Basic principles for Assessment of Bioactive Molecules-

B. Sesikeran MD FAMS
Chairman K-FFIG
Former Director
National Institute of Nutrition
Hyderabad

Functional Food- Nutraceutical

- **Functional Food-** Food or food ingredient which gives a additional health benefit

- **Nutraceutical**- Food ingredient which is used like a pharmaceutical – a product isolated or purified from foods that is generally sold in medicinal forms not usually associated with food. A nutraceutical is demonstrated to have a physiological benefit or provide protection against chronic disease- Health Canada

- A **dietary supplement** is a product that contains nutrients derived from food products that are concentrated in liquid or capsule form.

FSSAI definitions 1

- **Health supplement** is a category of foods, which consists of a concentrated source of nutrients (like proteins, minerals, vitamins, amino acids) and/or other ingredients with nutritional or physiological effects, singly or in combination, whose purpose is to supplement the normal diet.

FSSAI definitions 2

- **Ingredient** means plant or botanicals and their extracts, probiotics, prebiotics, and molecules/isolates as listed by Food Authority in its Schedule II, III and IV.

- **Nutraceutical** is a category of foods which consists of extracts, isolates and purified chemical compounds having a physiological benefit and help to maintain health.

- **Nutrient means** vitamins, minerals, amino acids and other nutrients as specified by Food Authority from time to time.

What is a Bioactive Molecule

- A naturally occuring molecule from any living system- plant/ animal/ fungi/ bacteria/ algae/terrestrial/ marine which has a demonstrable and measurable biological activity eg anti proliferative, anti oxidant, anti infective, growth promoting, cholesterol lowering etc

Definition- simplistic view

- Carrot - Food – vegetable
- Carrots prevent blindness due to Vitamin A deficiency – Functional food
- β carotene in carrot – pro vitamin A – Bioactive molecule
- β carotene supplements – Nutraceutical/ food supplement

Functional Component (bioactive molecules)	***Source***	***Health Benefit***
Alpha-carotene Beta-carotene	carrots fruits, vegetables	neutralize free radicals,
Lutein •	green vegetables	reduce risk of macular degeneration
Lycopene	tomato	reduce risk of prostate cancer
Insoluble Fibre	wheat bran •	reduce risk of breast or colon cancer
Beta-Glucan Soluble Fibre	oats psyllium	reduce risk of CVD ,,

Bioactives	***Food Source***	***Health benefit***
Omega-3 •	Fish and fish oils	reduce risk of CVD improve mental, visual functions
•Flavonoids Anthocyanidins	fruits	neutralize free radicals reduce cancer risk
Catechins	tea	,,
Flavanones	citrus	,,
Flavones	fruits/vegetables	,,

Functional component	Source	Health Benefit
stanol ester	corn, soy, wheat,	• inhibit cholesterol absorption
Fructo-oligosaccharides (FOS)	onion	Pre biotics
Lactobacillus	yogurt, other dairy	Gut health
Isoflavones: •Daidzein Genistein	soya- soy-based foods	menopause, CVD lower LDL
Lignans	flax, vegetables	,,
Proanthocyanidins	cranberries, cocoa,chocolate	improve urinary tract health reduce CVD ? Complications of DM

Bioactive Molecules in Indian Spices

SPICE	BIOACTIVE	FUNCTION
BLACK PEPPER	PIPERINE	AO,AI,LL, AC
CARDAMOM	LINALOOL	
CLOVES	EUGENOL	
CHILLI	CAPSAICIN	
CINNAMON	CINNAMALDEHYDE	
CORIANDER	D-LINALOOL	
CUMIN	CUMIN ALDEHYDE	
TURMERIC	CURCUMIN	
GINGER	GINGEROL	
MUSTARD	ALLYL ISO THIOCYANTE	
SAFFRON	SAFRANOL	
GARLIC	ALLICIN	

Historical Characterisation

- Source / sources of Bioactive Compound – Taxonomy of plant

  Eg Lycopene – Tomatoes, Red Carrots, Red Capsicum, Water melon

- History of Safe human use – traditional or Published data (FSSAI– 30yrs in country of origin OR 15 Yrs in India)

- Safe level of consumption / upper safe limit through food eg fenugreek was 6 Gms (Traditional) , as functional food – 20 gms

Physico Chemical Characterization

- Structure of the compound, molecular formula- Chemical fingerprint
- Available single or a family of molecules
- Category and variants eg Carotenoids/ Curcuminoids
- Molecular weight, solubility, stability etc
- Thermodynamic and spectral data
- Isolation of compound/ sample separation , synthesis
- Purity of final substance and impurities
- Preservation, storage , interactions with other substances

Pharmacokinetics

- Bioavailability
- Absorption
- Half life
- Accumulation in tissue
- Distribution
- Metabolism
- Excretion

Biological Activity

- Anti Oxidant/ anti Inflammatory / anti cancer etc
- Method of assessing and quantifying this activity and validated assay methods
- In vitro and In vivo methods
- ED 50
- Toxicokinetics

Biological Activity

- Adverse effect level –LOAEL / NOAEL
- Safe Upper Limit
- Acceptable Daily Intake
- Effect in physiological states like pregnancy, Children, Lactation

Bioinformatics

- Structure function similarities with other known molecules
- Compare in allergen database
- Toxicity Database and Drug interaction database

Identification of Biomarkers

- Identified and validated for their predictive value.

❖ Markers related to level of consumption and bioavailability–

Eg: Plasma levels of the bioactive molecule

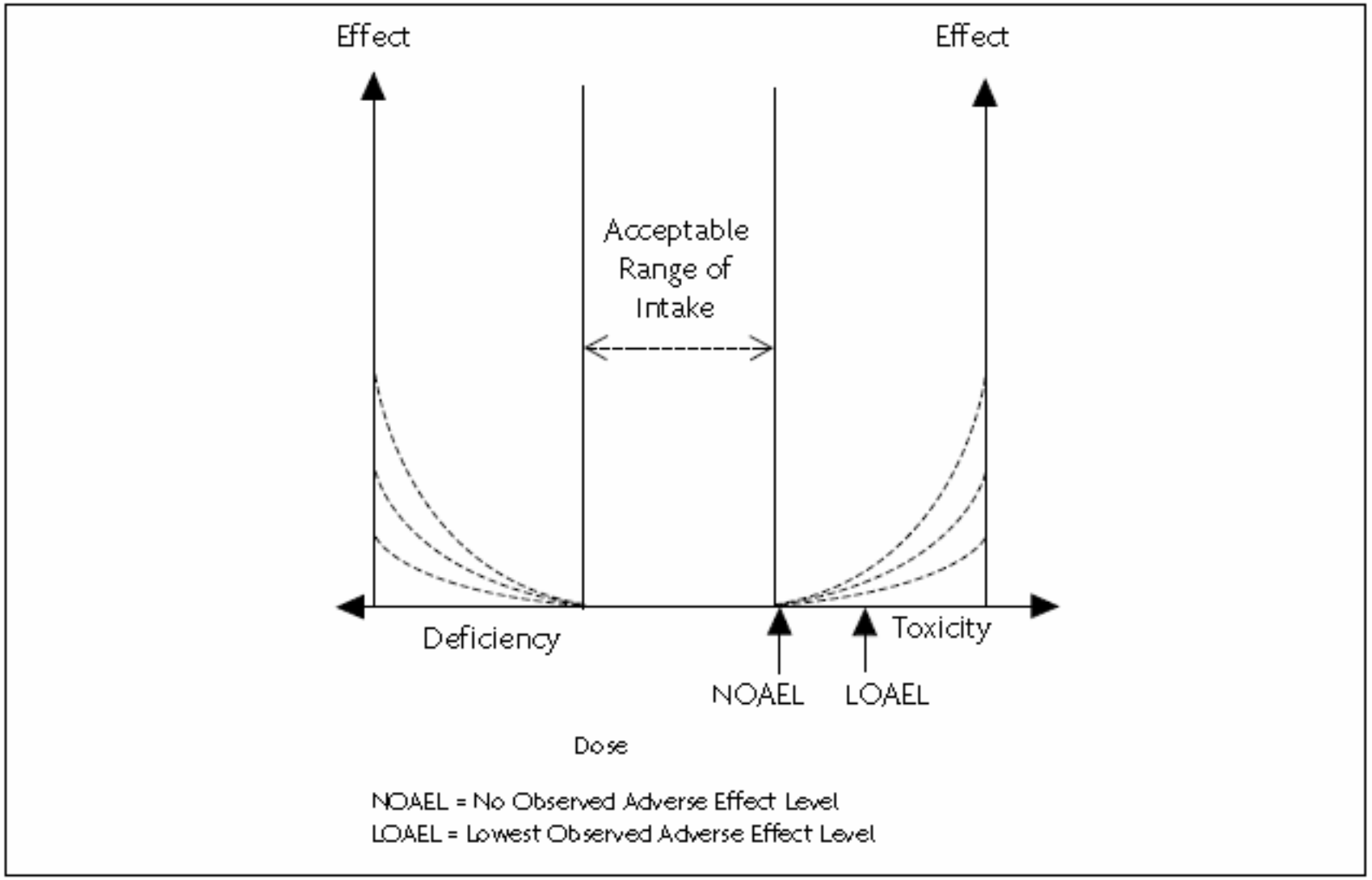
❖ Markers correlated to outcomes are indicator markers/ effect markers

Eg: Stanol consumption and Serum Cholesterol

❖ If the markers are related to risk of disease they are known as susceptibility markers.

Eg : Ratio of LDL cholesterol to Total Cholesterol and risk of CHD

Figure 1. Theoretical dose-response relationships in humans



Supplemental intake + Dietary and other known exposures = Estimated SUL (total)

Risk assessments

- Risk assessment :
- Hazard identification (Adverse effects)
- Hazard characterisation (including dose-response assessment);
- Exposure assessment
- Risk characterisation.
- Risk-benefit analysis

Extrapolating LOAEL to derive NOAEL (based on human data)

- If adverse effect is a biochemical change with no clinical or organ correlation – factor is 3
- Eg Serum transaminase levels were elevated at 30mg dose i.e LOAEL
- Then NOAEL will be 30/3 = 10 mg
- If adverse effect was a serious toxic change then NOAEL will be 30/10= 3 mg

Extrapolating NOAEL to derive SUL (based on ANIMAL data)

- If NOAEL is 10 mg
- SUL = 10 / 10 x 10 = 0.1 mg
- Factor of 10 for inter species variation
- Factor of 10 for inter individual variation

PRINCIPLES FOR ADDITION OF DIETARY ACTIVE COMPOUNDS IN FOODS

- Active compounds should be present at a level which will not result in either excess or insignificant intake
- Should be sufficient to exercise its beneficial effect
- Should not result in an adverse effect on the metabolism of any other nutrient
- Should be stable in food under customary conditions of packaging, storage, distribution and use
- Should be biologically available from the food
- Methods of measuring should be available

HUMAN STUDIES

- Data from other countries

  Target population – Indian men / women / children / elderly

- Comparative study

  Placebo Vs. Nutraceuticals

  Low dose Vs. High

  Traditional Vs. test

- Clear cut end points/outcomes: Biomarkers if validated.

SCIENTIFIC CONTRADICTIONS

- Vitamin E, betacarotene – benefits in case control
- Increased mortality and morbidity in RCT
- Long term selenium increases risk of type 2 DM
- Level of supplementation (200 µg – ½ SUL)
- Folic acid supplementation in question
- Health claims require validation and substantiation

Conclusion

- Translate new knowledge into product
- Ensure Safety and Quality
- Evaluate Efficacy – limited studies
- Make content Claim
- Evaluate product for specific health outcomes
- Make a product Claim

EVALUATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF ---------- AS A FOOD----------

Template

Required Information and data 1

Basis of Conclusion
Name and address of organization:
Name of substance:
Intended conditions of use:
Statutory Basis for GRAS conclusion:
Exemption from Premarket approval requirements:

Required Information and data 2

Part II- IDENTITY AND TECHNICAL INFORMATION

Description
Botanical identification
Specifications
Manufacturing Process
Biologically Active Constituents

Required Information and data 3

Part III- DIETARY EXPOSURE

Technical Effects
Intended Use Levels and Food Categories
Estimated Daily Intake from the Intended Use

Exposure Summary

Part IV- SELF LIMITING LEVELS OF USE
Part V- EXPERIENCE BASED ON COMMON USE IN FOOD

Required Information and data 4

Part VI- NARRATIVE
Data Pertaining to Safety
Common Knowledge of Safe Use
Toxicity Studies
Acute Toxicity Studies
Repeat-dose Toxicity Studies in Animals
Other Animal Studies-Carcinogencity, Mutagenicity

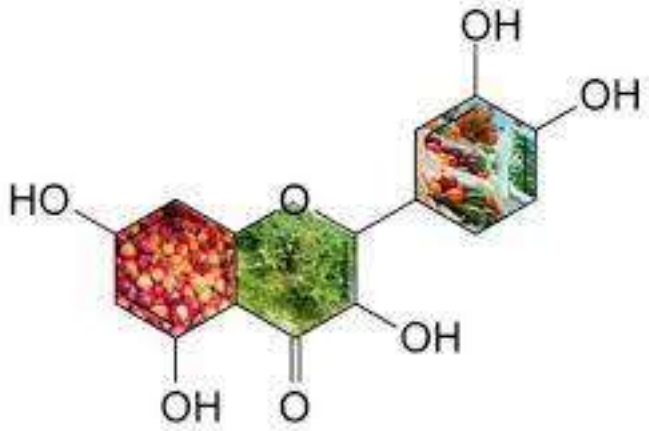
Required Information and data 5

Human Clinical Studies

Biochemical and Pharmacological Effects Expert Panel Review, Summary and Discussion

Expert Panel Conclusion

Part VII– SUPPORTING DOCUMENTS AND REFERENCES



Thank You