Hepato-toxic effect of diuron in albino rats

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Tumour initiating/promoting effect of diuron, a widely used substituted urea herbicide, was studied in rats using liver tumour model. Chronic exposure to diuron at a dose of 250 mg/kg body wt resulted in high mortality and weight loss in treated animals. The animals which received diuron + HCH treatment showed an increase in size and weight of liver as compared to controls. Liver tumours were not observed in any of the treated group whereas some significant histological changes were seen in diuron treated rat liver. Diuron thus has been found to be hepatotoxic albeit neither tumour initiating nor promoting in rat liver tumorigenesis assay system.

Diuron (chemical name: 3(3,4-dichlorophenyl)1,1, dimethyl urea) is a widely used herbicide. It causes irritation to eyes and mucous membrane of rabbits. Fetotoxicity and increased number of malformed fetuses following oral administration of diuron are reported in rats. Diuron has been found to be a tumour initiator in mouse skin carcinogenesis bioassay. Further, it is a suspected mutagen as it proved positive in Ames' test and testicular DNA biosynthesis assays. Diuron also induces micronuclei formation in mouse bone marrow cells and causes dominant lethal mutations in Swiss mice.

Hexachlorocyclohexane (HCH), a widely used insecticide is described as a hepatocarcinogen in the literature. It is also reported as a promoter of liver tumours in diethyl nitrosamine initiated rat liver and as a initiator (at the dose of 500 ppm for 4 months) in chilli extract promoted mouse liver tumorigenesis system.

The International Agency for Research on Cancer, Lyon, France, has considered diuron as one of the priority chemicals to be tested for its carcinogenic and mutagenic effects in experimental animals. Keeping in view its increased use as a herbicide in India and non availability of data on its liver tumour initiating or promoting activity, a study on the liver tumour initiating/promoting effect of diuron using 2-AAF initiated/HCH promoted rat liver tumorigenesis model was undertaken.

Diuron and hexachlorocyclohexane (HCH) were procured from Sigma Chemical Co., U.S.A. Other chemicals were procured locally and were of reagent grade.

The male albino rats of about 100 g body wt. (8 to 10 weeks old) were obtained from the animal colony of our Research Centre. Only 5 to 6 rats were housed in each cage and provided standard pellet diet and water ad libitum.

Preparation of diuron and HCH containing diet—The standard pellet diet was powdered using the mixer and grinder. 12.5 g diuron (technical grade) was dissolved in 500 ml of acetone. This solution was mixed with 5 kg of powdered diet and left overnight in open to dry. This gave the concentration of 2500 ppm which considering 10 g consumption of diet/100 g rat was equivalent to the dose of 250 mg/kg body wt. This dose was 7.5% of the reported oral LD50 value of diuron (3400 mg/kg body wt). Similarly, 2.5 g of technical grade HCH was dissolved in 500 ml of acetone and this solution was mixed with 5 kg powdered diet to have a final concentration of 500 ppm HCH in diet. These diets were prepared fresh fortnightly and used for all experiments.

The following groups show the experimental design. Each group consisted of 25 animals.

Diuron + HCH—Diuron containing diet was fed to the animals for 4 months and then HCH containing diet was provided for 8 months. Afterwards a normal diet was given till death / end of the experiment.

Diuron alone—Diuron containing diet was fed for 4 months and afterwards normal laboratory diet was given till death / end of the study.

HCH alone—HCH containing diet was fed for 8 months and afterwards normal laboratory diet was given till death / end of the study.
2-AACF + DIURON—2-Acetaminoflourene (200 ppm) was fed through diet for 19 days followed by diuron (500 ppm in diet for 8 months at the dose of 50 mg/kg body wt and afterwards normal-laboratory diet was given till death/end of the study.

2-Acetaminoflourene alone (2-AAF)—2-AAF was fed through diet (200 ppm) at a dose of 20 mg/kg body weight for 19 days and afterwards the normal diet was provided till death/end of the experiment.

Solvent control—The animals received solvent mixed diet till death/end of the experiment.

The animals which appeared weak and moribund were killed and were examined carefully for appearance of gross tumour in any tissue. Liver and lung were collected for routine histology. The 5 μm paraffin sections were cut and stained with Haemotoxylin and Eosin.

Treatment with diuron either alone or in combination with HCH for 8 months resulted in high mortality and weight loss of experimental animals as compared to untreated control groups. About 48% of the animals died in diuron + HCH treated group as compared to 40% in diuron treated or 10% in solvent treated control groups. The treatment of diuron + HCH resulted in hepatomegaly as compared to the corresponding control group. No liver tumour was observed in any of the treated/control groups (Table 1). The histology of the livers of diuron+HCH treated group showed fatty infiltrations of large number of cells. The animals which received the treatment of diuron or HCH also showed necrosis and similar fatty changes in the hepatic tissue.

The technical grade HCH is known to be hepatomegalic in laboratory animals, however the observation that HCH in combination with diuron further increases the liver weight synergistically is noteworthy. Further the observation that diuron alone or in combination with HCH or 2-AAF could not induce liver tumourigenesis is a significant finding and suggest that diuron is neither a tumour initiator nor promoter in the rat liver tumourigenesis assay model. These reports are although contrary to the reported clastogenic and tumour initiating effect of diuron in other experiment system albeit appear important because of the expected occupational hazards in the concerned agroindustries.

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References