Whole Food Testing

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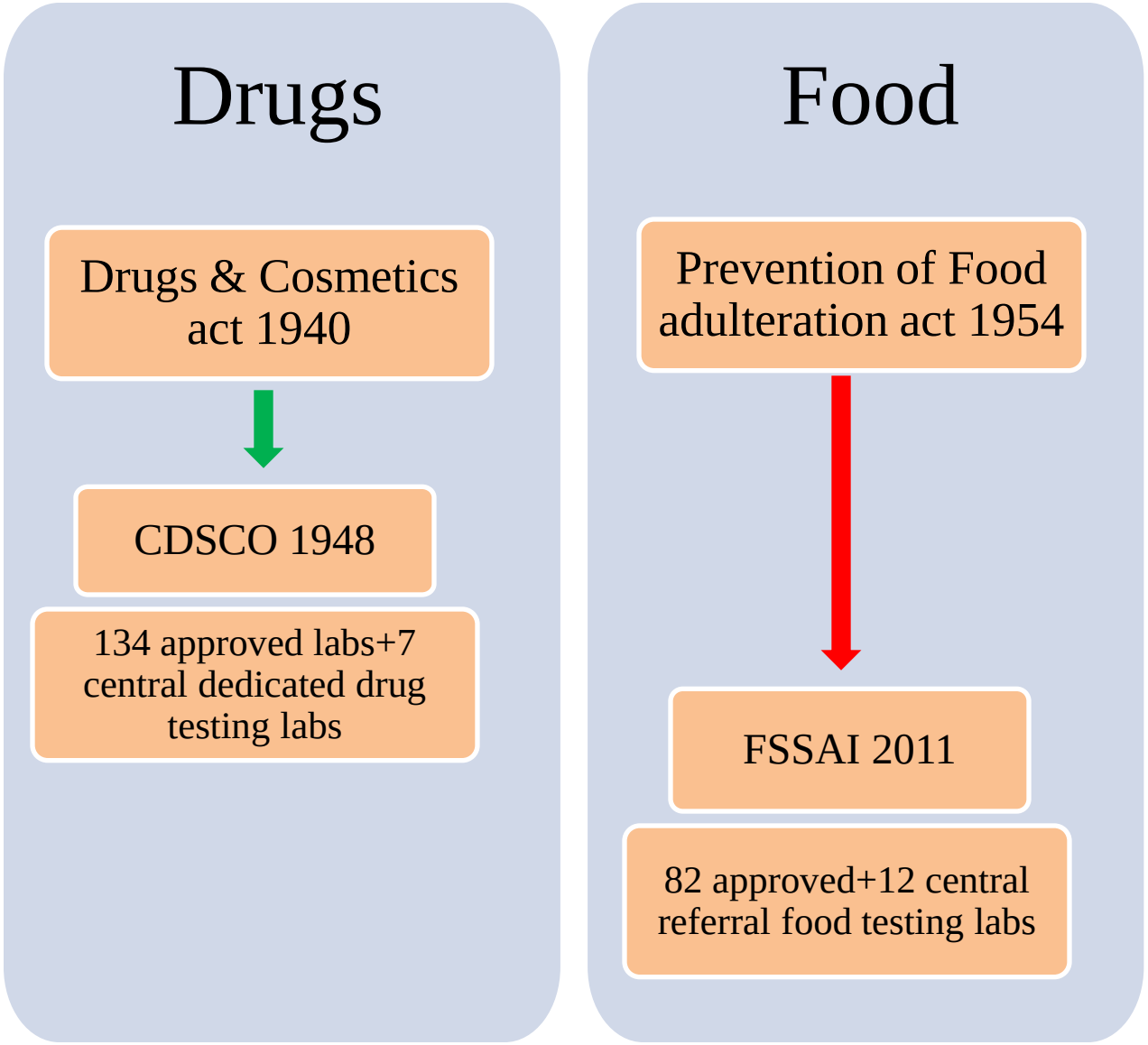
FOOD

Food Means any article used as food or drink for human consumption other than drug and also includes:

- Any article which enters or used in the composition or preparation of food
- Any flavoring matter or condiments
- Any other article notified by the Central Govt. e.g. packaged drinking water

Food Safety and Standards Act, 2006, India

“Drugs for the disease and food for the life. Imagine how good the regulations should be for the thing to which long term exposure occurs?”


Drugs
Drugs & Cosmetics act 1940
CDSCO 1948
134 approved labs+7 central dedicated drug testing labs
Food
Prevention of Food adulteration act 1954
FSSAI 2011
82 approved+12 central referral food testing labs

Why Food Safety?...

- Globalization of trade in food.
- Changing products, processes etc.
- Food handling practices
- More than 200 known diseases are transmitted through food[1]

"Food Safety is a hidden, often overlooked problem"

1. Mead et al., (1999). Food-related illness and death in the United States. . Emerg infect dis 5: 607-625.

Advances in Food/Food products

- Organic food
- Biotechnology-derived (BD) food
- Irradiated food
- Frozen food
- Freeze dried food
- Functional foods and Neutraceuticals

The term ‘Genetically Modified (GM)’ foods appears to be a misnomer because conventional techniques of plant, animal and microbial breeding also involve genetic modification, which is generally undefined. So, it is apt to use the term ‘biotechnology-derived (BD)’ foods.

Issues of health effects of BD-foods

➢ Toxicity of transgene *per se*

- Dietary DNA is not new to our digestive system and that derived from plant/animals is not toxic, in fact good for gut function[1,2].
- Foreign DNA is removed by hydrolysis during digestion, excision from the host genome, and targeted DNA methylation[3].

1. FAO/WHO (2000). Safety aspects of genetically modified foods of plant origin: Report of a Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology.;
2. Royal Society (2002). Genetically Modified Plants for Food Use and Human Health—An Update. Policy Document 4/02.
3. Doerfler, W. (1991). Patterns of DNA methylation—Evolutionary vestiges of foreign DNA inactivation as a host defense mechanism. Biol. Chem. Hoppe- Seyler 372: 557–564.

- **Toxicity of transgene-product in the whole food is to be considered on a case-by-case basis**

  •Transgene products - toxin (eg., Bt endotoxin, which is harmless in mammals), allergens (eg., Kiwi, which was once non allergic but now-a-days proved to be allergic).

Examples:

1) **StarLink corn**: To improve the insecticidal activity of Bt protein, Cry9C was created. It had negative impact on the health of humans and hence was not approved[1].

2) Nutritional quality of soybeans was sought to be improved using a brazil nut protein, the methionine rich 2S albumin. It failed because people allergic to Brazil nuts also reacted to the new soybean[2].

1. CDC (2001) Investigation of human health effecs associated with potential exposure to BD corn. A Report to the USFDA from the CDC
2. Nordlee *et al* (1996). Identification of a Brazil-nut allergen in transgenic soybeans. N Eng J Med 334: 688–692.

- **Unintended effects resulting from the insertion of transgene into the host genome**

  Current genetic engineering processes insert transgene randomly. Therefore, probability of pleiotropic and insertional mutagenic effects.

Examples:

1) *A1 gene* codes for salmon red color in Petunia flower. Genetic modification for improving the yield led to unexpected color patterns because of deletion of A1 gene/hypermethylation of its promoter[1].

2) Tryptophan-associated eosinophilia-myalgia syndrome (EMS): When a second BDO was used, the agent responsible for tryptophan-associated EMS increased in content but no novel toxicant was created[2].

1. Meyer et al. (1992). Endogenous and environmental factors influence 35S promoter methylation of a maize *A1* gene construct in transgenic Petunia and its color phenotype. Molec Gen Genet 231: 345–352.
2. Sullivan *et al*. (1996). EMS among non-L-tryptophan users and pre-epidemic cases. J Rheumatol 23: 1784–1787

- **Transfer of Antibiotic Resistance Marker Genes from the BD Food to Gut Microbes**[1]
  - •Genomic DNA is efficiently degraded in the gut
  - •Plant to microbe gene transfer rate is very low

- **Effect of transgene-product on non-target organisms**
  - • Bt affects not only the target insects - Lepidoptera, Coleoptera, or Diptera, but also the larvae of non-target insects like Monarch butterfly, which helps in pollination[2].

1. Royal Society (1998). BD plants for Food use. Policy Document 2/98
2. Losey *et al* (1999). Transgenic pollen harms monarch larvae. Nature 399: 214.

Misbelief about BD crops

- Potential adverse effects of BD varieties on indigenous flora and fauna brought into the public by anti-BD activists.

Bt gene product affects not only the target insects but also the larvae of non-target insects like Monarch butterfly, which helps in pollination. This has been observed at laboratory level but not at field level because of very limited synchrony between the time point of pollination and formation of larvae[1].

Gatehouse *et al*. (2002). The case of the monarch butterfly: A verdict is returned. Trends Genet 18: 249–251.

- Some academics have aligned with the ‘consumer interests’.

  Glyphosate is a broad-spectrum weedicide. Corn was engineered to be glyphosate-resistant. A study proved its carcinogenic property. This report was encashed by many BD activists. But later proved that the study was methodologically and statistically flawed leading to its retraction[1].

Because of all these, the public now mistrusts most mainstream sources of data on BD food despite the endorsements by the regulatory bodies –USFDA, USNAS, AMA, EC about their safety and the use of BD crops for decades.

1. Seralini *et al*. (2012). Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. Food Chem Toxicol 63:244

“In terms of risk, how a food crop is created is totally irrelevant –it is what is in the food that is important”

Issues of BD Food Testing

- Points to be focussed in Safety assessment of BD-foods[1]:
  - The whole food (food product) itself, rather than the process through which it is made.
  - "**Substantial Equivalence**"

  New BD food should be substantially equivalent in composition and nutritional characteristics to the existing food.
- Current safety testing concentrates on the toxicity of single chemicals rather than the whole food.
- No adequate animal models for certain toxic conditions e.g., Eosinophilia-Myalgia syndrome (EMS).

1. Hollingworth *et al*. (2003). The Safety of BD Foods produced through Biotechnology. Toxicol Sci 71:2-8

Whole food (WF) toxicity study design considerations

If the composition of the food and/or feed derived from BD plant is substantially modified, or if there are any indications for the potential occurrence of unintended effects based on the preceding molecular, compositional or phenotypic analyses, not only new constituents but also the whole food and feed derived from the BD plant should be tested.

Limitations of whole food (WF) animal studies in the safety assessment of BD crops

- Low toxicological power of whole food toxicity studies and flawed study design and failure resulted in numerous studies of BD crops as showing adverse effects

  eg., Transgenic potato containing *snowdrop lectin* gene affected parts of the rat intestine on feeding. Researchers have shown that some effects are due to lectin *per se* and the reason behind total toxicity could not be fully demonstrated.

  Limitations of the study

  - Potato glycoalkaloids which are toxic to monogastric animals were not measured.
  - Small sample size
  - Dietary deficiencies in the rats fed BD potato

Royal Society. (1999). Review of data on possible toxicity of GM potatoes. The Royal Society Ref 11/99.

➢ Suitable technique used for studying the safety

eg., Electron microscopic examination of the liver and pancreas revealed ultra structural anomalies in various studies of mice fed with 14% BD herbicide-tolerant soybean.

Limitations of the study:

- failure to control for possible litter effects
- inadequate methodological procedures to ensure an unbiased, quantitative assessment
- inappropriate statistical methods
- differences in the phytoestrogen content of the control and BD soybean-based feeds
- Electron microscopy of selected tissues is useful to elucidate a chemical's mechanism of action but it is not a recommended approach in OECD testing guidelines because the relatively small amount of tissue evaluated cannot be considered representative of the whole organ.

Williams AL, DeSesso JM. (2010). Genetically-modified soybeans – a critical evaluation of studies addressing potential ultrastructural changes associated with ingestion. Toxicologist 114: 1154.

- Whole food toxicity studies utilise inappropriate methods to detect unintended changes because of lower detection limits.

- Defined single substances can be dosed to laboratory animals at very large multiples of the expected human exposure, thus giving a large margin of safety. In contrast, foodstuffs are bulky, lead to satiation and can only be included in the diet at much lower multiples of expected human intakes.

- When testing whole foods, the possible highest concentration of the BD food and feed in the laboratory animal diet may be limited because of nutritional imbalance of the diet, or by the presence of compounds with a known toxicological profile.

BD food safety testing

Inspite of many issues with WF-feeding testing, most commonly used animal studies of BD food testing are:

- **42-day broiler chicken study:** performance parameters – mortality/survival, general health, weight gain, feed consumption, feed conversion, organ weights, etc. are measured. The study consisted of three 14-d phases (starter, grower, finisher)[1].

- **Sub chronic (90-day) rat study:** to detect potential toxicity. Apart from the parameters of above study, macroscopic and microscopic (histopathological) findings. Subchronic duration results are similar to those obtained after chronic exposure[2].

1. Herman *et al* (2009). Performance of broiler chickens fed diets containing DAS-68416-4 soybean meal. GM Crops 2: 169–175.
2. EFSA report, (2008). Safety and nutritional assessment of BD plants and derived food and feed: The role of animal feeding trials. Food Chem Toxicol 46:S2-70.

Triggers for the conduct of WF toxicity studies

- There is no evidence that the insertion of transgene from a non-toxic source into a BD crop has greater propensity to result in the *de novo* generation of novel toxic compounds

- Major challenge lies in identifying circumstances under which the WF testing is scientifically and ethically justified taking into consideration various factors such as:

  a) probability of recombinant DNA technology to produce a BD crop that will randomly produce novel, unintended toxic substances *de novo*

  b) implausibility of any such substances being highly toxic

  c) low concentration of unintended substances likely to be produced

  d) inherent limitations of WF toxicity studies

- However identifying cases under which WF testing is not needed is easier as WF toxicity studies are essentially bioassays and are inherently less sensitive than analytical chemistry techniques

- In addition use of a less sensitive WF testing to provide reassurance of the accuracy of more sensitive methods is difficult to justify.

“Necessity as the mother of acceptance of BD food”

Public will accept BD foods only when unmet needs are met by them

- In Philippines, vit A deficiency caused blindness, Beta carotene-enriched Golden Rice was accepted by the people because of its cheap rate compared to other vitamin A supplements[1].

- Genetic modification developed disease resistant varieties of papaya in Hawaii, where the non-BD crop was almost wiped out by ringspot virus.

- BD technology is also used in the orange groves of Florida, where the harvest is threatened by citrus greening disease[2].

1. (2013). Contrary to popular belief. Nat Biotechnol 31:767
2. July 27, 2013. The New York Times

Knowledge= Prevention