

## **Toxicological Effect of Disodium 6-Hydroxy-5-[(4-Sulfophenyl)azo]-2-Naphthalenesulfonate on Behavior of Albino Rats**

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**Abstract:** A food additive is substance that is not regarded as food but is added as cosmetic, preservative or processing aid. Food colorants are used in large amount to enhance the visual properties of food. Disodium 6-hydroxy-5-[(4-sulfophenyl) azo]-2-naphthalenesulfonate (Sunset Yellow) generally used in Bakery/biscuits industries. An attempt to study the effect of this dye on the behavior of rats is made in this paper. Tremors, convulsions, diarrhea, labored breathing, reduced food consumption, increase in liver weight; decrease in body weight, food avoidance, sluggishness and abnormal gait were observed in rats on exposure to sub-lethal dosage of Sunset Yellow. All the observation were found to be time and dose dependent

**Key words:** Food Additive • Coloring Agent • Albino Rat • Toxicity • Tremor • Convulsion • Diarrhea • Labored Breathing • Food Consumption • Sluggishness and Abnormal Gait

### **INTRODUCTION**

In present era drastic changes in the lifestyle of human being are observed. These changes have also affected their food habits. Nowadays, use of artificial colors to enhance the visual properties of food has increased. This attractive, palatable, flavored and colored food attracts human of all ages. There are reports on adverse effect of these colors, flavors, sweeteners and preservatives that are generally synthetic chemicals on human health [1-4].

Disodium 6-hydroxy-5-[(4-sulfophenyl)azo]-2-naphthalenesulfonate is a synthetic dyes commonly known as Sunset Yellow; Orange Yellow S; FD&C Yellow 6; C.I. 15985; E110 used in food coloring. It is water soluble and used in fermented foods which are heat treated. It is found in orange sodas, marzipan, Swiss rolls, apricot jam, citrus marmalade, lemon, sweets, beverage mix and packet soups, margarine, custard powders, packaged lemon gelatin desserts, energy drinks such as Lucozade, breadcrumbs, snack chips such as Doritos, packaged instant noodles, cheese sauce mixes and powdered marinades, bottled yellow and green food coloring, ice creams, pharmaceutical pills and prescription medicines, over-the-counter medicines (especially children's medicines) cake decorations and icings, squashes and other products

with artificial yellow, orange or red colors. It has been reported to damage kidney and adrenal gland when fed to rats and also found as carcinogenic [5, 6]. In the present investigation, we have tried to evaluate the toxic effects of this dye on the albino rats.

### **MATERIAL AND METHODS**

Normal healthy adult male and female albino rats weighing approximately 100-200 gm were selected for the experiments. They were procured from the local animal supplier of Gorakhpur U.P., India-273001. The animals were housed in polypropylene cages at room temperature 25°C±5°C. Rats were exposed to a photoperiod of 12 hours per day. The rats were acclimatized to laboratory conditions for 10 days and fed on rat pellets and water *ad libitum*. Each rat was weighed and assigned a number for convenience prior to the onset of the experiment. Rats were divided into four groups (A, B, C and D). Sunset yellow was administered orally at the dose level of 100mg/kg, 200mg/kg and 400mg/kg body weight for 28<sup>th</sup> days with one group of control rats. All the rats of control group were given vehicle (solvent) of similar dilution through oral route. The toxicity signs developed as a result of oral administration of sunset yellow were recorded on 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> day.

Clinical signs of toxicity; tremors, convulsion, diarrhea and labored breathing were observed. Food consumed by the rats under investigation was noted as gram/rat/day. Food avoidance was also observed. Abnormal gait of the rats was registered in all the sets. Assessment of body weight and relative liver weight, rats were weighed initially and after the experiment regimen. The relative change in body weight was determined. The rats were reweighed and sacrificed. The liver was dissected out from control and sub chronically treated rats on 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> days for organ weight. Liver weight was determined in relation to body weight as relative weight of liver.

**Statistical Analysis:** The data is expressed as mean±S.E. of four replicates. Student 't' test and two way ANOVA are applied for measurement of variation between control and treated groups.

## RESULTS

Diarrhea was not observed in control group but in the rats treated with sunset yellow 100 mg/kg, 200mg/kg and 400 mg/kg body weight; diarrhea was observed on 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> day in both male and female rats.

Tremor was not observed in control groups. In treated groups on exposure to 100 mg/kg body weight, tremor was noted at 28<sup>th</sup> day after treatment with sunset yellow in both male and female rats. On exposure to 200 mg/kg and 400 mg/kg body weight, tremor was observed on 21<sup>st</sup> and 28<sup>th</sup> day in both male and female rats.

Rats in control group did not show signs of convulsion. In treated groups on exposure to 100 mg/kg body weight of aqueous solution of sunset yellow, convulsion was observed at 28<sup>th</sup> day in male and female both rats. On exposure of 200 mg/kg and 400 mg/kg body weight of aqueous solution of sunset yellow, convulsion was observed at 21<sup>st</sup> and 28<sup>th</sup> day in both male and female rats.

Labored breathing was not observed in control rats. In the treated rat with the 100 mg/kg body weight, labored breathing was observed at 28<sup>th</sup> day in both male and female rats while on 200 mg/kg and 400 mg/kg body weight exposure to sunset yellow, labored breathing was observed at 21<sup>st</sup> and 28<sup>th</sup> day in both male and female.

Rats in control group had normal gait. On exposure to 100 mg/kg body weight of aqueous solution of sunset yellow, abnormal gait was observed at 21<sup>st</sup> and 28<sup>th</sup> day in both male and female rats. On exposure to 200 mg/kg and 400 mg/kg body weight of aqueous solution of sunset yellow abnormal gait was observed at 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> day after treatment in both male and female rats.

Rats in control group did not avoid food till the experiment regimen while the rats treated with of 100 mg/kg, 200 mg/kg and 400 mg/kg body weight sunset yellow solution; food avoidance was observed at 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> day. Maximum food avoidance was observed on 21<sup>st</sup> to 28<sup>th</sup> day in all the treated groups of rats. In control rats normal consumption of water was observed while the treated rats showed more consumption of water/liquid. Decrease in the food consumption in treated rats was observed as compared to control rat (Table 2).

Table 1: Toxic symptoms observed in rats on exposure to sub-chronic dosage of sunset yellow

Dose (mg/kg Body weight)	Days	Toxic Symptoms
100	7 <sup>th</sup>	Food avoidance, Diarrhea, More water consumption
	14 <sup>th</sup>	Food avoidance, Diarrhea, More water consumption
	21 <sup>st</sup>	Food avoidance, Diarrhea, Abnormal gait, More water consumption
	28 <sup>th</sup>	Food avoidance, Diarrhea, Convulsion, Abnormal gait, Tremor, Labored breathing, More water consumption
200	7 <sup>th</sup>	Food avoidance, Diarrhea, More water consumption
	14 <sup>th</sup>	Food avoidance, Diarrhea, Abnormal gait, More water consumption
	21 <sup>st</sup>	Food avoidance, Diarrhea, Convulsion, Abnormal gait, Tremor, Labored breathing, More water consumption
	28 <sup>th</sup>	Food avoidance, Diarrhea, Convulsion, Abnormal gait, Tremor, Labored breathing, More water consumption
400	7 <sup>th</sup>	Food avoidance, Diarrhea, More water consumption
	14 <sup>th</sup>	Food avoidance, Diarrhea, Abnormal gait, More water consumption
	21 <sup>st</sup>	Food avoidance, Diarrhea, Convulsion, Abnormal gait, Tremor, Labored breathing, More water consumption
	28 <sup>th</sup>	Food avoidance, Diarrhea, Convulsion, Abnormal gait, Tremor, Labored breathing, More water consumption

Table 2: Effect of different doses of aqueous solution of Sunset yellow on food consumption (g/day/rat) of the treated rats

Day	Control rats	Treated rat 100 mg/kg body weight	Treated rat 200 mg/kg body weight	Treated rat 400 mg/kg body weight
	Mean±	Mean± (Change in %)	Mean± (Change in %)	Mean± (Change in %)
7 <sup>th</sup>	15.9±0.092	15.80±0.092*	15.6±0.082*	15.4±0.071*
		0.63%	1.90%	3.15%
14 <sup>th</sup>	16.1±0.092	15.2±0.139*	14.7±0.070*	14.3±0.136*
		5.60%	8.70%	11.19%
21 <sup>st</sup>	16.3±0.108	14.65±0.65*	14.2±0.082*	13.95±0.065*
		10.13%	12.90%	14.42%
28 <sup>th</sup>	16.5±0.082	14.05±0.096*	13.4±0.092*	13.0±0.108**
		14.90%	18.80%	21.30%

\*indicates significant ( $p<0.05$ ) and \*\* indicates highly significant ( $p<0.01$ )

Table 3: Effect of different doses of aqueous solution of Sunset yellow on body weight of rats

Day	Control rats			Treated rats								
				100 mg/kg body wt			200 mg/kg body wt			400 mg/kg body wt		
	Pre Treat.	Posts treat.	Relative	Pre. Treat.	Post treat	Relative	Pre. Treat.	Post treat.	Relative	Pre.Treat.	Post treat	Relative
	Wt. Mean	Wt. Mean	change %	wt. Mean	wt. Mean	change %	wt. Mean	wt Mean	change %	wt. Mean	wt. Mean	change %
	±	±	↓	±	±	↓	±	±	↓	±	±	↓
7 <sup>th</sup>	149.00±0.912	152.50±0.289	2.40	154.00±0.409	151.50±0.50*	1.70	171.00±1.29	167.50±1.04*	2.05	187.00±1.23	180.50±0.552*	3.48
14 <sup>th</sup>	160.00±1.08	168.00±1.08	5.00	160.00±1.08	153.00±1.08*	4.40	175.00±1.08	162.00±0.707*	7.43	198.50±0.500	183.00±0.912	7.81
21 <sup>st</sup>	171.00±0.913	183.50±0.958	7.31	183.50±1.04	165.00±0.817*	10.10	183.00±0.912	164.00±0.707*	10.39	207.50±0.912	182.00±1.47	12.29
28 <sup>th</sup>	161.50±0.866	184.50±0.646	14.25	189.50±1.33	168.00±1.08*	11.35	96.50±0.65	171.00±1.19**	12.98	209.50±0.650	180.00±1.08**	14.09

\*indicates significant ( $p<0.05$ ) and \*\* indicates highly significant ( $p<0.01$ )

Table 4: Effect of different doses of aqueous solution of Sunset yellow on liver weight of treated rats

Day	Control rats			Treated rats								
				100 mg/kg body wt			200 mg/kg body wt			400 mg/kg body wt		
	Liver wt.	Body wt.	Relative	Liver wt.	Body wt.	Relative	Liver wt.	Body wt.	Relative	Liver wt.	Body wt.	Relative
	Mean±S.E.	Mean ± S.E.	Liver wt. %	Mean±S.E	Mean ± S.E.	Liver wt. %	Mean ± S.E	Mean ± S.E.	Liver wt. %	Mean ± S.E	Mean ± S.E.	Liver wt. %
7 <sup>th</sup>	4.75±0.029	152.50±0.289	3.10	4.70±0.051*	151.50±0.500	3.10	5.10±0.023*	167.50±1.04	3.05	8.05±0.054*	180.50±0.552	4.46
14 <sup>th</sup>	5.20±0.061	168.00±1.080	3.10	5.30±0.021*	153.00±1.080	3.47	5.45±0.021*	162.00±0.707	3.37	8.43±0.027	183.00±0.912	4.61
21 <sup>st</sup>	8.05±0.054	183.50±0.958	4.40	6.50±0.036*	165.00±0.817	4.00	5.65±0.021*	164.00±0.707	3.45	8.45±0.020	182.00±1.470	4.65
28 <sup>th</sup>	8.50±0.041	184.50±0.646	4.61	6.80±0.021**	168.00±1.080	4.05	6.60±0.036**	171.00±1.19	3.90	8.95±0.020**	180.00±1.080	4.98

\*indicates significant ( $p<0.05$ ) and \*\* indicates highly significant ( $p<0.01$ )

The body weight of treated rat show decrease in comparison to control rats (Table 3). Increase in liver weight of treated rat was observed in comparison to control rats (Table 4).

Statistical analysis indicates a significant ( $p<0.05$ ) decrease in the food consumption, body weight and an increase in liver weight in rats treated with 100 mg/kg, 200mg/kg and 400mg/kg body weight of sunset yellow respectively. Two way ANOVA indicates that variation in the dose and exposure time, significantly ( $p<0.01$ ) influences the consumption of food, body weight and liver weight in all group of treated rats (Tables 2, 3 and 4).

## DISCUSSION

The observed toxicity sign *viz.* tremor, food avoidance diarrhea, abnormal gait, convulsion and labored breathing etc. seems to be of muscarinic and nicotinic nature. They may also have arisen due to the inhibition of cholinesterase by sunset yellow. Similar signs and symptoms as a result of cholinesterase inhibition on exposure different pesticides in animals have also been reported [7-10]. Onset of tremors in the treated rat exposed to pesticides is generally reported due to depletion of calcium concentration [11, 12]. It is probable that sunset yellow affects the calcium metabolism and

leads to tremor in rats. More fluid/water intake was probably due to water imbalance caused by diarrhea.

Food avoidance with reduction in food consumption in the present study after aqueous administration may be attributed to the impairment in the functioning of the digestive system i.e. reduction in absorptive and digestive capacity of gastrointestinal tract (GIT) in rats. The findings of present study are in accordance to Shull and Cheeke [13], Kononen, *et al.* [14], Babu [15] and Ceron, *et al.* [16].

The abnormal gait in rats may be also attributed to the impaired neuromuscular physiology [17, 18].

The labored breathing on exposure to sunset yellow may lead to oxygen insufficiency which in turn disturbed the neuromuscular function that results in weakness of leg leading to abnormal gait in treated rats as observed in case of exposure to various pesticides by Fisher and Metcalf [19].

The decrease in body weight of rats may be due to reduced food consumption and diarrhea caused by sunset yellow. The loss in body weight is associated with the reduced food consumption in the present investigation and is in accordance with the finding of Georgiev, [20].

An increase in relative liver weight is generally observed when an animal is exposed to xenobiotics [21-23]. A significant increase in relative liver weight after sub chronic treatment of sunset yellow was recorded in present study. It may be assumed that intense muscles fasciculations (during convulsion and tremors) and labored breathing caused by sunset yellow leads to oxygen insufficiency that in turn may induce change in membrane permeability of the liver cell, leading to accumulation of total lipids and cholesterol in the hepatocytes and an increase in relative weight of liver in the rats under present investigation is in accordance to Gupta *et al.* [24].

An adverse effect on food consumption, increase in food avoidance, suggest an effect on the physiology and metabolism of gastrointestinal tract (GIT) of the animal on exposure to Sunset yellow. This results in weakening of body that further affects the behavior of the animal. Further effects on the metabolic process need to be investigated to know the exact mode of action of the Sunset yellow.

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