TOXICITY STUDIES OF OXY POWDER®-Revised

JOGENDER K. LALLA[1]*,MEENA U.SHAH[1] AND EDWARD F III GROUP[2]

[1]Mayfair Clinical Education and Research Centre, the Coordinating Research Centre, Sanskruti.20/701, 90 Feet Road, Thakur Complex, Kandivli (E),Mumbai- 400 101 (INDIA), Tel: 91-22-2870 1161, Fax: 91-22-2854 3643, email: j.k.lalla@gmail.com
[2]CEO, GLOBAL HEALING CENTER INC. (GHC) the Project Sponsor, 2040 North Loop West, Suite 108 Houston, Texas 77018, (USA)
Tel: (713) 476–001, Fax: (713) 476–0017, email:drgroup@ghchealth.com
Outsourced facilities:
(i) Microbiology Work: Micro Chem Laboratory, (NABL Certified for ISO 17025), 125, Vardhman Industrial Estate, Gokul Nagar, Thane (W)–400 601(Study Director: Dr. (Mrs) Deepa Bhajekar Tel: 22–2172 0631 Cell: 98701 76422, email: microchem@vsnl.net
(ii) Toxicology Studies: Indian Institute of Toxicology, Kim 2057, Sadashiv Peth, Vijaya Nagar Colony, Tilak Road, Pune–411 030 (Study Director, Dr.R.M.Bhide) Mobile: 98220 11159, email: iit@pn2.vsnl.net.in
[NOTE: OXY POWDER® is a Registered Trade Mark of Global Healing Centre Inc.]

Corresponding Author j.k.lalla@gmail.com

ABSTRACT

Objective:
The objective of this study was to assess the toxic effects of Oxy powder® ***in-vitro*** on four pathogenic microbes and a probiotic bacterium generally present in the human GI tract and to determine acute oral toxicity (LD_{50}) profile of Oxy Powder® in Sprague Dawley rats. The results shall be used in selecting doses in repeated dose toxicity study of Oxy Powder

BACKGROUND

(A) *In Vitro* Toxicity Study in Selected Microbial Cultures:
Host of plants, large number of drugs and few Nutraceuticals are reported to act as antimicrobial agents. **Oxy powder**®, a dietary supplement is used in patients suffering from chronic constipation and / or IBS; it may have antimicrobial action. This aspect has been experimentally assessed ***in vitro*** in selected microbes since it is extremely difficult to assess this in ***in vivo*** studies**.**
Guidelines followed:
Schedule Y in Drugs and Cosmetic Act (IInd Amendment) Rules, 2005, Ministry of Health and Family Welfare, Government of India,

TOXICITY STUDIES OF OXY POWDER®-Revised

Study design:
The study was designed to include 3 strains of pathogenic bacteria namely E. coli, Staph. aureus and Enterococcus faecalis, a yeast, Candida albicans and one Probiotic organism, Lactobacillus bifidigus**.** The experimental work performed under sterile conditions involved use of Cup plate technique with positive and negative controls. An Oxy powder® content of one capsule (715.5 mg) was dissolved in 60 mL sterile distilled water; citric acid solution prepared by dissolving 25 mg of the acid in 60 mL sterile distilled water was used as control. Separately, solution of **Oxy Powder**® and that of citric acid was added to the cups bored in inoculated media at five different intervals namely 0 minute, 15 minutes, 30 minutes, 45 minutes and 60 minutes. The plates were incubated at 37^{o}C for 72-96 hours.
The growth was recorded every 24 hours during incubation period.
Results & Conclusion:
At the given concentration of test substance, the **Oxy powder**® did not exhibit 'static' or 'cidal' activity against all the cultures employed indicating that **Oxy powder**® when used in the patients would not kill or inhibit the growth of microbes in GI tract.
(B) Acute Oral Toxicity of Oxy Powder in Sprague Dawley Rats:
US FDA does not evaluate safety, efficacy and quality of dietary supplement ingredients or products. Consumers often take dietary supplements at their own risk. Potential risks (including use of dietary supplements by children) are involved in the use of these products including contamination, adulteration, and dosage inconsistency. It becomes all the more important to establish safety, quality, effectiveness and tolerability of **Oxy Powder®.**
Guidelines followed:
(a) Schedule "Y" in Drugs and Cosmetics Act and Rules, 1988 (IInd Amendment, 2005), Ministry of Health and Family Welfare, Government of India. **(b)** OECD Guidelines for the Testing of Chemicals (No. 420, Section 4: Health Effects) "Acute Oral Toxicity - Fixed Dose Method" Adopted on 17th December 2001 **(c)** WHO GCP Guidelines (described in WHO's GLP Handbook) **(d)** UCSF IACUC POLICY–LABORATORY HOUSING AND STUDY AREAS FOR RESEARCH ANIMALS (May 2001, revised December 2003)
Study design:
The study was conducted by acclimatizing the animals for five days prior to dosing. They were maintained at temperature between 20 ^{o} & 24°C, relative humidity between 30% and 70%, 10 to 15 air changes per hour and 12 hours each of dark and light cycle. Each dose was administered as an aqueous suspension 'Per OS' at 10 mL/kg body weight
In the **Sighting study** performed in one female rat administered oxy powder at 2000 mg/kg body weight and subsequently 5000 mg/kg body weight in another, the animals survived., The **Main study** was then conducted at the dose level of 5000 mg/kg body weight in 4 female rats.. All four animals survived through the study period of 14 days.
End Points:
Gross pathological examination did not reveal any abnormalities. Necropsy examination did not reveal any gross abnormality; hence, histopathological examination was not carried out.
Conclusion:

TOXICITY STUDIES OF OXY POWDER®-Revised

Thus, Oxy Powder exhibited high degree of safety and the acute oral toxicity of Oxy Powder® in Sprague Dawley rats could be included in "Category 5" criteria of Globally Harmonised System.

KEYWORDS
GHC's Oxy Powder®, Dietary supplement – *In vitro* Toxicity to Microbes – Acute oral (LD50) Toxicity in Sprague Dawley Rats.

1.0 INTRODUCTION

Oxy powder®, a dietary supplement is used in patients suffering from chronic constipation and / or IBS. Its toxicity could not only be limited to the patients but also covers a wide variety of microbes inhabiting his GI tract. These include pathogenic bacteria, fungi and yeasts and strains of helpful probiotic bacteria belonging to Lactobacillus, Bifidobacteria, Streptococcus and other genera which produce B vitamins like biotin, niacin, folic acid and Vitamin B 6. Killing of these microbes would result in deficiency of these vitamins in the patient consuming **Oxy Powder**. There are no known side effects reported with the use of Probiotics (1) Many plants having tannins, sulphur compounds, terpenoids, alkaloids and flavonoids and few nutraceuticals have been found to possess antimicrobial activity (2).

It was therefore deemed necessary to experimentally assess toxicity to microbes at least in ***in vitro*** studies in selected microbes since it is extremely difficult to assess this in ***in vivo*** studies.

As regards toxicity to the patients consuming Nutraceuticals and Dietary supplements, US FDA does not evaluate safety, efficacy and quality of dietary supplement ingredients or products (3). Consumers who often assume that Dietary supplements are 'Safe" may be taking them at their own risk. Since dietary supplements are not subject to standardized quality control measures, contamination, adulteration, and dosage inconsistencies are common (3) Consequently, it becomes all the more important for **Edward Group-Global Healing Center Inc.**, the marketing company of **Oxy Powder**® to establish safety, quality, effectiveness and tolerability of the product which is freely used in the treatment of Chronic Constipation and Constipation – Predominant IBS.

1.1 Guidelines followed:

(A) *In Vitro* Toxicity Study in Selected Microbial Cultures:

Schedule Y in Drugs and Cosmetic Act (IInd Amendment) Rules, 2005, Ministry of Health and Family Welfare, Government of India, (4)

(B) ACUTE ORAL TOXICITY OF OXY POWDER® IN SPRAGUE DAWLEY RATS

(i) Schedule "Y" in Drugs and Cosmetics (Eighth Amendment, 2005) Rules 1988, Ministry of Health and Family Welfare, Government of India (4)

(ii) OECD Guidelines for the Testing of Chemicals (No. 420, Section 4: Health Effects) "Acute Oral Toxicity - Fixed Dose Method" Adopted on 17th December 2001(5)

(iii) WHO GCP Guidelines (described in WHO's GLP Handbook) (6)

(iv) UCSF IACUC POLICY– LABORATORY HOUSING AND STUDY AREAS FOR RESEARCH ANIMALS (May 2001, revised December 2003) (7)

1.2 Profile of Oxy Powder Capsules®

TOXICITY STUDIES OF OXY POWDER®-Revised

Composition:
Each Oxy Powder capsule® (Av Wt. of contents-715.5 mg) contains 685 mg Ozonated Magnesium Oxides, 5.5 mg Bis-carboxyethyl germanium sesquioxide (Ge-132) and 25 mg Natural Citric Acid filled in Kosher Certified "00" Vegetarian Capsules.

Mechanism of Action:
The Ozonated Magnesium Oxide releases nascent oxygen in acidic pH of stomach aided by Citric acid included in the formulation to achieve pH 4 (8) in the GI tract. Germanium element in native form is highly toxic but Ge–132 is reported to be totally non toxic in mice, rats (9, 10) and humans (11). ADME of Ge-132 has been reported (12, 13) Although Ge –132 is non toxic and safe when taken orally, yet, by way of abundant precaution and to rule out toxicity due to ***in vivo*** **interactions**, this study was undertaken.

1.3 Justifications:

1.31 *In Vitro* toxicity study in selected microbial cultures-Rationale for selection of microbes:

The organisms selected included 3 species of pathogenic bacteria namely Escherichia coli, Staphylococcus aureus and Enterococcus faecalis; Candida albicans, yeast, and a Probiotic organism, **Lactobacillus bifidigus**. They make up most of the flora in the colon. Staphylococcus aureus, E.coli and Candida albicans are "recognized pathogens" by B.P., USP/ NF, and other Pharmacopoeias of the world. [14] Virulence of Staphylococcus aureus (15), Escherichia coli, (16, 17), Candida albicans (18) and Enterococcus faecalis (19) causing gastroenteritis resulting in nausea, vomiting, diarrhoea, and abdominal pain as well as other diseases is well documented in the standard books of Medical Microbiology.

Lactobacillus bifidigus: Probiotic bacteria inhabit human G.I.tract, favourably alter the intestinal microflora balance, inhibit the growth of harmful bacteria, promote good digestion, boost immune function and increase resistance to infection (20). .Lactobacillus bifidigus, a probiotic bacterium is G+ve anaerobic bacilli inhabiting colon. It is associated with a lower incidence of allergies (21) and also prevents some forms of tumour growth (22). Candida infection of the vaginal tract and mouth (Thrush) respond very favourably to Lactobacillus bifidigus. The latter destroys the pathogenic organisms through production of lactic acid, hydrogen peroxide, enzymes and natural antibacterial substance, 'Bacteriocin' thereby helping to establish a healthy intestinal environment. Lactobacillus bifidigus improves bowel function by aiding peristalsis and results in the production of a softer, smoother stool.

Since Oxy Powder® is used in the treatment of Chronic Constipation and Constipation– Predominant IBS, it is important to see that it does not have any toxicity towards Lactobacillus bifidigus. For assessing this aspect, Lactobacillus bifidigus was selected.

1.32 Acute Oral Toxicity of Oxy Powder® in Sprague Dawley Rats

(a) Rationale for Selection of Sprague Dawley Rat as Test System:

One of the recommended rodent species by the regulatory authorities for conducting preclinical toxicity studies among rodents is Sprague Dawley Rat, as it is a sensitive species for expression of toxic responses.

1) Rat is recommended rodent species for conducting acute toxicity studies as per OECD guidelines.
2) Availability of the historical control data at the testing facility.

(b) Route of Administration and Reason for Choice

TOXICITY STUDIES OF OXY POWDER®-Revised

Oral route of administration is the proposed therapeutic route of administration in human being.

2.0 EXPERIMENTAL PROCEDURES, OBSERVATIONS, RESULTS, DISCUSSION AND CONCLUSIONS:

These are separately grouped and described for **(A)** ***In Vitro*** toxicity study in selected microbial cultures and **(B)** Acute oral toxicity of Oxy Powder® in Sprague Dawley Rats.

(A) *In Vitro* toxicity study in selected microbial cultures:

2.1 Materials and Methods:

(a) Test Substance: (i) Oxy Powder capsules® (Marketed as by Global Healing Center, Inc. USA). (ii) Citric acid as an associate component.

(b)Test System: The study was designed to include 3 species of pathogenic bacteria namely Escherichia coli, Staphylococcus aureus and Enterobacter faecalis*;* one species of yeast, Candida albicans and one species of probiotic bacterium, Lactobacillus bifidigus.

Note: These test results are specifically limited to in vitro conditions. No testing was done with actual humans suffering from diseases caused by these pathogenic organisms.

3.0 General Experimental Procedure:

The experimental work involved use of Agar cup plate technique in four sets, one for the test substance, second for citric acid as control and the other two to act as 'positive' and 'negative controls'. The Petri dishes were prepared and used employing standard microbiological procedures. All the operations were performed under sterile conditions to prevent microbial contamination.

(a) Preparation and use of solution of test substance (Oxy Powder®):

(i) Contents of one capsule of Oxy Powder (715.5 mg) were dissolved in 60 mL sterile distilled water giving solution representing 11.93 mg. Oxy Powder®. /mL (ii) 25 mg citric acid was dissolved in 60 mL sterile distilled water giving 0.42 mg /mL citric acid solution to be used as a control (since citric acid present in Oxy Powder® capsule could also inhibit growth of microbes).

(b) Use of solution of test substance and observation:

Separately, solutions of **Oxy Powder**® and Citric acid [Section 3.0 (a) (i)] were added to the cups bored in inoculated media at five different intervals namely 0 minute, 15 minutes, 30 minutes, 45 minutes and 60 minutes. The plates were incubated at 37^{O}C for 72-96 hours. The growth was recorded every 24 hours up to 96 hours during incubation period.

4.0 Observations:

No inhibition was observed at the given concentration of Oxy Powder® against the five test cultures Escherichia coli, Staphylococcus aureus, Enterobacter faecalis, Candida albicans and Lactobacillus bifidigus, at the given time intervals. The control solution of citric acid as well as +ve and –ve controls did not inhibit the growth of five cultures

5.0 Discussion and Conclusion:

The results indicated that Oxy Powder® used as 1.193% w/v solution during the incubation period in the *in vitro* study was non-toxic to the test cultures.

It is very encouraging to see that the Probiotic organism Lactobacillus bifidigus is also not killed by Oxy powder® indicating thereby that this Probiotic organism would survive *in vivo* in large intestine and colon region when Oxy powder® is used in the patients suffering from chronic constipation or IBS. Other

normal physiological processes including generation of B complex factors by the Probiotic organisms shall continue in routine and would avoid administration of B complex factors externally to the patients suffering from constipation and undergoing treatment with Oxy powder

(B) Acute oral toxicity of Oxy Powder® in Sprague Dawley Rats

6.0 MATERIALS AND METHODS

6.1 Test Substance: Oxy powder® capsules (Marketed by Global Healing Center, Inc., USA)

6.2 Test System:

Animals: Female Sprague Dawley Rat **Age:** 5-8 weeks **Body weight:** 129 ± 5 g

Source: I.I.T. Animal house

No. of animals per dose: Sighting study: One and Main study: Four

6.3 Test Conditions:

Acclimatization: Five days prior to dosing.

Veterinary examination: Before allocation of animals to different doses after completion of acclimatization period.

Identification of animals: By cage number and individual marking on fur.

Diet: Pelleted feed *ad libitum* supplied by Nav Maharashtra Chakan Oil Mills Ltd, Pune

Water: Aqua guard potable water in glass bottles *ad libitum*

Housing & Environment:

Sighting study: One animal per polypropylene cage provided with bedding of husk.

Main study Maximum 5 animals per each polypropylene cage provided with bedding of husk.

Temperature between 20 ^{o} & 24°C, **Relative humidity** between 30% and 70%, **Air changes** 10 to 15 per hour and **Dark and light cycle** each of 12 hours.

6.4 Selection of Doses:

Table 1 includes Dose Selection for Sighting study and for the Main study. Based on the results of sighting study, dose for the main study was selected.

Dose volume : 10 mL/kg.

Vehicle : Distilled water

TOXICITY STUDIES OF OXY POWDER®-Revised

Table 1 Dose Selection

Sighting study			Main study		
Dose (mg/kg body weight)	No. of animals	Mortality	Dose (mg/kg bodyweight)	No. of animals	Mortality
2000	1	0/1	—	—	—
5000	1	0/1	5000	4	0/4

NOTE: *Based on the results of sighting study, dose for the main study was selected*

Preparation of dose – Procedure:
The test substance was suspended in distilled water to obtain concentrations of 200.0 mg/mL and 500.0 mg/mL strength of suspensions. The test substance was administered in the dose volume of 10 mL/kg body weight**.**
The suspension formulation was prepared fresh on the day of dosing.
6.5 Randomization and Numbering of Animals: Eleven healthy female rats, acclimatized to laboratory conditions for 5 days prior to dosing were used in this study. Animals were randomly assigned to the cages and the individual animal was fur marked with picric acid. The females were nulliparous and non-pregnant
6.6 Preparation of Animals:
The rats were deprived of feed for 16 hours before and 3 hours after administration of the test substance. Water was not withheld during this period
6.7 Experimental Procedure:
The test substance, suspended in distilled water was administered by gavage to rats using a ball-tipped intubation needle (18 gauge) fitted on to a syringe as per SOP on Test Article/Substance (TA/S) administration - Gavage/Intubation.
6.8 Allocation of animals:

Allocation of Rats for the Sighting study and the Main study are shown in **Table 2**.

TOXICITY STUDIES OF OXY POWDER®-Revised

Table 2 Allocation of Rats

(i) Sighting study

Species/strain	Group No.	Animal Nos. Female	Dose (mg/kg)	Concentration (mg/ml)	Route
Rats/Sprague Dawley	I	1	2000	200	Oral
	II	1	5000	500	

(ii) Main study:

Species/strain	Group No.	Animal Nos. Female	Dose (mg/kg)	Concentration (mg/ml)	Route
Rats/Sprague Dawley	I	4	5000	500	Oral

7.0 OBSERVATIONS:

7.1 Clinical Signs of Intoxication:

Observations of clinical signs were made at 10 minutes, 30 minutes, 60 minutes, 2 hours, 4 hours and 6 hours after dosing on day1and once daily thereafter for 14 days approximately at the same time. Cage side observations included changes in the skin, fur, eyes, mucous membrane, respiratory, circulatory, autonomic, and central nervous system changes, somatomotor activity and behavioural pattern. Occurrence of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma, if any, was also recorded

Individual Animal-Clinical Signs of Intoxication are included in **Table 3**.

Table 3
Individual Animal- Clinical Signs of Intoxication

Sighting study:

Group No.	Dose mg/kg	Observed Signs	Total Number of Animals	Animal Nos.	Period of signs in days from - to

I	2000	Nil	1	1	1 - 14
II	5000	Nil	1	1	1 - 14

Main study:

Group No.	Dose mg/kg	Observed Signs	Total Number of Animals	Animal No.	Period of signs in days from - to
I	5000	Nil	1	2	1 - 14
		Nil	1	3	1 - 14
		Nil	1	4	1 - 14
		Nil	1	5	1 - 14

7.2 Observation for Mortality:

The results of animals observed for mortality twice daily were recorded. Individual Animal-Clinical Signs of Mortality are included in **Table 4**.

Table 4
Individual Animal- Mortality Record

Sighting study:

Group No.	Dose mg/kg	Animal No.	Mortality Absolute%	Mortality Relative %
I	2000	1	0	0
II	5000	1	0	0

Main study:

Group No.	Dose mg/kg	Animal No.	Mortality Absolute%	Mortality Relative %
I	5000	2	0	0
		3	0	0
		4	0	0
		5	0	0

TOXICITY STUDIES OF OXY POWDER®-Revised

7.3 Body Weight: Individual animal body weight was recorded following the period of fasting on day 0, weekly thereafter for 13 days and at termination of study on the 14th day. Changes in the body weight were recorded. Individual Animal-Mean Body Weight and % Body Weight gain are included in **Table 5**.

Table 5
Individual Animal-Mean Body Weight and Percent Body Weight Gain

Sighting study:

Group No.	Dose (mg/kg body weight)	Body weight Day 1 (g)	Body weight Day 7 (g)	% body weight gain day 1-7	Body weight Day 14 (g)	% body weight gain day 7- 14	% body weight gain day 1- 14
I	2000	124.50	142.50	14.46	165.50	16.14	32.93
II	5000	133.20	158.20	18.77	181.40	14.66	36.19
rise in wt due to↑dose	150%	8.7(7%)	15.7(11%)	4.31	15.9(9.6%)	1.48	3.26

Main study:

Group I Animal No.	Dose (mg/kg body weight)	Body weight Day 1 (g)	Body weight Day 7 (g)	% body weight gain day 1-7	Body weight Day 14 (g)	% body weight gain day 7- 14	% body weight gain day 1- 14
2	5000	137.8	155.3	12.70	174.8	12.56	26.85
3	5000	125.4	146.9	17.15	168.0	14.36	33.97
4	5000	133.0	151.2	13.68	170.0	12.43	27.82
5	5000	124.1	146.6	18.13	167.4	14.19	34.89
Average	5000	130.1	150.0	15.4	170.0	13.3	30.8

7.4 Gross Pathology:
Macroscopic examination was performed on animals sacrificed at the end of the observation period of 14 days. Individual Animal- Gross Pathology findings are included in **Table 6**.

TOXICITY STUDIES OF OXY POWDER®-Revised

Table 6
Individual Animal- Gross Pathology Findings

Sighting study:

Animal No.	Site and lesion observed	Group	Dose (mg/kg body weight)	Fate	Gross Pathology Findings
1	NAD	I	2000	TS	NAD
1	NAD	II	5000	TS	NAD

Main Study:

Animal No.	Site and lesion observed	Group	Dose (mg/kg body weight)	Fate	Gross Pathology Findings
2	NAD	II	5000	TS	NAD
3	NAD	II	5000	TS	NAD
4	NAD	II	5000	TS	NAD
5	NAD	II	5000	TS	NAD

TS = Terminal sacrifice
NAD = No abnormality detected

7.5 Histopathology:
Histopathology was not done since Necropsy examination did not reveal any gross abnormality;

8.0 RESULTS

8.1 Clinical Signs of Toxicity and Mortality:
(a) Sighting study – Group I and Group II
One rat each treated at the dose level of 2000 mg/kg and 5000 mg/kg body weight respectively did not show any signs of toxicity and survived the study period of 14 days. .
(b) Main study – Group I
All 4 rats treated at the dose level of 5000 mg/kg body weight did not exhibit any signs of toxicity during the study period of 14 days. No mortality was seen during study period of 14 days.
8.2 Body Weight:
The Mean Body Weight and Percent Body Weight Gain for Sighting study and for the Main study indicate that there was continuous rise in weight of rats, rise being higher with higher dose.
8.3 Gross Pathology Findings:
Macroscopic examination of animals sacrificed at termination revealed no abnormalities.

8.4 Summary of Mortality Record:
During 14 days period of study, no mortality was recorded in **Sighting** as well as **Main** study.
8.5 Summary of Clinical Signs of Intoxication:
During 14 days period of study, no signs of Intoxication were observed in any rat in both studies.

9.0 Discussion

As stated above, US FDA does not evaluate safety, efficacy and quality of dietary supplement ingredients or products (3). It thus becomes responsibility of Dietary supplement producer or marketing organization to establish these attributes for their products before delivering them to the patients and general public and protecting them from using unsafe, ineffective and substandard products. **Global Healing Center (GHC)** undertook complete care and evaluation of Quality, Safety, Efficacy and Tolerability of Oxy Powder® marketed by GHC.
Acute Oral Toxicity Studies conducted under controlled conditions of use of animal species their age, body weight, their housing, product dose and volume administration, quality of diet and water, monitoring, and strictly following the guidelines to ensure validity of results.
As regards the results,

- There was gain in the body weight of experimental rats observed in sighting at both the dose levels as well as in the main study. In the latter study, with administration of 5000 mg/kg body weight to 4 animals, weight increase post administration of Oxy Powder was 15.41% between 1- 7 days, 13.39% between 7- 14 days and 30.88% between 1- 14 days.
- Macroscopic examination of animals sacrificed at termination revealed no abnormalities.
- During 14 days period of study, no mortality was recorded in **Sighting** as well as **Main** study.
- During 14 days period of study, no signs of Intoxication were observed in any rat in both the studies.

10.0 CONCLUSION

On the basis of the results of this preliminary study, it is evident that Oxy Powder formulation exhibited high degree of safety even at dose level of 5000 mg/kg body weight. The acute oral toxicity of Oxy Powder® in Sprague Dawley rats could be included in "Category 5" criteria of Globally Harmonised System. Further work on sub chronic toxicity study for 28 days is being published as Paper-2.

11. REFERENCES

(1) Robert E.C. Wildman, "Handbook of nutraceuticals and functional foods", 2nd ed., 2006, CRC Press, P 76.
(2) Natasha Trenev, 'Probiotic for Life', Alive, issue 257, March 2004 (www.alive.com/3502a5a2.php?)

TOXICITY STUDIES OF OXY POWDER®-Revised

(3) New York State Task Force on Life & the Law, "Dietary Supplements, Balancing Consumer Choice & Safety" p.39, Oct.2005; New York State Department of Health, Albany, NY 12237, Available at http:// www. health.state.ny.us/nysdoh/task force]

(4) Schedule "Y" in Drugs and Cosmetics (Eighth Amendment) Rules 1988, Ministry of Health and Family Welfare, Government of India.

5) OECD Guidelines for the Testing of Chemicals (No. 407, Section 4: Health Effects) "Repeated Dose 28-Day Oral Toxicity in Rodents" (Adopted on 12 May 198 1 and Updated on 27 July 1995.)

(6) UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases (TDR)"HANDBOOK OF GOOD LABORATORY PRACTICE (GLP)**,** Quality practices for regulated non-clinical research and development"

(7) UCSF IACUC POLICY– Laboratory Housing and Study Areas for Research Animals (Adopted on May 2001, Revised December 2003).

(8) Independent Laboratory Tests on Oxygen Based Colon Cleansers; Scientific Analysis of Commercial Products for Oxidative Ability" quoted In 'Oxy powder TESTED AGAINST COMPETITION COMES OUT #1 IN LAB TESTS' at http://www.greenwillowtree.com/Page.bok

(9) Asai K., "Miracle Cure: Organic Germanium", pp 59-66, Japan Publications Inc., 1980. (Through www.regenerativenutrition.com/content).

(10) Samochowiec L and Wojcicki J. Basic pharmacological properties of Sanumgerman. In: 1st Int. Conf. on Germanium, Hanover; October 1984. (Lekim & Samochowiec eds., Semmelweis-Verlag, 1985 (available in Sandra Goodman," The health and life enhancer (GERMANIUM)", CHAPTER 11)

(11) Miyao K, Onishi T, Asai K. Tomizawa S & Suzuki F. Toxicology and Phase I Studies on a Novel Organogermanium Compound, Ge-132. Current Chemotherapy & Infectious Disease. Proceedings. 11th ICC & 19th ICAAC, Amer. Soc. Microbiology, 80-82. 1980

(12) Lekim D. & Kehlbeck H. 1985. "The biological activity of germanium" in 1st Int. Conf. on Germanium. Hanover, Oct. 1984. Lekim & Samochowiec, eds. Semmelweis-Verlag.

(13) Mizushima M, Satoh H, and Miyao K. Some pharmacological and clinical aspects of a novel organic germanium compound Ge-132. In: 1st Int. Conf. on Germanium. Hanover, Oct. 1984. Lekim & Samochowiec, eds. Semmelweis-Verlag. 1985. GE132; PHAR. NER.

(14) "Typical Pharmaceutical Materials Required by Official Compendia to ensure Freedom from Specific Objectionable Microorganisms" USP XXI / NFXVI, United States Pharmacopoeial Convention, Rockville, MD, 1980.

(15) Staphylococcus aureus, Wikipedia, the free encyclopedia.htm & Toxic shock syndrome (TSS). (TSS available at http://en.wikipedia.org/wiki/Toxic shock syndrome)

(16) Rolhion N and Darfeuille-Michaud A (2007)."Adherent-invasive Escherichia coli in inflammatory bowel disease". Inflammatory Bowel Dis. **13** (10): 1277–83

(17) Todar, K., (2008), "Pathogenic E. coli". Online Textbook of Bacteriology**.** University of Wisconsin-Madison Department of Bacteriology.

(18) Ryan KJ, Ray CG (editors) (2004). Sherris Medical Microbiology (4th ed.), McGraw Hill. pp. 294–5. [ISBN 0-8385-8529-9].

(19) Pelletier LL (1996). Microbiology of the Circulatory System in*:* Baron's Medical Microbiology (Baron S et al, eds.) (4th edition), University of Texas Medical Branch. [ISBN 0-9631172-1-1].
(20) Swiss Herbal Remedies Limited, Canada (http://www.swissnatural.ca/company.asp) (Product list available at www.zooscape.com/cgi-bin/maitred/GreenCanyon/questp100462).
(21) Björkstén B, Sepp E, Julge K, et al. 2001. Allergy development and the intestinal microflora during the first year of life. Journal of Allergy and Clinical Immunology, Volume 108, Issue 4, Pages 516-520.
(22) Guarner F and Malagelada JR., 2003. Gut flora in health and disease. The Lancet, Volume 361, Issue 9356, 8 February 2003, Pages 512-519.