# Physico-Chemical Study of Some Commercially Available Energy Drinks in Bangladesh and Its Human Health Risk Evaluation



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## **Outline of the Presentation**

- Definition of Energy Drinks
- Effect of Energy Drinks on Human Health
- Objective of The Study
- Methodology
- Results and Discussion
- Human Health Risk Assessment
- Conclusion

# **Energy Drinks**

Energy drinks are non-alcoholic beverages claimed to give extra burst of energy for daily obligation and promote wakefulness, maintain alertness, and provide cognitive and mood enhancement.

They are carbonated beverages that contain significant quantities of sugar and caffeine as well as blends of exotic herbal extracts, B vitamins and amino acids meant to give consumers short term boost in energy and increase mental alertness.

# **Ingredients found in Energy Drinks**

- Common ingredients:
- Caffeine
- Ginseng
- Taurine
- Bitter orange
- -Glucose
- Additional ingredients:
- Acidity Regulators
- Vitamins (Niacin, Pantothenic Acid, B<sub>6</sub>, B<sub>12</sub>)
- Flavorings
- Colours (Caramel, Riboflavin)



# **Energy Drinks**



Fig 1: Energy Drinks Available in Bangladesh

## **How Energy Drinks Work**

caffeine enters the bloodstream within 10 minutes of consuming an energy drink, triggering a rise in heart rate and blood pressure.



Over the next 15-45 minutes, caffeine levels in the bloodstream peak. As a result, an individual will feel more alert and experience improved concentration.



All of the caffeine is absorbed within 30-50 minutes of consuming an energy drink and the liver responds to this by soaking up more sugar into the bloodstream.



Blood pressure is "significantly higher" for up to six hours after drinking the liquids



It will take around 6-7 hours for the body to achieve a 50% reduction in the amount of caffeine in the bloodstream - known as the "half-life"



It takes an 12-24 hours for the body to completely remove the caffeine from the bloodstream, though this does depend on individual factors. The withdrawal effect of caffeine include headache, irritability, constipation etc.

## **Effects of Energy Drinks**

The potential risks associated with energy drink consumption include:

- Caffeine overdose (palpitations, high blood pressure, nausea and vomiting, convulsions and, in some cases, even death)
- Type 2 diabetes as high consumption of caffeine reduces insulin sensitivity
- Late miscarriages, low birth weight and still-births in pregnant women
- Neurological and cardiovascular system effects in children and adolescent
- Sensation-seeking behaviour
- Use and dependence on other harmful substances
- Poor dental health, obesity

# **Objective of the Project**

- Analysis of the physical parameters (pH, EC, TDS, TA etc.) as well as heavy metal contents present in energy drinks.
- ❖ The concentration of different heavy metals will be compared by the drinking water standards of WHO, USEPA, BIS and Bangladesh National Standards.
- Assessment the health risks of heavy metals associated with the consumption of energy drinks in Bangladesh.

## **Methodology**

The investigation of heavy metals in energy drinks is very important in the point of view of science as well as human health. The present research work is associated with the following areas of interest:

- (1) 13 energy drinks samples were collected from different shops of Dhaka City and they were stored in a refrigerator at 4°C.
- (2) The samples were kept open for 24 hours to remove all the CO<sub>2</sub> in it.
- (3) Various physical properties such as pH, Electrical Conductivity (EC), Total Dissolved Solids (TDS), Total Acidity (TA) etc. were measured.

## **Methodology**

- (4) The samples were digested for heavy metals analysis (Fig 2). The method was repeated for three times for each of the metal to get an accurate and precise result.
  - (5) All chemicals used for digestion like HNO<sub>3</sub> and HCl for aqua-regia and the standards for heavy metals were analytical grade and obtained from Merck-Germany and Wako, Japan respectively.
- (6) Digested samples were analyzed for heavy metals like Fe, Mn, Cu, Zn, Ni, Cd and Cr by Flame Atomic Absorption Spectrophotometer (Shimadzu AA6800).
- (7) The concentration of heavy metals were compared by different drinking water standards and from the metallic concentration health risk factors were evaluated.

Fig 2: Digestion Method of Energy Drinks

25.0 mL sample was pipette out and placed in a 125 mL conical flask.

The conical flask was put down on a digital hot plate at 200°C for 45-50 minutes to reduce the volume.

The reduced sample was placed in porcelain crucible with a little wash with De-ionized water and heated at 200°C until all the water has gone and the sample turn black.

The sample was again transferred into a 125 mL conical flask and it was heated at 90°C to reduce the volume.

The sample was cooled and 10 mL of Aqua-Regia was added to it.

The crucibles were placed in a muffle furnace and heated for 4 hours at 450 - 500°C

When the volume of the sample reduced to 2 mL (near dryness), then the heating was stopped and cooled down for filtering

The sample was filtered using Whatman 42 filter paper in a 25 mL volumetric flask and the volume was up to mark using de-ionized water

### **Instrumentation**

- The instruments used in this research project are
- pH meter (Orion 4 star, Thermo-scientific)
- EC meter (EC 214, Hanna instruments)
- TDS measurement machine (SD 320 con, Lovibond)
- Analytical Balance (CP 225 D, Sartorius)
- Digital Hot plate (Lab Companion)
- Muffle Furnace (Memmert)
- Flame Atomic Absorption Spectrophotometer (AA-6800, SHIMADDZU corporation, Japan)

Table 1: The statistical data of Physico-chemical parameters of Energy Drinks

Parameters	рН	EC (μS/cm)	TDS (ppm)	TA (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)	Cr (ppm)	Cd (ppm)	Ni (ppm)
Max	4.352	1080	756	0.70	5.704	0.04	6.544	0.103	0.55	0.023	0.098
Min	2.819	345	241.5	0.08	0.981	0.016	0.049	0.042	0.012	0.008	0.036
Mean	3.464	634.308	444.015	0.374	2.487	0.027	1.102	0.076	0.088	0.015	0.066
Standard Deviation	0.5565	241.2369	168.8658	0.1877	1.4488	0.0077	1.8450	0.0147	0.1410	0.0046	0.0202

#### Table-2: Physical Parameters of Energy Drinks

Name	Sample ID	Manufacturer	Colour	Temp	рН	EC	TDS	TA
				(°C)		(µS/cm)	(ppm)	(%)
Power	PR	Mymensingh Agro Limited	Dark Orange	23.4	3.027	414	289.80	0.42
Speed	SP	Akiz Food limited (AFBL)	Orange	23.3	2.930	466	326.20	0.61
Braver	BR	Pran Foods Limited	Golden Yellow	23.2	3.011	511	357.70	0.23
Wild Brew	WB	Akiz Food limited (AFBL)	Light Orange	23.8	4.041	534	373.80	0.08
Oscar	OS	Mymensingh Agro Limited	Light Orange	24.0	4.031	820	574.00	0.28
Houston	HS	Akiz Food limited (AFBL)	Green	23.7	2.819	430	301.00	0.41
Bull Dozer	BD	Pran Foods Limited	Deep Orange	24.0	3.270	345	241.50	0.34
Black Horse	ВН	Globe Soft Limited	Yellow	23.1	2.944	573	401.10	0.43
Royal Tiger	RT	Globe Soft Limited	Greenish Yellow	23.5	3.060	469	328.30	0.29
3 Horses	TH	Imported (Germany)	Light Yellow	23.4	4.352	718	502.60	0.20
Red Bull	RB	Imported (Austria)	Light Yellow	23.6	3.483	1010	707.00	0.65
Hollander	HL	Imported (Germany)	Golden Yellow	23.8	3.872	1080	756.00	0.70
Bavaria	BV	Imported (Holland)	Yellow	23.9	4.197	876	613.20	0.22
WHO Std (2017)					6.5-8.5		1000.00	0.39-1.1
		BD Std (2009)			6.5-8.5	2000	1000.00	

Sample ID	Fe	Mn	Zn	Cu	Cr	Cd	Ni
	(ppm)						
PR	5.704	0.040	0.078	0.061	0.017	0.013	0.065
SP	4.415	0.025	0.235	0.082	0.067	0.020	0.079
BR	4.107	0.022	0.114	0.081	0.042	0.023	0.097
WB	1.325	0.020	0.454	0.103	0.020	0.019	0.098
os	3.186	0.028	1.346	0.083	0.072	0.021	0.076
HS	1.566	0.032	6.544	0.042	0.095	0.015	0.051
BD	2.174	0.023	0.622	0.080	0.052	0.011	0.058
BH	2.279	0.027	0.071	0.065	0.250	0.018	0.047
RT	1.576	0.016	0.049	0.070	0.085	0.014	0.043
TH	1.872	0.021	0.072	0.085	0.012	0.010	0.055
RB	0.981	0.019	1.925	0.084	0.040	0.012	0.087
HL	1.060	0.039	2.761	0.083	0.047	0.008	0.062
BV	2.089	0.035	0.061	0.072	0.049	0.016	0.036
WHO Std (2017)	0.30	0.40	5.00	2.00	0.05	0.003	0.07
BD Std (2009)	1.00	0.10	5.00	1.00	0.05	0.005	0.10
USEPA (2014)	0.30	0.05	5.00	1.30	0.10	0.005	0.10

#### Table 3:

Concentration

of Heavy

Metals in

Energy

Drinks

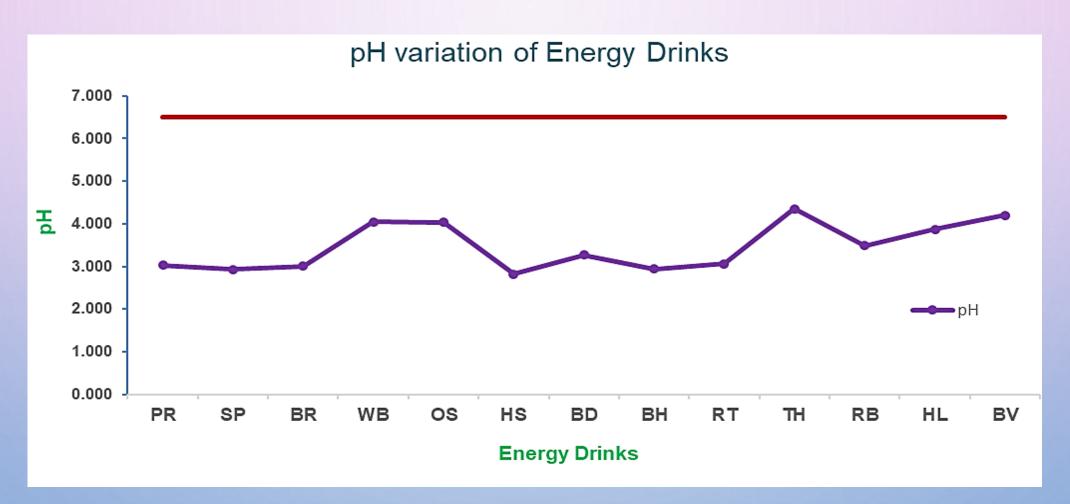


Fig 3: The pH variation of Energy drinks compared by standard

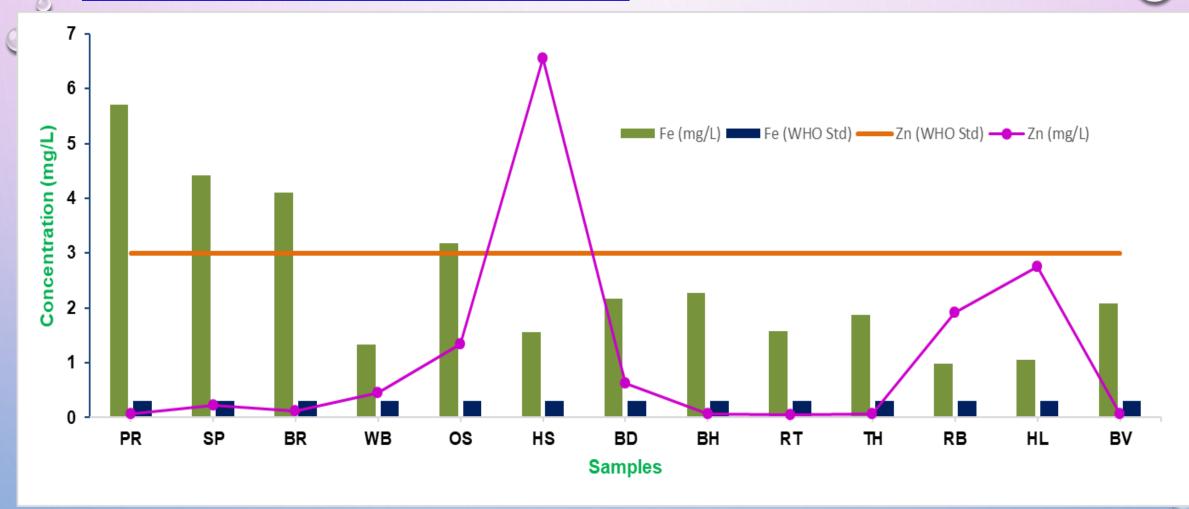


Fig 4: The Fe and Zn concentration of Energy Drinks

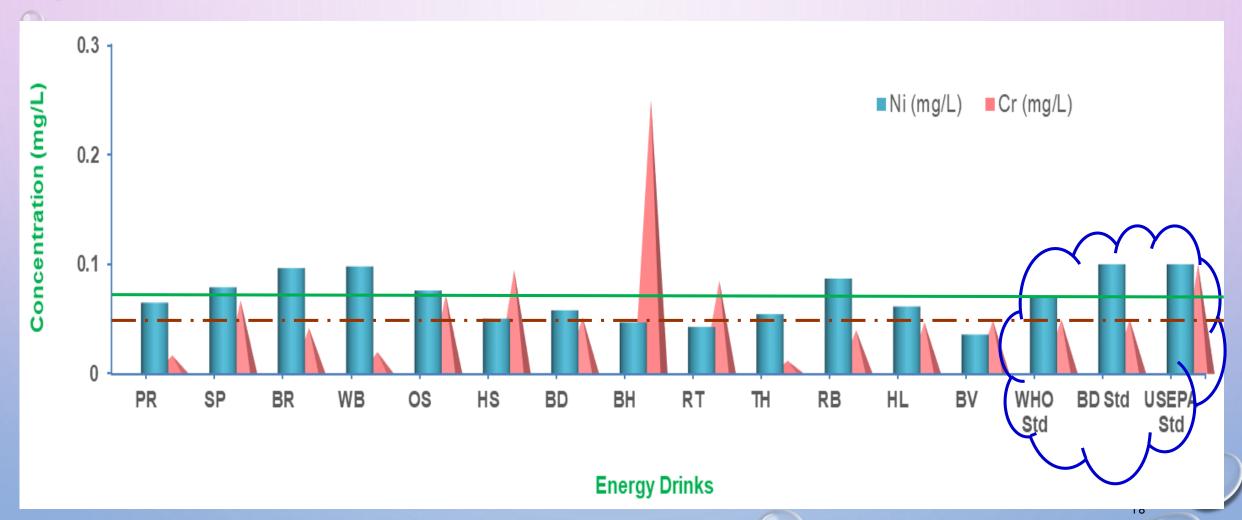


Fig 5: Comparison of Ni and Cr concentration of Energy Drinks with different standards

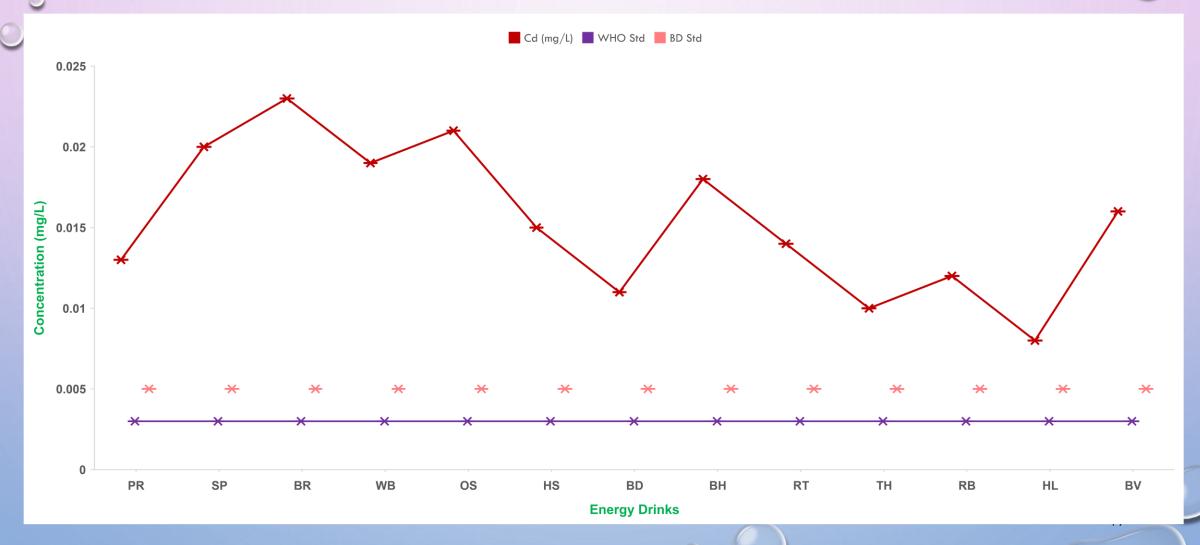


Fig 6: Cd in Energy Drinks and its comparison with standards.

#### Table 4: Correlation analysis of different parameters in Energy Drinks

			,		- p				
Variables	рН	TDS	Fe	Mn	Zn	Cu	Cr	Cd	Ni
рН	1								
TDS	0.6094	1							
Fe	-0.3115	-0.4104	1						
Mn	0.0097	0.2014	0.3607	1					
Zn	-0.2415	0.0814	-0.3462	0.2866	1				
Cu	0.6561	0.3549	-0.2372	-0.4349	-0.5034	1			
Cr	-0.3863	-0.1157	-0.0564	0.0015	-0.0810	-0.3136	1		
Cd	-0.1443	-0.2516	0.3554	-0.2049	-0.1780	0.0716	0.2312	1	
Ni	0.0686	0.0622	0.1534	-0.3098	-0.0687	0.6015	-0.3265	0.4111	1

#### **Human Health Risk Assessment**

According to the definition of United States Environmental Protection Agency (USEPA), A human health risk assessment is the process to estimate the nature and probability of adverse health effects in humans who may be exposed to chemicals in contaminated environmental media, now or in the future.

The Non-carcinogenic (adverse effect on health but not cause cancer) risk assessment can be done by evaluating Acceptable Daily Intake (ADI), Hazard Quotient (HQ) and Hazard Index (HI) for Oral Ingestion. The carcinogenic risk is assessed by Cancer Risk (CR) for Oral Ingestion.

## Chronic Daily Intake (CDI)

Acceptable daily intake or ADI is a measure of the amount of a specific substance in food or drinking water that can be ingested (orally) on a daily basis over a lifetime without an appreciable health risk. It can be calculated by the equation below and the unit is mg/kg/day.

$$CDI = (C \times IR)/BW$$

Here, C is the heavy metal concentration (mg/L), IR is daily ingestion rate of drinking water (L/d), BW is the average body weight (kg).

#### Table 5: Chronic Daily Intake (CDI) of Heavy Metals in Energy Drinks

Sample ID	Unit	Fe (ADI)	Mn (ADI)	Zn (ADI)	Cu (ADI)	Ni (ADI)	Cr (ADI)	Cd (ADI)
PR	mg/kg/day	0.04753	0.00033	0.00065	0.00051	0.00054	0.00014	0.00011
SP	mg/kg/day	0.03679	0.00021	0.00196	0.00068	0.00066	0.00056	0.00017
BR	mg/kg/day	0.03423	0.00018	0.00095	0.00068	0.00080	0.00035	0.00019
WB	mg/kg/day	0.01104	0.00017	0.00378	0.00086	0.00082	0.00017	0.00016
OS	mg/kg/day	0.02655	0.00023	0.01122	0.00069	0.00063	0.00060	0.00018
HS	mg/kg/day	0.01305	0.00027	0.05453	0.00035	0.00042	0.00079	0.00013
BD	mg/kg/day	0.01812	0.00019	0.00518	0.00067	0.00048	0.00043	0.00009
ВН	mg/kg/day	0.01899	0.00023	0.00059	0.00054	0.00039	0.00458	0.00015
RT	mg/kg/day	0.01313	0.00013	0.00041	0.00058	0.00036	0.00071	0.00012
TH	mg/kg/day	0.01560	0.00018	0.00060	0.00071	0.00045	0.00010	0.00008
RB	mg/kg/day	0.00818	0.00016	0.01604	0.00070	0.00073	0.00033	0.00010
HL	mg/kg/day	0.00883	0.00033	0.02301	0.00069	0.00051	0.00039	0.00007
BV	mg/kg/day	0.01741	0.00029	0.00051	0.00060	0.00030	0.00041	0.00013
WHO CDI	mg/kg/day	0.018	0.002	0.011	0.060	0.070	0.035	0.006

#### **Hazard Quotient (HQ):**

The ratio of the potential exposure to a substance and the level at which no adverse effects are expected (calculated as the exposure divided by the appropriate chronic or acute value). A hazard quotient of 1 or lower means adverse non cancer effects are unlikely, and thus can be considered to have negligible hazard.

#### **Hazard Index (HI):**

The sum of hazard quotients for toxics that affect the same target organ or organ system. The result of HI should be below 1 to avoid adverse effect on health.

$$HI_{ing} = \sum HQ_{ing}$$

#### Calculation of Hazard Quotient (HQ) for Oral Ingestion

According to USEPA (2011), Hazard quotient for oral ingestion can be calculated by the

following equation:

$$HQ_{ing} = \frac{C_{water} \times IR \times EF \times ED}{AT \times BW \times R_f D_{oral}}$$

\*\*Oral reference Dose R<sub>f</sub>D of Cu: 0.0371,

Zn: 0.30, Pb: 0.0014, Cd: 0.0005, Cr: 0.003,

Ni: 0.02, Fe: 0.7, Mn: 0.024, mg/kg/d.

Exposure Factors	Unit	Values
C <sub>water</sub> (Metal concentration in water)	mg/L	
IR (Ingestion Rate)	L/day	0.5
EF (Exposure Frequency)	Day/year	156
ED (Exposure Duration)	Year	30
AT (Average Time)	Days	4680
BW (Average Body Weight)	Kg	60
R <sub>f</sub> D (Oral Reference Dose)	mg/kg/d	**

#### Table 6: Hazard Quotient and Hazard Index of Energy Drinks

Sample ID	Fe (HQ <sub>ing</sub> )	Mn (HQ <sub>ing</sub> )	Zn (HQ <sub>ing</sub> )	Cu (HQ <sub>ing</sub> )	Ni (HQ <sub>ing</sub> )	Cr (HQ <sub>ing</sub> )	Cd (HQ <sub>ing</sub> )	$\mathbf{HI}_{\mathrm{ing}}$
PR	0.0679	0.0139	0.0022	0.0137	0.0271	0.0472	0.2167	0.3886
SP	0.0526	0.0087	0.0065	0.0184	0.0329	0.1861	0.3333	0.6385
BR	0.0489	0.0076	0.0032	0.0182	0.0402	0.1167	0.3833	0.6181
WB	0.0158	0.0069	0.0126	0.0231	0.0408	0.0556	0.3167	0.4715
os	0.0379	0.0097	0.0374	0.0186	0.0317	0.2000	0.3500	0.6853
HS	0.0186	0.0111	0.1818	0.0094	0.0210	0.2639	0.2500	0.7559
BD	0.0259	0.0080	0.0173	0.0180	0.0242	0.1444	0.1833	0.4211
ВН	0.0271	0.0094	0.0020	0.0146	0.0196	1.5278	0.3000	1.9004
RT	0.0188	0.0056	0.0014	0.0157	0.0179	0.2361	0.2333	0.5288
ТН	0.0223	0.0073	0.0020	0.0191	0.0227	0.0333	0.1667	0.2734
RB	0.0117	0.0066	0.0535	0.0189	0.0363	0.1111	0.2000	0.4380
HL	0.0126	0.0135	0.0767	0.0186	0.0256	0.1306	0.1333	0.4110
BV	0.0249	0.0122	0.0017	0.0162	0.0150	0.1361	0.2667	0.4727
$\mathbf{HI}_{\mathrm{ing}}$	0.3849	0.1205	0.3981	0.2226	0.3550	3.1889	3.3333	$\Sigma$ HI= 8.0033

#### Carcinogenic Risk for Oral Ingestion

Carcinogenic health risks are expressed by their cancer slope factor (CSF) which converts the estimated exposure through inhalation or ingestion via intake of metals into incremental risk of an individual developing cancer over time. The range of carcinogenic risks (CR<sub>ing</sub>) acceptable or tolerable is 1.0E-06 to 1.0E-04 (USEPA 2011). The equation is as follows:

$$CR_{ing} = \frac{C_{water} \times IR \times EF \times ED}{AT \times BW} \times CSF$$

Cancer Slope Factor (CSF) for Cd and Cr is 6,3 and 0.5 mg/kg/day respectively.

Exposure Factors	Unit	Values
C <sub>water</sub> (Metal concentration in water)	mg/L	
IR (Ingestion Rate)	L/day	2.2
EF (Exposure Frequency)	Day/year	365
ED (Exposure Duration)	Year	70
AT (Average Time)	Days	25,550
BW (Average Body Weight)	Kg	60
CSF (Cancer Slope Factor)	mg/kg/d	**

#### **Carcinogenic Risk for Oral Ingestion**

Sample ID	Cr (CR <sub>ing</sub> )	Cd (CR <sub>ing</sub> )
PR	7.083E-05	6.825E-04
SP	2.792E-04	1.050E-03
BR	1.750E-04	1.208E-03
WB	8.333E-05	9.975E-04
os	3.000E-04	1.103E-03
HS	3.958E-04	7.875E-04
BD	2.167E-04	5.775E-04
вн	2.292E-03	9.450E-04
RT	3.542E-04	7.350E-04
тн	5.000E-05	5.250E-04
RB	1.667E-04	6.300E-04
HL	1.958E-04	4.200E-04
BV	2.042E-04	8.400E-04

Table 7: Carcinogenic Risk

assessment of Cd and Cr in

different energy drinks

## **Conclusion**

- All the physical parameters like pH, EC, TDS and Total Acidity (TA) has been measured and it has been seen that pH of all the energy drinks are relatively very low which is not suitable for our heath, specially our teeth. TDS in some drinks are also higher in some samples indicating the presence of high concentration of metals and other particles.
- ✓ As energy drinks contain lots of sugar and acid together it caused tooth decay. It occurs by bacteria in the mouth using sugar from drinks and acids that dissolve the enamel and damage the teeth. Regular loss of enamel can lead to cavities and exposure of the inner layers of the tooth that may become sensitive and painful.
- ✓ Heavy metals like Fe, Mn, Cu, Zn, Ni, Cd and Cr were measured by Atomic Absorption Spectrophotometer and it was found that Fe and Cd concentration in all the energy drinks samples were much higher than WHO standard value. Cr and Ni concentration in most of the samples were also above the standards as well.

## Conclusion

- For the assessment of non-carcinogenic risk; Chronic Daily Intake (CDI), Hazard Quotient (HQ) and Hazard Index (HI) were evaluated and the CDI of all metals were within safety limit (except few for Fe).
- ✓ The Hazard Index of **Cr and Cd** were much higher than 1 (3.19 and 3.33 respectively). Also for sample BH the HI is higher than 1. **The total summation of HI is 8.00** indicating some severe heath affect of these energy drinks on regular consumption. From the HI calculation the increasing non-carcinogenic threat for oral ingestion is **TH<PR<HL<BD<RB<WB<BV<RT<BR<SP<OS <HS<BH.**
- ✓ The carcinogenic risk factor was also calculated for Cr and Cd but it was found higher than the standard limit (1×10<sup>-6</sup> to 1×10<sup>-4</sup>) for all the samples for Cd and of most samples for Cr. This high carcinogenic factor indicate the cancer risk from these drinks for long time of exposure or intake.
- ✓ So, these drinks can be taken occasionally, not in a regular basis and if possible in a diluted way to save the teeth as well as the health.

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