samples randomly (equal probability)
  - Using random number table or statistical software generating random number
- It is not always easy
  - e.g. innermost of the warehouse

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Types of random sampling

Cluster sampling (single stage cluster sampling)
- ✓ dividing the population into sub-groups (or clusters)
- ✓ a clusters is randomly chosen.

Multistage random sampling
- ✓ dividing the population into sub-groups (or clusters)
- ✓ one or more clusters are randomly chosen
- ✓ element within the chosen cluster is sampled.

Stratified random Sampling
- ✓ dividing the population into homogeneous (based on some characteristic) sub-groups (or strata)
- ✓ Simple random sampling is performed within each stratus.

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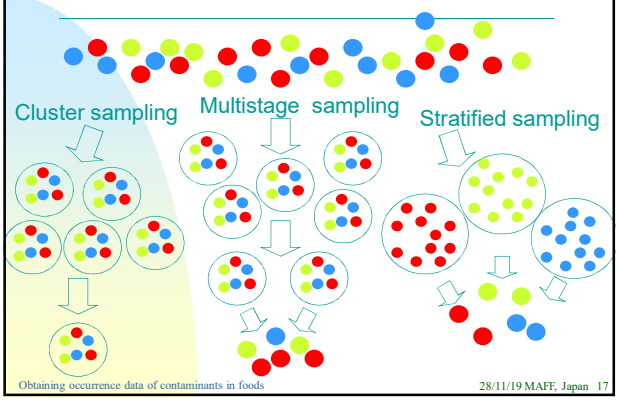
Types of random sampling



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2. Sampling for surveillance to obtain occurrence data

How to decide sample size for surveillance to obtain occurrence data?

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Surveillance and sampling plan

What do we want to know?

- Nation-wide contamination or regional contamination?
- seasonal difference or annual average?
- Average or high percentile?

By selecting appropriate sampling plan, we want a subset of a statistical population that accurately reflects the members of the entire population.

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Mean and Median (1)

mean

- $(x_1+x_2+\ldots x_n)/\mathbf{N}$
- sensitive to extreme values
- For datasets including <LOQ values, calculations are needed to estimate upper, lower and medium boundary mean values

median

- the middle value in a set of values arranged in order of size:
- the average of the two middle values if **N** is even.
- a robust measure of central tendency

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Percentile (%ile)

- The values are ranked in ascending order, i.e. from smallest to largest.
- For example, the 20th percentile is the value below which 20% of the observations may be found.
  - 25th percentile (25%ile): the first quartile (Q_1)
  - 50th percentile (50%ile): median or the second quartile (Q_2)
  - 75th percentile (75%ile) : the third quartile (Q_3).

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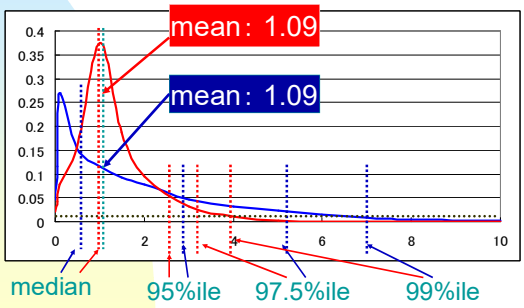
Two distributions, same mean



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Surveillance of contaminants

- Identification of food group(s) to be investigated further
  - preliminary survey (e.g. 10 to 20 samples for various kinds of food)
  - 2 samples for each food are not sufficient
- Identification of high percentile values
  - High percentile values (e.g. 95th, 97.5th, 99th percentiles) are necessary for setting ML or exposure assessment, etc.
  - CCCF – normally 2-3% of violation rate

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Sample size for surveillance on contaminants

- Need to consider cost for surveillance
- For 97.5th percentile, at least one above 97.5th percentile of the population in a occurrence data is necessary.
- Need to consider minimum number of samples to know high percentile value with 95% confidence level
- Impossible to analyze all food samples.
- Impossible to know maximum value.

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Using the concept of binomial distribution

- Probability that one independent data (i.e. sample size 1) is larger than 90th percentile value of a population.

  (90th percentile value of a population means 90% of data are smaller than that value.)

  (1-0.9) = 0.1 (=10%)

- EXAMPLE: Probability that at least one data larger than 90th percentile of a population is found in a dataset with 95% confidence.
  1－(Probability that all values in a dataset, size *n*, are smaller than 90th percentile of population)
  $1-0.9^n = 0.95 \Rightarrow n = \ln(1-0.95)/\ln 0.9$

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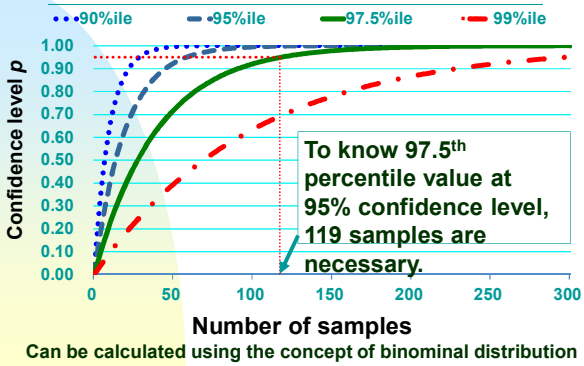
Precentile, No. of sample, and Confidence Level



Can be calculated using the concept of binominal distribution

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3. Sampling for conformity assessment in regulation

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Sampling inspection vs 100% inspection

➢**Sampling inspection**
- ✓ All lot inspection (samples taken from all lots) by using huge cost is impractical.
- → Statistical sampling plan is needed.

➢100% inspection is impossible (except visual inspection)
- ✓testing is usually destructive
- ✓the cost of 100% inspection is very high; and
- ✓100% inspection takes too long.

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Two types of sampling plan

Sampling plan: a scheme defining the number of items to collect and the number of non-confirming items required in a sample

➢Attribute Sampling Plan
➢Variable Sampling Plan
- ➢Quality characteristics are measured in a continuous scale (e.g. concentrations mg/kg)

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Interpretation of testing results

- If samples are representative of the lot, testing result ≤ ML means the whole lot conforms with the ML
- Actually, the above is not always true.

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Example: sampling plan for the analysis of OTA in wheat

- **FAO Mycotoxin Sampling Tool**
  - http://tools.fstools.org/mycotoxins/
- Regulatory Limit (10 ug/kg)
- Laboratory sample size – 2 kg
- Test portion – 5 g
- Accept / reject limit – 10 ng/kg

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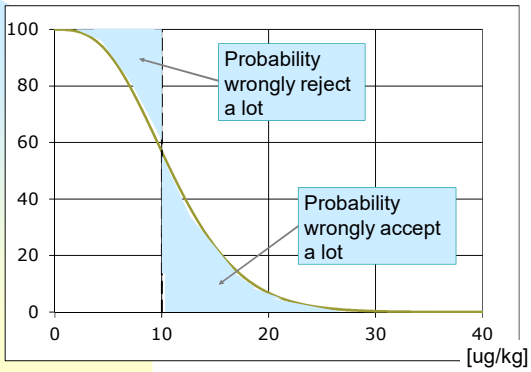
OC curve: OTA in wheat



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5. Validation of analytical method

Use of Validated Analytical method

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Inadequate data (example)

5.3 Distribution

The data from Brazil and Uruguay could not be used as they had been obtained by analytical methods with high LODs, ranging from 5 µg/kg to 50 µg/kg, which are inadequate to detect and measure ochratoxin A at 0.94 µg/kg and 0.19 µg/kg, the weighted mean concen-trations found in Europe and the USA in cereals and cereal products, respectively.

(JECFA 47, 2001)

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Validated method

"If you don't use validated methods, only you will know how good your numbers are."

AOAC International

***Validated Test Method*:**
An accepted test method for which validation studies have been completed to determine the accuracy and reliability of this method for a specific purpose.

Reference:
ICCVAM Guidelines for the nomination and submission of new, revised and alternative

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Method Validation Study

- Validated method is a method tested in many laboratories on the same samples, showing that the method is rugged enough to produce comparable results in different labs, with different operators.

Collaboration study, Ring-test- trial

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Validation Study Protocol and Guidelines

- IUPAC
  Protocol for the Design, Conduct and Interpretation of Method-Performance Studies. Pure & Appl. Chem., 67(2), 331-343 (1995) http://www.iupac.org/publications/pac/67/2/0331/
- AOAC International
  AOAC Official Methods of Analysis (2002). Interlaboratory Collaborative Study, Appendix D: Guidelines for Collaborative Study Procedures To Validate Characteristics of a Method of Analysis http://www.aoac.org/vmeth/guidelines.htm
- ISO 5725-2:1994
  Accuracy (trueness and precision) of measurement methods and results -- Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method

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Recommended methods in Codex

General Methods of Analysis for Contaminants (CODEX STAN 228-2001)

Recommended Methods of Analysis and Sampling *(CODEX STAN 234-1999)*

Special foods	Linoleate (in the form of glycerides)	AOAC 922.06; 969.33; 963.22	Acid hydrolysis, preparation of methyl esters and gas chromatography	II
Special foods	Linoleate (in the form of glycerides)	AOAC 922.06; 979.19	Acid hydrolysis and spectrophotometry	III
Special foods	Loss on drying (milk based)	AOAC 925.23 -ISO 6731|IDF 21	Gravimetry	I
Special foods	Nicotinamide for foods not based on milk	AOAC 961.14	Colorimetry	II
Special foods	Nicotinamide for milk-based foods	AOAC 944.13	Microbioassay	II
Special foods	Pantothenic acid/enriched foods	AOAC 945.74	Microbioassay	II
Special foods	Pantothenic acid/non-enriched foods	*The Analyst* 89 (1964):1, 3-6, ibid. 232 US Dept Agr., *Agr. Handbook* 97 (1965)	Microbioassay	IV
Special foods	Phosphorous	AOAC 986.24	Colorimetry	II

Type I: Defining methods
Type II: Reference methods
Type III: Alternative approved methods
Type IV: Tentative method - most likely not validated!

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Performance characteristics

- Limit of detection (LOD)
  - The lowest concentration that show the presence of analyte
  - The concentration cannot be determined.
- Limit of quantification (LOQ)
  - The lowest concentration that can be measured
  - Generally 2-3.3x higher than LOD
- There are many definitions and ways of calculation

⇒ Analytical results should be accompanied with the information of LOD and LOQ

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Performance characteristics

- Recovery
  - The percentage of analytical value of spiked sample
- Relative Standard Deviation
  - Standard deviation divided by mean
  - For evaluation of variability (“uncertainty”) of the method

- RSD_R - between laboratories
- RSD_i - same laboratory but different days
- RSD_r - same laboratory on the same day

Generally larger

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What is Measurement Uncertainty?

- DOES NOT mean the analytical value is uncertain
- Estimated range in which the true value would be found
- Showing reliability of the results
- Estimated by experimental data and statistical evaluation
- MU can be estimated from RSD_R

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Measurement Uncertainty in Codex

Definition

Measurement uncertainty:

Non-negative parameter characterizing the dispersion of the values being attributed to a measurand, based on the information used.

Expanded measurement uncertainty:

product of a combined standard measurement uncertainty and a factor larger than the number one

Guidelines on Analytical Terminology (CAC/GL 72-2009)

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Performance characteristics

- HorRat
  - RSD divided by the value calculated by Horwitz equation
  - Generally,
    - For RSD_R: 0.5 ≦ HorRat ≦ 2
    - For RSD_r : 0.3 ≦ HorRat ≦ 1.3

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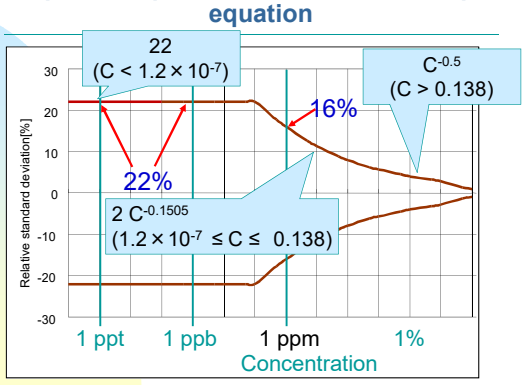
Graphical expression of Horwitz/Thompson equation



Thompson, M., Analyst, 125, 385-386 (2000)

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Criteria approach

- Identify method performance characteristics based on existing method validation data and establish criteria for evaluating acceptable method of analysis

⇒ Laboratory can select a method that meets the criteria

⇒ Laboratory has flexibility on analytical method.

Reference: Codex Procedural Manual

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Examples for numeric values for the criteria

Applicability:	The method has to be applicable for the specified provision, specified commodity and the specified level(s) (maximum and/or minimum) (ML). Expressed in terms of the reproducibility standard deviation (s_R) or in terms of LOD and LOQ.
Minimum applicable range:	For ML ≥ 0.1 mg/kg, [ML - 3 s_R , ML + 3 s_R] For ML < 0.1 mg/kg, [ML - 2 s_R , ML + 2 s_R] s_R* = standard deviation of reproducibility

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Examples for numeric values for the criteria

LOD:	For ML ≥ 0.1 mg/kg, LOD ≤ ML · 1/10 For ML < 0.1 mg/kg, LOD ≤ ML · 1/5
LOQ	For ML ≥ 0.1 mg/kg, LOQ ≤ ML · 1/5 For ML < 0.1 mg/kg, LOQ ≤ ML · 2/5
Precision:	For ML ≥ 0.1 mg/kg, HorRat value ≤ 2 For ML < 0.1 mg/kg, the RSD_R < 44%. RSD_R= relative standard deviation of reproducibility. RSD_R ≤ 2. $PRSD_R$

* The s_R should be calculated from the Horwitz / Thompson equation

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6. Requirement for laboratories

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Codex guidelines for Import and Export Control testing laboratories

- Compliance with the general criteria in ISO/IEC 17025 → maintain compliance
- Internal quality control procedures
- Participation in proficiency testing for related combination of analyte/matrix
- Use of validated analytical method

Guidelines for the Assessment of the Competence of Testing Laboratories Involved in the Import and Export Control of Food (*CAC/GL 27-1997*)

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Food Control Laboratory Management: Recommendations

Protocols and Guidelines adopted in Codex

- International Harmonized Protocol for the Proficiency Testing of Testing of (Chemical) Analytical Laboratories
  Pure & Appl. Chem., 65 (1993) 2132-2144
- Protocol for the Design, Conduct and Interpretation of Method Performance Studies
  Pure & Appl. Chem., 67 (1995) 331-343
- Harmonized Guidelines for Internal Quality Control in Analytical Chemistry Laboratories
  Pure & Appl. Chem., 67 (1995) 649-666

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Exercise 2

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Exercise 2

Exercise 2 Minimum number of samples

Calculate minimum sample size obtaining at least one value higher than 98th percentile with 95% confidence

(i.e. violation rate of 2%)

N.B. Confidence level at 95% is generally used in Codex and developed countries

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Exercise 2

Exercise 2 Sample size for surveillance

Calculate minimum sample size obtaining at least one value higher than 98th percentile with 95% confidence

Percentile | Min. sample size | confidence

$$1 - P^n = p$$

$$n = \frac{\ln(1-p)}{\ln P}$$

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Exercise 3

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Data analysis using occurrence data

Purpose:

- To estimate population (e.g. nation-wide situation) from data
- For further consideration
  - setting maximum level
  - evaluating effectiveness of risk management measures
  - trend analysis

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Datasets

- The larger the sample size is, the better results you will get
- May be good to combine available datasets if appropriate.
- How to determine whether multiple datasets can be combined?
  - Is it appropriate to assume these samples come from the same population?
  - Are these data statistically not different?

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Kruskal-Wallis H test

- Non-parametric – datasets need not follow any specific distribution
- Comparison of 2 or more datasets
- Result: Datasets are significantly different or not significantly different at a certain confidence level
  - Null hypothesis H_0
    -> the distributions of both populations are equal.
  - The alternative hypothesis H_1
    -> the distributions are not equal.

NB: For 2 datasets, it is called “Mann-Whitney U test”

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Exercise 3.1

- Check if DATA1 and DATA2 are significantly different (confidence level=95%).
  - If they are not significantly different, you can combine these datasets. Then, calculate mean, median, maximum, minimum and sample standard deviation for the combined dataset.
  - If they are significantly different, you cannot combine them. Then, calculate means, medians, maximum, minimum and sample standard deviation for DATA1 and DATA2.

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U-test table

n1\n2	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
2											0	0	0	1	1	1	1	1	2
3							0	0	1	1	2	2	3	3	4	4	5	5	5
4					0	0	1	2	3	3	4	5	5	6	6	7	8	9	10
5				0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
6			0	1	2	3	4	5	7	8	9	11	12	13	15	16	18	19	20
7			0	2	3	4	5	7	8	9	11	12	14	16	17	19	21	22	24
8		0	1	3	4	6	7	9	11	12	14	16	17	19	21	22	24	26	27
9		0	2	4	5	7	9	11	13	15	17	19	21	23	25	27	29	31	33
10		1	3	5	7	9	11	13	16	18	20	23	25	27	30	32	35	37	39
11		1	3	6	8	11	13	16	18	21	24	26	29	32	35	37	40	43	46
12	0	2	4	7	9	12	15	18	21	24	27	30	33	36	39	43	46	49	52
13	0	2	5	8	11	14	17	20	24	27	30	34	37	41	44	48	51	55	58
14	0	3	6	9	12	16	19	23	26	30	34	38	41	45	49	53	57	61	65
15	1	3	6	10	13	17	21	25	29	33	37	41	46	50	54	58	62	67	71
16	1	4	7	11	15	19	23	27	32	36	41	45	50	54	59	64	68	73	78
17	1	4	8	12	16	21	25	30	35	39	44	49	54	59	64	69	74	79	84
18	1	5	9	13	18	22	27	32	37	43	48	53	58	64	69	74	80	85	90
19	1	5	9	14	19	24	29	35	40	46	51	57	62	68	74	80	85	91	97
20	2	5	10	15	20	26	31	37	43	49	55	61	67	73	79	85	91	97	103

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What do you do if many analytical values are < LOQ ?

- There are many kinds of approach – the approach taken should be documented
- One approach: use both LB and UB datasets
  - Lower bound (LB) – replacing the results below LOQ with 0
  - Upper bound (UB) – replacing the results below LOQ with LOQ

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Relevant Excel functions

statistics	Excel function
Mean	average(range)
Median	median(range)
Maximum	max(range)
Minimum	min(range)
Sample standard deviation	stdev.s(range)
Parent standard deviation	stdev.p(range)

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Exercise 3.2

Creating a frequency table, histogram

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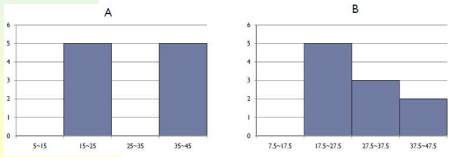
Number of bins and class interval of histogram

- Shape of histogram varies by bin width
  - Too small or too large bin size gives not enough information on distribution.
- There is no "best" number of bins, and different bin sizes/interval can reveal different features of the data.



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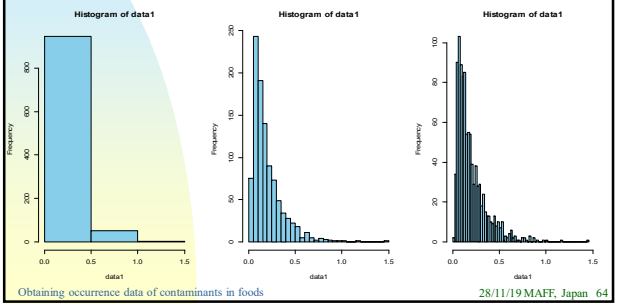
Effect of bin size using same dataset

Try to make histograms with various bin size

Too large bin width | Good bin width | Too small bin width



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Histogram

1. Purpose
   to graphically summarize the distribution of a data set
2. Steps for drawing histogram
   - Frequency table
     - decide class interval or bin size, usually ten or more
     - need to consider border value to include lower or upper class
   - Calculating relative frequency, cumulative frequency
   - Making bar plot with no gap width between each bar

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Excel function

"frequency" function

① Enter the bin numbers (upper level)
(In this case, they are A1:A5)

② Select columns for the result (B1:B5)

③ type "=frequency([range of data], [bin numbers])

④ CTRL+SHIFT+ENTER

0.03	92
0.06	87
0.09	49
0.12	28
0.15	19
0.18	10

	A	B	C
1	0.2	177	
2	0.4	79	
3	0.6	29	
4	0.8	10	
5	1	5	
6			
7			

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Exercise 3.3

Calculation of high percentile

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Exercise 3.3

For risk management,
- High concentration sample
- High exposure

may be of interest.

-> High percentile

1) Calculation with actual data – use Excel function
2) Modelling the actual data – use @Risk etc.

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Exercise 3.3

- Calculate
  - 50 %ile (median)
  - 95 %ile
  - 97.5 %ile
  - 99 %ile

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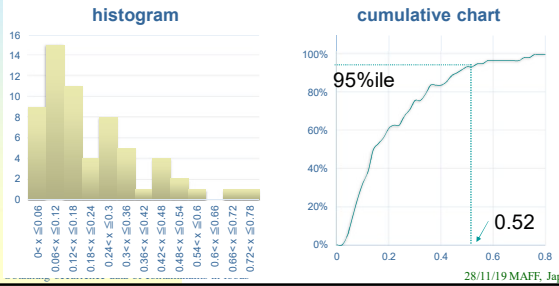
Cumulative chart

- Useful for displaying the current totals of several different series of data in one comparative visual model



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Summary– important points

- Data analysis is critical for risk management.
- Exercise by yourself for better understanding.
- In actual situation, collaboration with government scientists, laboratory analytical chemists, and statisticians is needed.

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