

Total Diet Study (TDS) on Total Aflatoxins (B1, B2, G1 and G2) Consumed through Selected Foods from Selected Clusters of Nepal

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Presentation Layout

- * Introduction**
- * Research design including sampling**
- * Results & Discussion with key findings**
- * Conclusions**
- * Recommendations**



What is Total Diet Study (TDS) ?

- ❖ A Total Diet Study (TDS) has been used as a tool for estimating the level of dietary exposure to certain chemical contaminants coming from different food sources among general population since 1960's.
- ❖ It is recognized as a cost effective approach to estimating dietary exposure of contaminants by the World Health Organization (WHO) in 1978.

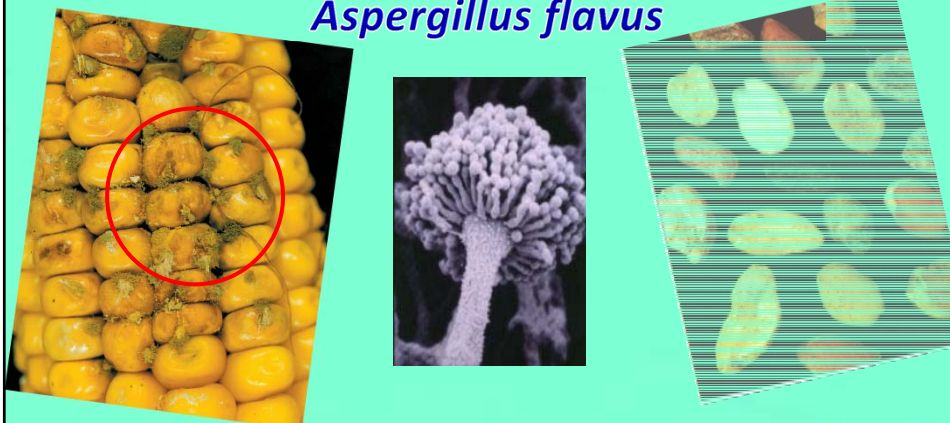


What is Total aflatoxins?

- ❖ Aflatoxins B₁, B₂, G₁ and G₂ has been defined as total aflatoxins (TA) by Codex Alimentarius Commission (CAC), which are naturally occurring toxin compounds produced as metabolites by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* growing on a variety of food and feed products under favorable temperature and humidity.
- ❖ Likewise, aflatoxins M₁ and M₂ are the secondary metabolites of G₁ and G₂ , which are found in animal's milk and meat.
- ❖ Similarly, the other most common mycotoxins are ochratoxins, tricothecenes, zearalenone and fumonisins.

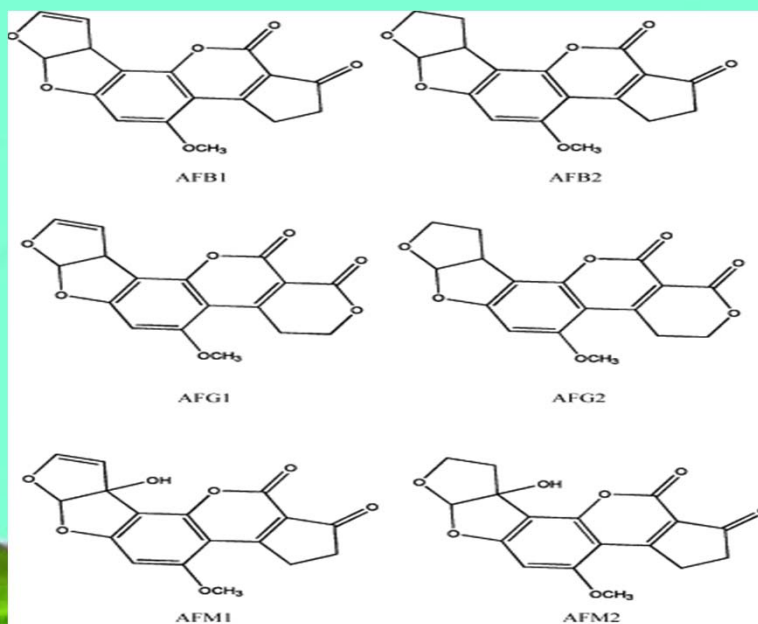


Mould Infested Maize and EMS of *Aspergillus flavus*



Aflatoxins are potentially hazardous to human and animals having strong immunosuppressive, mutagenic, teratogenic and carcinogenic effects (Hussein and Jeffrey, 2001).

Chemical Structures of Aflatoxins



MRL for total aflatoxins in foods

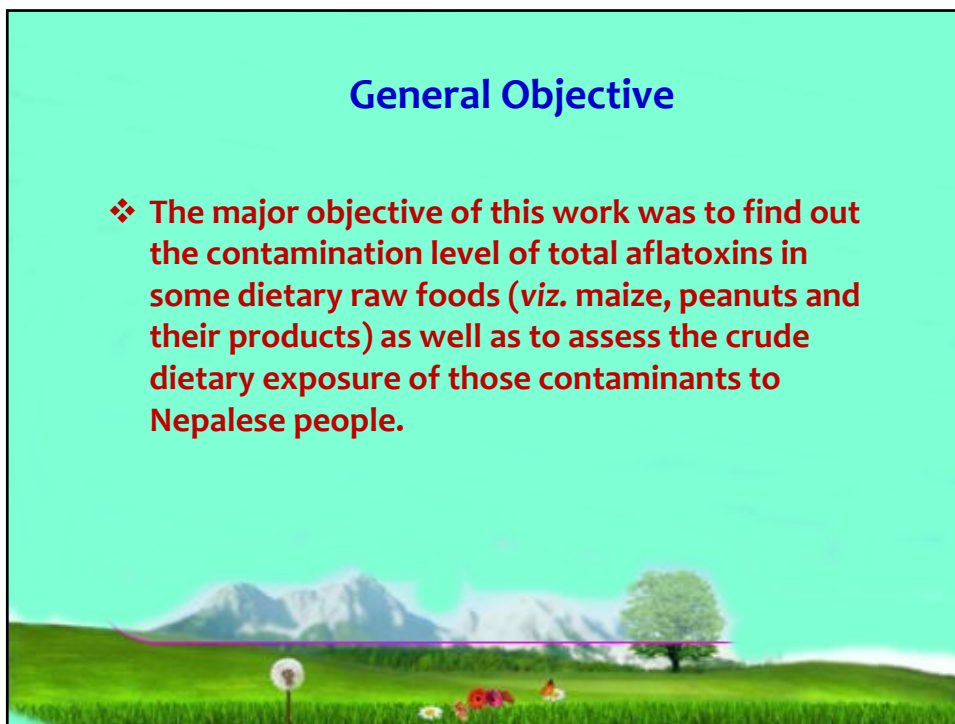
Agency	Food	MRL	Reference
GoN, Nepal	Peanuts, beans and other cereals products	20 μ g/kg	Food Standard, (2067)
CAC	Peanuts	15 μ g/kg	Codex Standard 193-1995
CAC	Brazil nut, Almond, Pistachio	10 μ g/kg	Codex Standard 193-1995
EU	Peanut/Groundnut	15 μ g/kg for unprocessed 4 μ g/kg for processed	for (EC) No 1881/2006
EU	Almond	10 μ g/kg for direct human consumption	(EC) No 1881/2006
PFA, India	Beans and cereals	30 μ g/kg	PFA, (2004)
FDA	All products except milk designed for human	20 μ g/kg	Cornell University (2013)

Mycotoxins contamination in some foods

Country	Commodities	Mycotoxin	Level of presence	Reference
Syria	Different milks	AFM1	>20 to 765 ng/l.	Ghanem & Orfi, (2009)
Iran	Human breast milk	AFM1	0.3–26.7 ng/kg).	Sadeghi <i>et al.</i> , (2009)
Tunisia	Tunisian foods	OTA	3.5 \pm 5.3 ng/g	Ghali <i>et al.</i> , (2008)
		Zearalenone	10.4 \pm 11.8 ng/g.	
Athens	Breakfast cereals	AFB1	mean 1.42 ng/g	Villa and Markaki (2009).
		OTA	Mean 0.18 ng/g	
Iran	Pistachio	AFB1	9- 45.7ng/g	Sheibani, Ghaziaskar, (2009).
		AFB2	ND- 8ng/g	
Nepal	Ground nuts	AFB1	3.78-39.69ppb	Rai and Chaudhari, (2011)
Pakistan	Buffaloes' milk	AFM1	0.027 μ g/ L	Hussain <i>et al.</i> , (2008)
	Cow's milk		0.044 μ g/L	

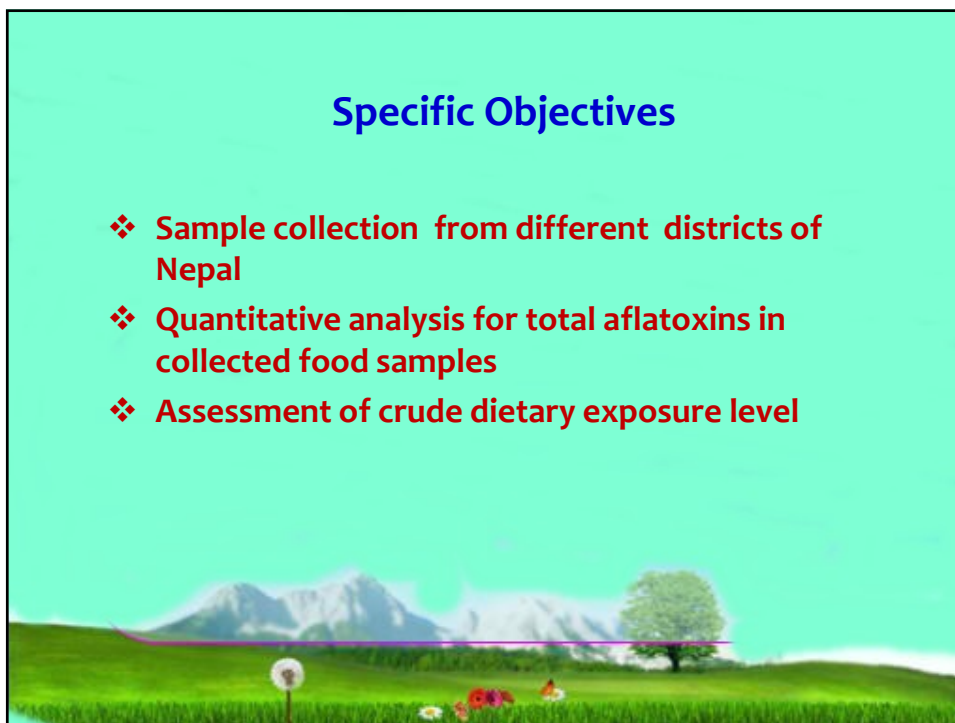
General Objective

- ❖ The major objective of this work was to find out the contamination level of total aflatoxins in some dietary raw foods (*viz.* maize, peanuts and their products) as well as to assess the crude dietary exposure of those contaminants to Nepalese people.



Specific Objectives

- ❖ Sample collection from different districts of Nepal
- ❖ Quantitative analysis for total aflatoxins in collected food samples
- ❖ Assessment of crude dietary exposure level



Limitations of study

- ❖ This study did not include the effects of storage condition for raw foods in sampling area.
- ❖ The result is in the form of combine aflatoxins (B1, B2, G1 and G2) termed as total aflatoxins so that the individual aflatoxin was not determined in food samples.



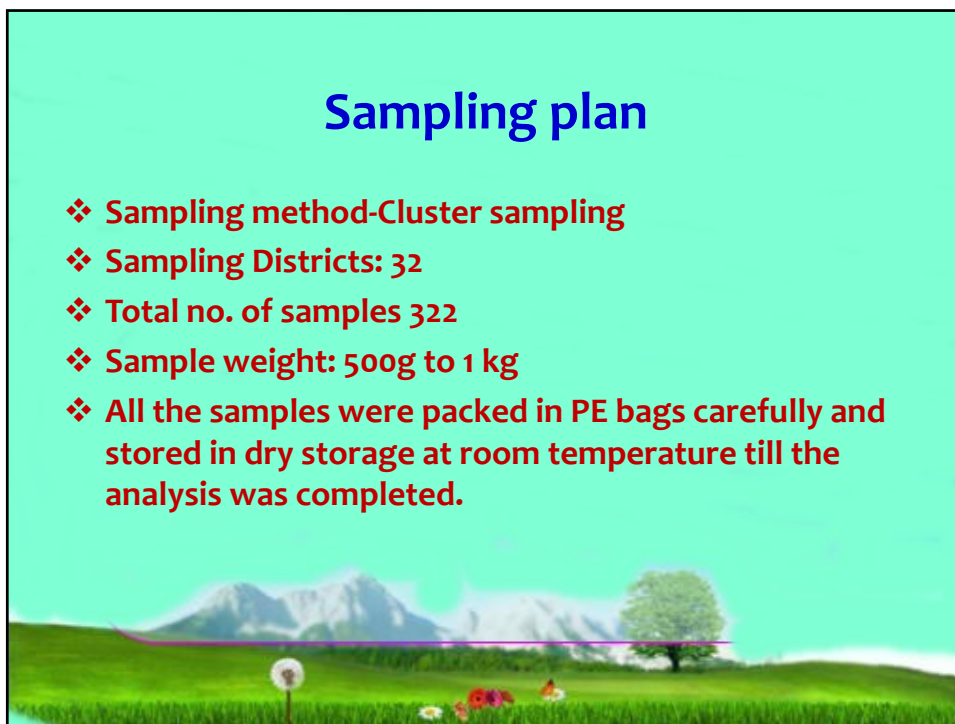
Food Samples

- ❖ Maize
- ❖ Peanuts
- ❖ Maize based products (e.g. corn flakes, suzi grits, Lito, popcorn etc.),
- ❖ Peanut/nut based products (e.g. Dalmoth, peanut butter, nut cracker, protease etc.),
- ❖ Legumes (e.g. soybean, beans etc.) and
- ❖ Others (e.g. Uwa, wheat, buckwheat, etc.).

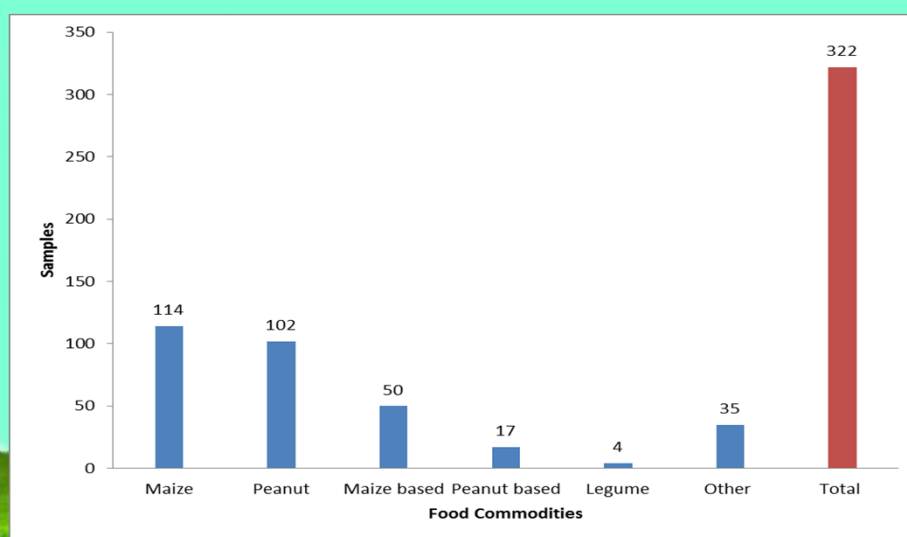


Sampling plan

- ❖ Sampling method-Cluster sampling
- ❖ Sampling Districts: 32
- ❖ Total no. of samples 322
- ❖ Sample weight: 500g to 1 kg
- ❖ All the samples were packed in PE bags carefully and stored in dry storage at room temperature till the analysis was completed.



Commodity wise sampling plan



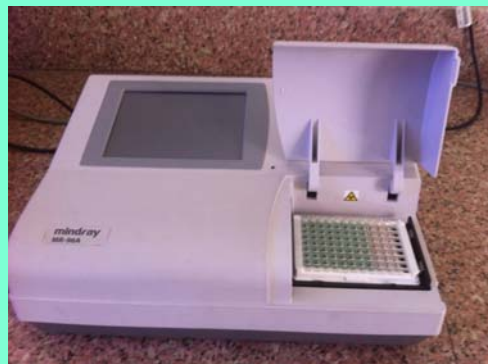
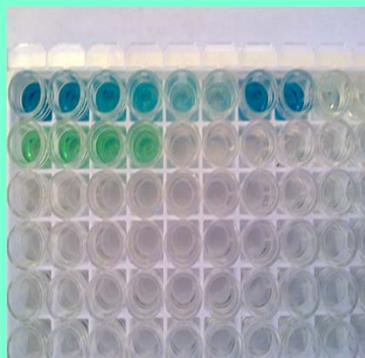
Region wise Sampling plan

Region	Food commodities						Total
	Maize	Peanut	Maize Based products	Peanut/ Nuts based products	Legumes	Others	
Eastern	26	30	9	5	3	2	75
Central	38	36	12	0	1	1	88
Western	18	16	13	5	0	21	73
Mid-western	19	10	7	1	0	8	45
Far western	13	10	9	6	0	3	41
Sub total	114	102	50	17	4	35	322

Analytical method

- ❖ Enzyme-linked Immunosorbent assay (ELISA) HELICA Total Aflatoxins Assay (USA), was used for the Quantitative detection for aflatoxins B₁, B₂, G₁ and G₂.
- ❖ LOD <1.0 ppb (µg/kg)

ELISA Kit Reader



Sample extraction and analysis

Grind sample to the particle size of fine (50% passes through a 20 mesh screen).

Weight 20g of sieved portion of the then add 100ml of the Extraction Solvent (70% methanol: 30% DW) in a 250ml stoppered Volumetric flask

All the samples were mixed and stirred manually for a minimum of 2 minutes.

Allow the particulate matter to settle, then filter 5 - 10ml of the extract through a Whatman No 1 filter paper and collect the clear filtrate to be tested. The sample is now ready for testing

Read the optical density (OD) of each micro well with a micro titer plate reader using a 450nm filter

Sample preparation



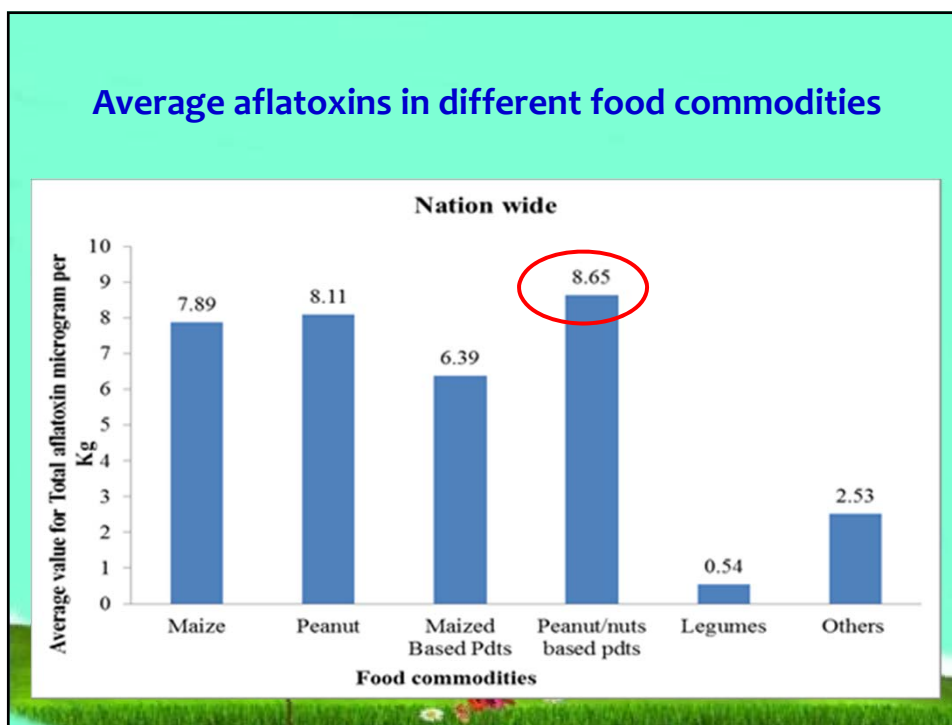
Presence of Total aflatoxins in food samples

S.N.	TDS foods	Contamination No.	% of Contamination
1	Maize	80	70.17
2	Peanuts	74	72.54
3	Maize based products	40	80
4	Peanut based products	14	82.35
5	Legumems	3	75
6	Others	8	22.85

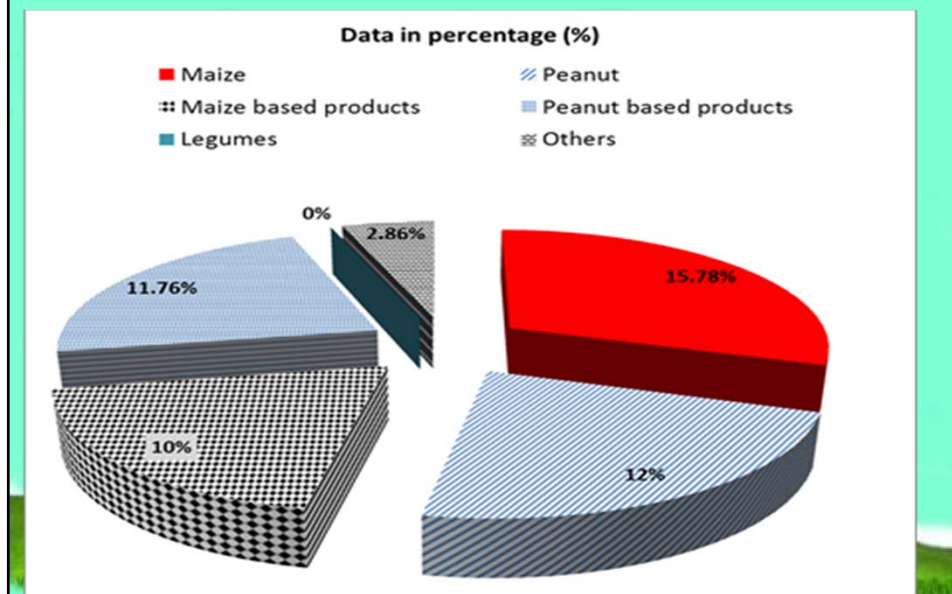
Total aflatoxins in different region of Nepal

Regions	Maize	Peanut	Maized Based Pdts	Peanut/nuts based pdts	Legumes	Others
Eastern	nd to 17.4	nd to 35.7	1 to 13.4	6.3 to 16	1 to 2.6	nd
Central region	nd to 30	nd to 24.5	1.1 to 23.1	-	nd	nd
Western region	nd to 30	nd to 30	nd to 30	nd to 30	-	nd to 12.3
Midwestern	nd to 27	nd to 5.8	nd to 9.6	10.2	-	nd to 4.5
Far western	1 to 30	1.9 to 21	nd to 25	nd to 20.5	-	nd to 22.1

Average aflatoxins in different food commodities



TA exceeding the mandatory limit of GoN



Crude dietary exposure of TA

Food Commodities	ICMR RDA (g/d)	Total average aflatoxin (µg/ kg of food)	Crude aflatoxin consumption (µg/ day)	Reference weight in Kg	
				Male (µg/kg b.w./day) 60kg***	Female (µg/kg b.w./day) 50kg***
Maize	183	7.89	1.44	0.024	0.029
Peanut*	40	8.11	0.32	0.005	0.006
Maize based products	183	6.39	1.17	0.019	0.023
Peanut based products*	40	8.65	0.34	0.006	0.006
Legumes	26	0.54	0.014	0.0002	0.0003
Others**	183	2.53	0.463	0.008	0.0093

* Taken from balanced diet for normal women (high cost diet) (Swaminathan, 1991)

**Others products means mostly cereal based other than maize

*** Reference weight taken from Gopalan *et al.*, (2004)

Crude dietary intake of TA(Region wise)											
Food Commodities	ICMR RDA (g/day)	Region***									
		Eastern		Central		Western		Midwestern		Far western	
		Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
		60kg	50kg	60kg	50kg	60kg	50kg	60kg	50kg	60kg	50kg
Maize	183	0.012	0.014	0.024	0.029	0.024	0.028	0.025	0.029	0.045	0.054
Peanut*	40	0.007	0.009	0.003	0.003	0.007	0.009	0.0005	0.0006	0.007	0.009
Maize based products	183	0.027	0.032	0.013	0.016	0.013	0.016	0.015	0.018	0.029	0.036
Peanut based products*	40	0.007	0.009	0	0	0.005	0.006	0.007	0.008	0.004	0.004
Legumes	26	0.0006	0.0008	0	0	0	0	0	0	0	0
Others**	183	0	0	0	0	0.016	0.019	0.001	0.001	0.008	0.009

* Taken from balanced diet for normal women (high cost diet) (Swaminathan, 1991)
 **Others products means mostly cereal based other than maize
 *** Reference weight taken from Gopalan *et al.*, (2004)
 0= No total aflatoxin found in samples or no sample taken
 ^Results are in ($\mu\text{g}/\text{kg}$ b.w./day)

Key findings

- ❖ Among 322 raw food samples, TA was found in 68.01% of samples; however, the average TA level did not cross the mandatory standard for food (20ppb) or for feed (30ppb).
- ❖ Over 80% of peanut, maize and their products were found to be contaminated with total aflatoxins.



Key findings contd..

- * Interestingly, 18.32% of raw food sample showed higher concentration than the MRL of GoN, where maize, peanut, their products and other contributed as 15.78%, 11.76%, 11.76%, 2.86% respectively.
- * Crude dietary exposure of TA was found to be highest in Far western region ($0.045\mu\text{g}/\text{kg}$ b. w./day for male and $0.054\mu\text{g}/\text{kg}$ b. w./day for female), while Eastern region showed the lowest in raw maize.

Conclusions

- * The average content of total aflatoxins in different food samples and their dietary exposure to human was found to be within the safe limit; however, 68% samples were found to be contaminated in different concentrations.
- * Moreover, about 18% were found to be heavily contaminated with TA.
- * Therefore, a strategic intervention program is required at policy level of government to control such contaminants in food commodities.

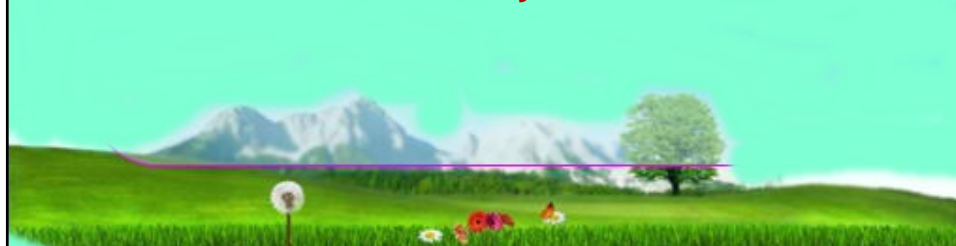
Recommendations

- * **National Reference Recommended Dietary Allowance (RDA) should be established.**
- * **the government side as well as UN agency such as WHO, FAO could work jointly in future to establish the National RDA in Nepal.**
- * **Since, this is a preliminary work on TDS in Nepal, scopes for other contaminants as well as other food commodities (raw and cooked state) should be covered in future studies plan.**



Recommendations contd..

- * **Analytical competency is most vital part of TDS work; therefore government as well as development partners should prioritize on building up of facility and competency for instrumental analysis for all kinds of contaminants in country.**



Acknowledgements

- * We would like to acknowledge WHO, country office for Nepal for supporting this program in 2012.
- * We are also grateful to DFTQC for giving this opportunity to our team.



Thank you !!!

