

## ACUTE TOXICITY AND GROSS BEHAVIOURAL EFFECTS OF INDOXACARB IN LABORATORY ANIMALS

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### ABSTRACT

Indoxacarb is the first commercial insecticide of the oxadiazine group used to protect vegetables from a number of pests. The maximum tolerated oral dose (MTD) was determined in mice and rats as 500 mg/kg and 600 mg/kg, respectively. The hyperexcitation, intermittent clonic convulsions, vigorous rolling, head tilt, salivation, increase heart rate and respiration, restlessness, loss of grip strength and biting behaviour were common symptoms of toxicity. Indoxacarb showed delayed toxicity in mice and rats when single dose administered MTD group was kept for 14 days. Some mice and rats died during this duration of 14 days and rest showed decrease in body weight. Haematological examination performed on 14<sup>th</sup> day of single dose acute toxicity study showed a decrease in haemoglobin content, leucocyte counts and erythrocyte counts suggesting some adverse effects on blood cells of mice.

**Key words:** Indoxacarb, MTD, haematology, body weight, gross behaviour

Indoxacarb is the first broad-spectrum insecticide of the oxadiazine group which chemically is (s)-methyle-7-chloro-2,3,4a,5-tetrahydro-2-[methoxy carbonyl(4-trifluoro methoxy phenyl)carbamoyl] indeno[1,2-e][1,3,4]oxadiazine-4a carboxylate and is used on vegetables to control lepidopteran and other insect pests having sucking mouthparts (Harder *et al.*, 1997, Anon, 1998, Wing *et al.*, 1998, Liu *et al.*, 2002). It has been bracketed under reduced risk product by the Environment Protection Agency (Anon, 1998). Some preliminary toxicological information on this compound has been reported and the systematic toxicological study has not been conducted so far. Therefore, study on acute toxicity and gross behavioural effects of Indoxacarb in adult male Wistar albino rats and Swiss albino mice has been undertaken.

### MATERIALS AND METHODS

The studies were conducted in male mice and rats on the commercially available formulation product of indoxacarb (Kingdixa 145 SC)

manufactured by Gharda Chemicals Ltd., India. Swiss albino male mice weighing between 25-35 g and Wistar albino male rats weighing between 125-175 g were used in this study.

Maximal tolerated dose (MTD) of indoxacarb was determined in mice and rats by the method described by Moser and Padilla (1997). For determining pilot dose range small group comprising of 2 to 3 animals were used for single oral doses each of 750, 600, 550, 500, 450 and 400 mg/kg in mice and 1000, 900, 800, 700, 600 and 500 mg/kg in rats which included a few lethal doses. The animals were fasted overnight with only water being provided *ad libitum*. The MTD was considered as the dose that produced clear observable signs of toxicity without any lethality. Subsequently, MTD was verified by administering close doses in 3 larger group of animals each comprising of 10 animals which led to final selection of acute oral dose which resulted in manifestation of maximum toxic symptoms with either no mortality or to the maximum of 10 per cent. The mice and rats of MTD group were subjected to observation for 14 days post administration. Gross observable behavioural effects were observed daily for 14

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days in case of mice and six days in case of rats as all the rats died within a period of 7 days. Body weight changes in mice and rats were recorded by weighing individual animal on alternate days. Blood samples of mice were also collected for haematological studies. Necropsies were performed on these animals at the end of experiments. The results were expressed as Mean  $\pm$  S.E. and results were analyzed by using unpaired student's *t*-test as described by Panse and Sukhatme (1978).

## RESULTS AND DISCUSSION

The MTD of indoxacarb was found to be 500 mg/kg in mice and 600 mg/kg in rats. Sarver (1996) reported oral median lethal dose ( $LD_{50}$ ) of indoxacarb technical grade as 1730 and 267 mg/kg in male and female rats, respectively. The acute oral  $LD_{50}$  has been reported to be in excess of 3000 mg/kg (Mulder *et al.*, 1975, Jacobson, 1990).

Indoxacarb treated rats and mice showed a dose dependent onset and severity of toxic symptoms. Grossly observable symptoms after indoxacarb administration started with staggering gait, motor incoordination and prostrations. Other major symptoms observed were intermittent clonic convulsions, vigorous rolling, head tilt, salivation, increase heart rate and respiration, restlessness, loss of grip strength and biting behavior. Gross observable symptoms started between 30 to 45 min in mice whereas these were seen in 40 - 60 min in rats following the administration of indoxacarb. The difference in time of onset may be due to difference in absorption of indoxacarb from the gastrointestinal tract of the two species. At higher doses the death of animals could be attributed to severe central nervous system toxicity (like motor incoordination, clonic convulsions and head tilt). Frame (1997a) also observed the cause of death as CNS disorders, heart inflammation or necrosis with DPX-JW 062-106 (1 part active (DPX-KN128 S-enantiomer) and 1 part inactive (IN-KN127 R-enantiomer) enantiomer of indoxacarb). Thus, indoxacarb seems primarily to be a neurotoxic insecticide. In the MTD group, the peak effect induced by indoxacarb was seen

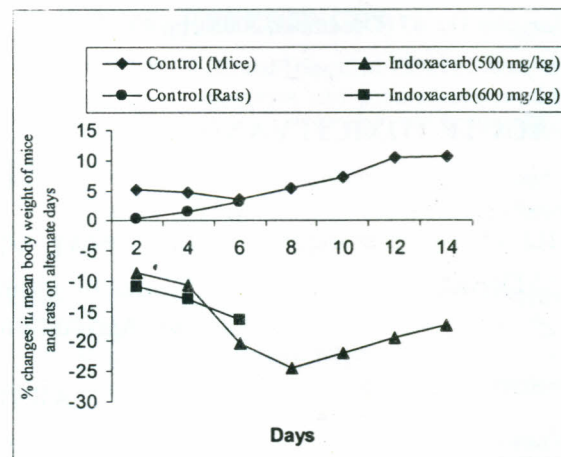


Fig 1. Effect of acute toxicity of indoxacarb on body weight of mice and rats.

within 1 to 2 hours after oral administration in mice but in rats peak toxicity appeared on the second day. Animals were observed daily upto 14 days for their body weight gain, normal activity and feed consumption. The body weight recorded for 14 days was decreased (Fig 1) and may be due to reduced feed consumption. The maximum decrease in body weight was observed to the extent of 25% in mice on 8<sup>th</sup> day and 15% in rats on 6<sup>th</sup> day. During the study one mouse died each at 12<sup>th</sup> and 13<sup>th</sup> day of the administration whereas all rats died within a period of 7 days after indoxacarb administration. The death of animals on different days after single dose administration strongly suggests that indoxacarb produces delayed toxicity in rodents.

Table 1

Effect of indoxacarb (500mg/kg, P.O.) on various haematological parameters in mice

Parameters	Control*	Treatment**
Hb (g/dl)	13.4 $\pm$ 0.2 <sup>a</sup>	11.1 $\pm$ 0.2 <sup>b</sup>
TEC (x 10 <sup>6</sup> )	9.2 $\pm$ 0.6 <sup>a</sup>	7.3 $\pm$ 0.3 <sup>b</sup>
TLC (x 10 <sup>3</sup> )	12.9 $\pm$ 1.5 <sup>a</sup>	7.6 $\pm$ 1.4 <sup>b</sup>
Lymphocytes (%)	65.5 $\pm$ 0.8 <sup>a</sup>	60.6 $\pm$ 1.5 <sup>b</sup>
Neutrophils (%)	30.5 $\pm$ 0.6 <sup>a</sup>	35.5 $\pm$ 1.5 <sup>b</sup>
Monocytes (%)	3.8 $\pm$ 0.4 <sup>a</sup>	3.5 $\pm$ 0.4 <sup>a</sup>
Eosinophils (%)	0.1 $\pm$ 0.1 <sup>a</sup>	0.2 $\pm$ 0.1 <sup>a</sup>

\* Six animals were used

\*\* Eight animals were used

Values are mean  $\pm$  S.E.

Values bearing common superscripts within a row of a parameter do not differ significantly at 5% level of significance.



Rats seem to be more susceptible than mice to indoxacarb toxicity. Necropsy findings did not show any gross changes or lesions in the vital organs except lungs and liver being slightly congested.

The data of haematological studies, performed in mice on 14<sup>th</sup> day of the study are presented in the Table 1. Indoxacarb decreased the haemoglobin content, leukocyte and erythrocyte counts in mice. These effects could be due to adverse effects of insecticide on bone marrow or direct destruction of blood cells. However, a similar decrease in haemoglobin content, and erythrocyte counts was also observed in subchronic toxicity of rats and dogs (Malek, 1997, Mertens, 1997, Frame, 1997b), which has observed the cause of death of rats as bone marrow atrophy, splenic lymphoid depletion and thymic necrosis.

Indoxacarb was found to cause significant lymphocytopenia and neutrophilia in acute toxicity in mice and rats. These findings suggested that indoxacarb may act as stressor agent and cause release of glucocorticoids which further cause lymphocytopenia and neutrophilia. Chemical stressors stimulate the release of ACTH and cause lymphocytopenia and neutrophilia (Haynes and Murad, 1980). The results suggest that indoxacarb has some adverse effect on blood cells. The above findings in mice and rats suggest that indoxacarb produces delayed toxicity involving central nervous system and blood cells.

## REFERENCES

- Anon, (1998). Avaunt <sup>TM</sup>, insect control agent. Technical Bulletin H 79164 (Wilmington: DuPont).
- Frame, S.R. (1997a) Oncogenicity study with DPX-JW062-106 (50% DPX-KN128, 50% DPX-KN127) eighteen-month feeding study in mice. Unpublished report No. HLR 799-96 from DuPont Haskell Laboratory, Newark, Delaware, USA. Submitted to WHO by E.I. du Pont de Nemours and Company, Wilmington, Delaware, USA. ([http://whqlibdoc.who.int/publications/2006/9241665211\\_12\\_eng.pdf](http://whqlibdoc.who.int/publications/2006/9241665211_12_eng.pdf)).
- Frame, S.R. (1997b). Oncogenicity study with DPX-JW062-106 (50% DPX-KN128, 50% DPX-KN127) two-year feeding study in rats. Unpublished report No. HLR 1174-96, RV1 from DuPont Haskell Laboratory, Newark, Delaware, USA. Submitted to WHO by E.I. du Pont de Nemours and Company, Wilmington, Delaware, USA. ([http://whqlibdoc.who.int/publications/2006/9241665211\\_12\\_eng.pdf](http://whqlibdoc.who.int/publications/2006/9241665211_12_eng.pdf)).
- Harder, H.H., Riley, S. L., McCann, S.F. and Irving, S.N. (1997). DPX-MP062: A novel broad-spectrum, environmentally soft, insect control compound. In P. Dugger and D. Richter (eds), Proceedings of Beltwide Cotton Conferences, New Orleans. LA, USA, 6-10 January 1997 (Memphis: National Cotton Council). pp. 48-50.
- Haynes, R.C. Jr. and Murad, F. (1980). In: Goodman and Gilman's. The Pharmacological basis of Therapeutics. (6<sup>th</sup> edn.), MacMillan Publishing Co. Inc., New York. pp. 1466-1498.
- Jacobson, R.M. (1990). A new class of insecticidal dihydropyrazoles. In: Crombie, L. (Edt.), Recent Advances in the Chemistry of Insect Control I. Royal Society of Chemistry, London, pp. 206-211.
- Liu, T.X., Sparks, Jr. A.N., Chen, W., Liang, G.M. and Brister, C. (2002). Toxicity, Persistence and efficacy of Indoxacarb on cabbage looper (Lepidoptera: Noctuidae) on cabbage. *Economic Entomol.* **92**: 360-367.
- Malek, D.E. (1997). Subchronic oral toxicity: 90-day study with DPX-JW062-106 (50% DPX-KN128, 50% DPX-KN127) feeding study in rats. Unpublished report No. HLR 751-93, RV2 from DuPont Haskell Laboratory, Newark. ([http://whqlibdoc.who.int/publications/2006/9241665211\\_12\\_eng.pdf](http://whqlibdoc.who.int/publications/2006/9241665211_12_eng.pdf)).
- Mertens, J.J.W.M. (1997). Subchronic oral toxicity: 90-day study with DPX-JW062-106 (50% DPX-KN128, 50% DPX-KN127) feeding study in dogs. Unpublished report No. HLO 494-95, RV3 from WIL Research Laboratories, Inc., Ashland, Ohio, USA. Submitted to WHO by E.I. du Pont. ([http://whqlibdoc.who.int/publications/2006/9241665211\\_12\\_eng.pdf](http://whqlibdoc.who.int/publications/2006/9241665211_12_eng.pdf)).
- Moser, V.C and Padilla, S. (1997). Age and gender-related differences in the time course of behavioural and biochemical effects produced by oral chlorpyrifos in rats. *Toxicol. Appl. Pharmacol.* **149**: 107-119.
- Mulder, R., Wellinga, K. and van Daalen, J.J. (1975). A new class of insecticides. *Naturwissenschaften* **62**: 531-532.
- Pansee, V.G and Sukhatme, D.V. (1978). Statistical Method for Agricultural Workers, (3<sup>rd</sup> edn.), ICAR, New Delhi.
- Sarver, J.W. (1996) Acute oral toxicity study with DPX-MP062 technical (approximately 75% DPX-KN128 and 25% DPX-KN127) in male and female rats. Unpublished report No. HLR 910-96 from DuPont Haskell Laboratory, Newark, Delaware, USA. Submitted to WHO by E.I. Du Pont de Nemours and Company, Wilmington, Delaware, USA. ([http://whqlibdoc.who.int/publications/2006/9241665211\\_12\\_eng.pdf](http://whqlibdoc.who.int/publications/2006/9241665211_12_eng.pdf)).
- Wing, K.D., Schnee, M.E., Sacher, M. and Connair, M. (1998). A novel oxadiazine insecticide is bioactivated in lepidopteran larvae. *Archive Insect Biochem. Physiol.* **37**: 91-103.