

# Dose and Time Related Effects of Lindane ( $\gamma$ -Hexachlorocyclohexane) on Hair Growth in Rabbit

Liaqat Ali, Muhammad Aslam, Khalid Mahmood Akhtar, Sandus Tariq

---

## Abstract

**Background:** One of the most effective 2<sup>nd</sup> line scabicial agent lindane ( $\gamma$ -HCH) is still used in many countries. It is moderately hazardous and causes severe physiological dysfunction in various organ systems. **Method:** The effects of ( $\gamma$ -HCH) on hair growth of male oryctolagus Cuniculus Rabbit was studied in variable doses of 5,10,15 and 25mg/kg body weight for short term (up to 14 days) and also long term (up to 30 days) exposure. In addition general conditions and also some hematoenzymatic parameters were recorded in unexposed and exposed (treated) animals. **Results:** There was gradual increase in loss of hair with increasing dose of Lindane. With 5mg/kg body weight mild hair loss in neck and trunk, 10mg/kg body weight mild patchy loss

of hair in neck & trunk region, 15 mg/kg body weight marked patchy loss of hair in neck region & moderate patchy loss of hair in trunk region, 20mg/kg body weight patches of almost complete loss of hair all over the body was observed. In addition, dose and time related anorexia, diarrhea, behavioral changes (weakness, restlessness, excitement and hyperactivity), bleeding tendency (observed while taking blood) and enzymatic changes (significantly increased serum alkaline phosphatase and SGPT) were also observed. **Conclusion:** The pharmacological use of  $\gamma$ -HCH, even as a 2<sup>nd</sup> line scabicial agent should be strictly banned owing to its toxic effect on various systems of body. **Key words:** Lindane, scabicial agent, hair growth, hematoenzymatic parameters.

---

## INTRODUCTION

Lindane is man-made organochlorine, a chemical variant of hexachlorocyclohexane (HCH). Hexachlorocyclohexane (HCH) is a cyclic compound. The position of the Cl atoms gives this cyclic compound eight isomeric forms. The  $\gamma$ -isomer of hexachlorocyclohexane ( $\gamma$ -HCH) is a halogenated organic insecticide which at one time was used widely throughout the world.<sup>1,2</sup>

### Corresponding Author:

Dr. Liaqat Ali

Professor of Anatomy

UM&DC Faisalabad

Phone No. +92333-6521948

E-mail. [drliqat\\_ali@yahoo.com](mailto:drliqat_ali@yahoo.com)

It was first introduced as a scabicial agent for human use in the 1950s<sup>3</sup> and later on it was used topically as a prescription medication for treatment of human infestations of head lice and scabies.<sup>4</sup> The WHO classified lindane as "Moderately Hazardous".<sup>5</sup> Lindane causes severe physiological dysfunction in various organ systems by enhancing oxidative stress by interacting with the cell membrane, triggering the generation of Reactive Oxygen Species (ROS) and altering the level of antioxidant molecules.<sup>6,7,8</sup>

A variety of adverse reactions to lindane pharmaceuticals have been reported ranging from

skin irritation, burning sensations, itching, dryness, rash, seizures and rarely death.<sup>5,9</sup> Exposure to large amounts of lindane can affect the nervous system, producing a range of symptoms from headache and dizziness to seizures, convulsions and more rarely death.<sup>10,11,12</sup> Prenatal exposure has been associated with altered thyroid hormone levels and affects brain development.<sup>13</sup>

In 2003, the FDA issued a “black box” public health warning for lindane treatments, reemphasizing that lindane should only be used as second line therapy. Second line of treatment means that it can only be used/prescribed when other “first line” treatment have failed or cannot be used.<sup>14,15</sup> The black box warning emphasizes that lindane should not be used on premature infants and individuals with known uncontrolled seizure disorders, and should be used with caution in infants, children, the elderly, and individuals with other skin conditions (e.g. dermatitis, psoriasis) and people who weigh less than 110lbs(50kg), as they may be at risk of serious neurotoxicity.<sup>4</sup>

## MATERIALS AND METHODS

Fifty adult male rabbits (*Oryctolagus cuniculus*) of approximately same ages (9 months) were divided into groups A,B,C,D and E, each comprising of ten animals. Group A animals were kept as control, and given empty capsules. Group B,C,D and E animals were given lindane ( $\gamma$ -HCH) orally in capsules with the help of vaginal speculum. One capsule containing lindane with dose level 5,10,15 and 20mg/kg body weight was given once daily for 4 weeks to B,C,D and E group animals respectively.

Status of hair growth was observed during the experimental study on weekly basis in the morning before giving feed. In addition, general physical conditions, including behavioral changes and body weight of each animal were recorded. Blood samples from the marginal ear vein of each animal were collected weekly for some hematoenzymatic parameters.

## RESULTS

Hairs of control group A animals were shiny, significant growth was noticed and there was no detectable hair loss up till the end of experiment.

Among group B animals, mild hair loss in neck region was noted during the 4<sup>th</sup> week of experiment. (Fig.1)

Mild but definite patchy loss on the trunk and neck regions was observed during last week in the C group animals. (Fig.2)

Detectable hair loss was seen during the 3<sup>rd</sup> week in group D animals over the trunk and neck regions, which became more marked and patchy toward the terminal stage of experiment. (Fig.3)

Detectable hair loss was observed even during the 2<sup>nd</sup> week in case of group E animals, that became marked and patchy during 3<sup>rd</sup> week (Fig.4) and in 4<sup>th</sup> week it was much more marked and diffused. (Fig.5)

**Figure-1**  
**External appearance of rabbit treated with 5 mg/kg (group B) of Lindane. Mild hair loss in neck & trunk region**



**Figure-2**  
External appearance of rabbit treated with 10 mg/kg (group C) of Lindane. Mild patchy loss of hair in neck & trunk region



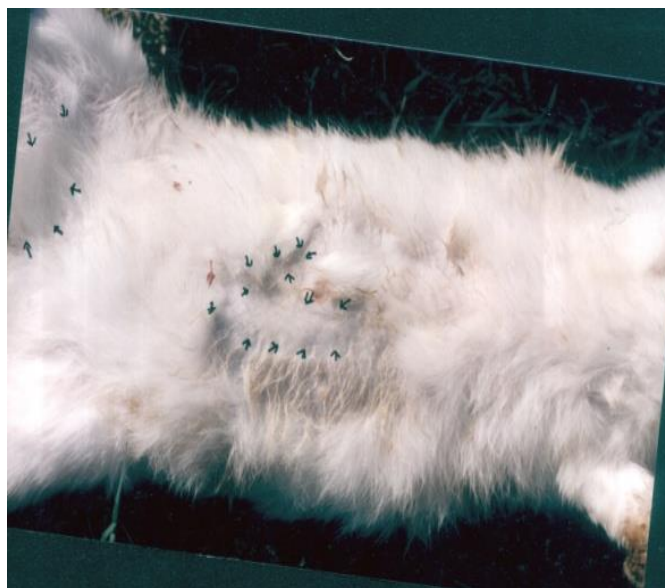
**Figure-4**  
External appearance of rabbit treated with 20 mg/kg (group E) of Lindane at the end of 3<sup>rd</sup> week. Marked & more diffuse loss of hair all over the body



**Figure-3**  
External appearance of rabbit treated with 15 mg/kg (group D) of Lindane. Marked patchy loss of hair in neck region & moderate patchy loss of hair in trunk region



**Figure-5**  
External appearance of rabbit treated with 20 mg/kg (group E) of Lindane at the end of 4<sup>th</sup> week. Patches of almost complete loss of hair on trunk



**Table-1**  
**Effect of Lindane ( $\gamma$ -HCH) on mean body weight of rabbit**

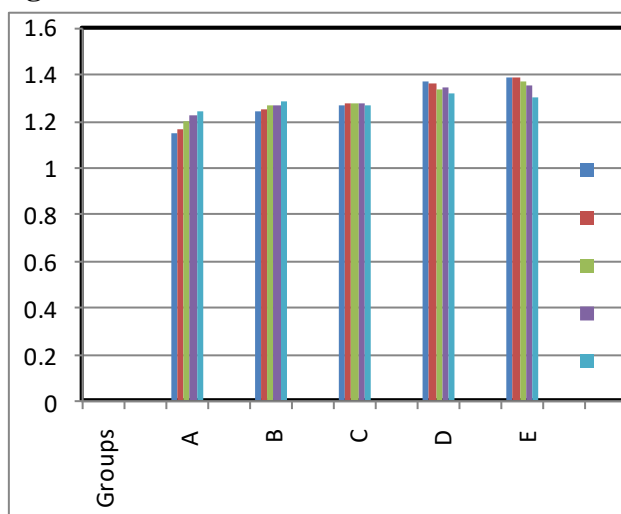
		Weight (kg)					
Groups		One Week	1 <sup>st</sup> Week	2 <sup>nd</sup> Week	3 <sup>rd</sup> Week	4 <sup>th</sup> Week	Growth rate (% again/day)
A	Mean	1.1423	1.1597	1.1970	1.2196	1.2432	0.2944
	$\pm$ SE	0.0017	0.0015	0.0024	0.0024	0.0016	0.0061
B	Mean	1.2445	1.2511	1.2631	1.2697	1.2794	0.0935**
	$\pm$ SE	0.0018	0.0028	0.0027	0.0035	0.0031	0.0101
C	Mean	1.2700	1.2750	1.2729	1.2707	1.2651	-0.015***
	$\pm$ SE	0.0030	0.0037	0.0034	0.0036	0.0029	0.0050
D	Mean	1.3700	1.3570	1.3309	1.3410	1.3200	-0.121***
	$\pm$ SE	0.0035	0.0033	0.0034	0.0083	0.0021	0.0061
E	Mean	1.3900	1.3840	1.3730	1.3510	1.3000	-0.216***
	$\pm$ SE	0.0033	0.0036	0.0032	0.0036	0.0033	0.0113

For statistical significance control group has been compared to treated group.

Student's t-test ( $P < 0.05 = *$ ,  $P < 0.01 = **$ ,  $P < 0.001 = ***$ )

Graph showing the effect of lindane ( $\gamma$ HCH) on growth rate of rabbit.

**Figure-6**



- Zero week
- 1<sup>st</sup> week
- 2<sup>nd</sup> week
- 3<sup>rd</sup> week
- 4<sup>th</sup> week

**Table-2**  
**Dose related effects of Lindane ( $\gamma$  HCH) on some hematoenzymatic parameters of rabbit**

Group	Serum Alkaline Phosphatase U/I				Serum Glutamic Pyruvic Transaminase U/I			
	1 <sup>st</sup> week	2 <sup>nd</sup> week	3 <sup>rd</sup> week	4 <sup>th</sup> week	1 <sup>st</sup> week	2 <sup>nd</sup> week	3 <sup>rd</sup> week	4 <sup>th</sup> week
A	49.13 $\pm$ 1.0	50.25 $\pm$ 0.3	50.30 $\pm$ 0.23	49.80 $\pm$ 1.5	41.87 $\pm$ 1.30	40.25 $\pm$ 0.8	40.50 $\pm$ 1.18	40.25 $\pm$ 0.98
B	52.0 $\pm$ 2.08	53 $\pm$ 0.47	54.50 $\pm$ 0.54	*57 $\pm$ 0.63	41 $\pm$ 0.44	43 $\pm$ 0.82	*45 $\pm$ 0.64	**50 $\pm$ 1.05
C	55.0 $\pm$ 0.86	*57 $\pm$ 0.64	*60 $\pm$ 1.22	**70 $\pm$ 1.26	43 $\pm$ 0.53	*45 $\pm$ 1.05	**55 $\pm$ 0.59	**60 $\pm$ 1.22
D	*60.0 $\pm$ 1.22	**80 $\pm$ 1.06	**100 $\pm$ 1.07	**110 $\pm$ 1.26	*45 $\pm$ 1.01	**50 $\pm$ 0.42	**60 $\pm$ 1.43	**69 $\pm$ 0.39
E	*62.0 $\pm$ 0.44	**85 $\pm$ 0.66	**104 $\pm$ 0.81	**114 $\pm$ 0.73	**47 $\pm$ 0.42	**52 $\pm$ 0.53	**65.05 $\pm$ 0.74	**79.5 $\pm$ 0.42

For statistical significance control group has been compared to treated group/student's t-test ( $p < 0.01 = * = p < 0.001 = **$ )

### PHYSIOLOGICAL CONDITIONS

Group A animals were generally healthy and active. Mild degree of restlessness and hyperactivity were noticed among group B animals during the last 4<sup>th</sup> week of experiment.

Mild degree of weakness, restlessness, excitement, hypersensitivity and reactivity were observed during 3<sup>rd</sup> week among C group animals, and these changes become more apparent at the end of 4<sup>th</sup> week.

---

In group D animals behavioral changes were realized even at the end of 2<sup>nd</sup> week, and these became more significant towards the end of experiment.

The behavioral changes were apparent even during early 2<sup>nd</sup> week among the E-group animals, and these became much more significant during 3<sup>rd</sup> week, and towards the end of experiment marked weakness, restlessness, excitement, hypersensitivity and hyperactivity were observed in all animals of the Group E

### **GIT Disturbances**

No observable change was noticed regarding food intake in group-A animals.

Reduced food intake (anorexia) and mild diarrhea was noticed among the group-B animals during 4<sup>th</sup> week of experiment.

There was dose and time related anorexia and diarrhea in group C, D and E animals during the experimental study period.

### **Body Weight**

In group A animals, there was generalized trend towards gain in body weight and at the end of experiment all animals gained significant body weight.

There was insignificant increase of body weight among the group-B animals at the end of experiment.

There was slight decrease in body weight of C-group animals at the end of experiment.

There was significant decrease in body weight of D-group animals and this decrease was more significant in Group-E animals at the end of study (Table-1)

### **Hematoenzymatic Parameters**

All parameters investigated remained constant in control group A animals throughout the experiment.

Serum alkaline phosphatase and SGPT level were increased significantly even in 1<sup>st</sup> week. Further increase followed in dose and time

related/dependent pattern and at the end of experiment significant increase was observed even in lowest dose treated group-B animals (Table-2) There was also dose and time related bleeding tendency that was noticed while taking blood samples.

## **DISCUSSION**

### **Hair loss**

This could be due to the deposition of lindane ( $\gamma$ -HCH) subcutaneously damaging the hair follicles<sup>16</sup> and since,  $\gamma$ -HCH has strong tendency to deposit subcutaneously<sup>17</sup>, it could directly damage the hair follicle, thence the hair loss.

Decreased blood supply to hair follicle caused by damage to blood capillaries could be the other cause of hair loss.<sup>18</sup>

### **Behavioral Changes**

These changes could be result of direct drug action on CNS.<sup>17</sup>

Lindane is a neurotoxin that interfering with the GABA receptor-chloride channel complex,<sup>19,20,21</sup> could produce a range of symptoms from headache and dizziness to seizures, convulsions and more rarely death.<sup>10,11</sup>

### **GIT Disturbances**

Lindane has direct action on the satiety center in CNS<sup>17</sup> and it is also excreted in saliva with very nauseating metallic taste<sup>22</sup>, thus causing the loss of appetite (anorexia). Diarrhea could be due to direct action of lindane on Enteric Nervous System of the GIT.<sup>17</sup>

### **Body Weight**

The cause of reduced weight could be due to anorexia<sup>23</sup>, enhanced protein degradation along with decreased synthesis and GIT disturbances.<sup>24</sup>

### **Hematoenzymatic Changes**

Lindane has been found to increase lipid per oxidation (LPO) and decrease glutathioine (GSH) level, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), glutathione reductase (GR), quinine reductase activity and protein.<sup>25</sup>

---

The level of serum alkaline phosphatase is always considered to be the index for obstructive and degenerative hepatic disease. Increase serum levels of transaminase (SGPT & SGOT) have been described to be associated with cell necrosis of many different tissues.<sup>26</sup>

Liver is a metabolically versatile organ responsible for the regulation of internal chemical environments<sup>12</sup>, the primary site for detoxification<sup>5</sup> and it is major organ of antioxidant defense system<sup>27</sup>. Lindane hepatotoxicity would be reflected by wide variety of changes in serum level of alkaline phosphatase, SGPT and SGOT.<sup>26</sup> In this study, lindane proved to be a highly toxic chemical that produced variety of changes even with smaller (5mg/kg of body weight) dose. Since it is a persistent pollutant<sup>1</sup> and further it can get into body by oral route (eating lindane contaminated food like plants, meat and dairy products), pulmonary route (breathing in work places) and through skin (through use of soaps, lotion, or shampoo containing lindane, that help treat and control head and body lice and scabies)<sup>28</sup>. The chances of accumulation in body fat and subsequent toxicity are more in/among communities in the developing countries (like Pakistan), where the environmental protection and food and drug administration agencies might not be as effective as in developed countries.

## CONCLUSION

Lindane is highly toxic. Keeping in view of its highly toxic nature and its rapid absorption through all major routes<sup>28</sup> (oral, pulmonary and skin), its (lindane) use as a pesticide must be strictly banned (as in developed countries)<sup>4</sup> and its pharmaceutical use must only be allowed under strict medical advice.

## REFERENCES

1. Okai M, Kubota K, Fukuda M, Nagata Y, Nagata K, Tanokura M. Crystallization and preliminary X-ray analysis of gamma-hexachlorocyclohexanedehydrochlorinase Lin A from *Sphingobium japonicum* UT26. *Acta Crystallogr Sect F Struct Biol Cryst Commun.* 2009; 65: 822-4.
2. Brandenberger H, Maes RAA, editors. *Analytical toxicology: for clinical, forensic and pharmaceutical chemists.* Berlin; New York: de Gruyter: 1997.
3. Nolan K, Kamrath J, Levitt J. Lindane toxicity: a comprehensive review of the medical literature. *Pediatr Dermatol.* 2012; 29: 141-6.
4. Humphreys EH, Janssen S, Heil A, Hiatt P, Solomon G, Miller MD. Outcomes of the California ban on pharmaceutical lindane: clinical and ecologic impacts. *Environ Health Perspect.* 2008; 3: 297-302.
5. Padma VV, Lalitha G, Shirony NP, Baskaran R. Effect of quercetin against lindane induced alterations in the serum and hepatic tissue lipids in wistar rats. *Asian Pac J Trop Biomed.* 2012; 02: 910-5.
6. Saravanan M, Prabhu Kumar K, Ramesh M. Haematological and biochemical responses of freshwater teleost fish *Cyprinus carpio* (Actinopterygii: Cypriniformes) during acute and chronic sublethal exposure to lindane. *Pesticide Biochem Physiol.* 2011; 100: 206-211.
7. Vijaya Padma V, Sowmya P, Arun Felix T, Baskaran R, Poornima P. Protective effect of gallic acid against lindane induced toxicity in experimental rats. *Food Chem Toxicol.* 2011; 49: 991-998.
8. Bano M, Bhatt D. Neuroprotective role of a novel combination of certain antioxidants on lindane induced toxicity in cerebrum of mice. *Res J Agri Bio Sci* 2007; 03: 664-669.
9. Thomson Micromedex. 2006. Lindane topical. In: Volume 1: Drug Information for the Healthcare Professional. 26th ed. Greenwood

- 
- Village, CO: Thomson Micromedex, 1940–1943.
10. Harnic D Rasic- Markovic A, Djuric D, Susic V, Stanojlovic O. The Role of nitric oxide in convulsions induced by lindane in rats. *Food chem. Toxicol.* 2011; 49: 947-954.
  11. Centers for Disease Control and Prevention (CDC). Unintentional topical Lindane ingestions-United States, 1998-2003. *MMWR Morb. Mortal.* 2005; 54: 533-5.
  12. Zucchini-Pascal N, de Sousa G, Rahmani R. Lindane and cell death: At the crossroads between apoptosis, necrosis and autophagy *Toxicol.* 2009; 256: 32-41.
  13. Alvarez-Pedrerol M, Ribas-Fito N, Torrent M, Carrizo D, Garcia-Esteban R, Grimalt JO, et al. Thyroid disruption at birth due to prenatal exposure to beta-hexachlorocyclohexane. *Environ Int.* 2008; 34: 737.
  14. McCarthy JS, Kemp DJ, Walton SF, Currie BJ. Scabies: more than just an irritation. *Postgrad Med J.* 2004; 80: 382-7.
  15. Thomas DR, McCarroll L, Roberts R, Karunaratne p, Roberts C, Casey D, et al. Surveillance of insecticide resistance in head lice using biochemical and molecular methods. *Arch Dis Child.* 2006; 91: 777-9.
  16. Anand M, Gulati A, Gopal K, Gupta GS, Khanna RN, Rays PK, et al. Hypertention and myocarditis in rabbits exposed to r HCH and endosulfan. *Vet Hum Toxicol.* 1990; 32: 521-523.
  17. Negherbon WO. Insecticides In: Hand book of toxicology. Vol III. W. B. Saunder's Company: Philadelphia; 1956.
  18. Dikhith TS, Raizada RB, Sriastiva MK. Long Term dietary study and development of no observed effect level of technical r HCH to rats. *J Toxicol Environ Health.* 1991; 34: 495-507.

## AUTHORS

- **Prof. Dr. Liaqat Ali**  
Professor of Anatomy  
UM & DC Faisalabad
- **Dr. Muhammad Aslam**  
Associate Professor of Forensic Medicine  
UM & DC Faisalabad
- **Prof. Dr. Khalid Mahmood Akhter**  
Professor of Anatomy  
Independent Medical College Faisalabad
- **Dr. Sundus Tariq**  
Assistant Professor of Physiology  
UM & DC Faisalabad

Submitted for Publication:	06-05-2013
Accepted for Publication:	20-01-2014
After minor revisions	