قسم : طب الحيوان - كلية الطب البيطرى - جامعة الزقازيق
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استحداث أورام بواسطة مادة الداى فلوبنز ميرون مباشرة أو خلال التعرض عبر السخد فى الفئران البيضاء

فوزى شعبان ، سمية السيد ، مجدى قدرى ، أنيسة مصطفى ، على حيدر

تم تحديد الجرعة متوسطة السمية للداى فلوبنزيرون فى الفئران البيضاء جنس العجوزا . وبترك الحيوانات التى لم تنفق من خلال تحديد الجرعة متوسطة السمية لمدة احدى عشر شهرا ثم ذبحت وأثبت الفحص الهستوباثولوجى وجود ورم حليمى قشرى خلوى فى المعدة وورم سرطانى قشرى خلوى بوليجونى فى الرئتين وقد ثبت خلال هذه الدراسة أن نسل الحيوانات التى أعطيت الجرعة متوسطة السمية فى اليوم الثامن عشر من الحمل ظهور ورم سرطانى كبدى خلوى متنقل الى الرئه فى الذكور والاناث بعد ثلاثة أشهر من عمر النسل .

تم الكشف ومدى توزيع الداى فلوبنزيرون خلال الأنسجة وامكانية مروره خلال السخد بواسطة استعمال التحليل اللونى الطبقى الرقيق مع جهاز مقياس الضوء الطبقى وجهاز تحليل الاشعة تحت الحمراء .

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DIRECT AND TRANSPLACENTAL INDUCTION OF TUMOR IN RATS AFTER SINGIE EXPOSURE TO DIFLUBENZURON INSECTICIDE

(With 1 Table & 7 Figures)

By

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SUMMARY

The oral LD50 of Diflubenzuron was determined in Agoza strain albino rats, survived animals were left and slaughtered after eleven months. Post mortem and histopathological examination revealed the presence of squamous cell carcinoma in the stomach and polygonal cell carcinoma of the lungs. The offspring from mothers administered the LD50 dose at the 18th day of pregnancy developed hepatocellular carcinoma which metastasis in their lungs. Detection, tissue distribution and evidence of transplacental transmission of diflubenzuron were investigated by TLC, spectrophotometric and Infra-red spectrscopic techniques.

INTRODUCTION

It is now an established fact, that during the last two or three decades malignant tumors have became one of the main causes of mortality among childern under fifteen in most of the industrial developed countries (ARIEL and PACK, 1960; MARSDEN and STEWARD, 1968). The previous conclusion depends also upon the WHO figures published in 1967, 1970, and according to international statistics of cancer in childhood (IVANKOVIC, 1972) the placental transfere of chemical attract our interst to study the effect of diflubenzuron insecticide which is now used in a large scale in Egypt as an insecticide against cotton and fruit culture pest and may be used in the future for control of mosquites and flies, either alone or in combination with other insecticides.

MATERIAL AND METHODS

Experimental Animals:

Adult male and female Agoza strain rats with a body weight of 180 to 200 g had been purchased from breeding colony of Ministry of Health, Cairo. All animals were housed in stainless-steel cages with hard-wood shaving as beeding, semipurified powdered diets and water were available ad libitum. Vaginal smear examined daily and females at esterus stage housed with one male, the day on which spermatozoa were found in the vaginal smear designated the first day of pregnancy, at this time, the male was removed from the cage.

Insecticide and Method of Administration:

Diflubenzuron,a member of the benzoyl phenyl urea group of insecticides (Dimiline, TH 6040, N "4 chlorophenyl" amino carbonyl 2, 6 - difluorobenzamide) received from Philips Thilips technical office in Cairo as 25% wettable powder. Insecticide was given via gastric intubation using rat stomach tube (Huber Hil Fiker,Swizerland).

Experimental Procedures:

1- Determination of LD50: The oral LD50 of Diflubenzuron was carried out according to Lichtfield and Wilcoxon method (1949). Histopatchological examination was carried out on died animals, while living, slaughtered after eleven months and histopathological studies were carried out on different tissues.

2- Effects of diflurobenzuron on pregnant mothers foetuses, placenta and offsprings: fourty one pregnant mother administered the LD50 dose via intragastric intubation at the 18th day of gastation and the following investigations were carried out on living mothers and their offsprings:-

   a- To study the effect of diflubenzuron on placenta, as mothers eat placenta just after delivery, six, mothers rendomely chosen after 24 hours post administration, their placenta were taken just after delivery subjected to histopathological examination.

   b- To avoid milk factor as mean of transmission fourty featuses were taken just after delivery, half foetuses subjected to histopathological examination while the other used for detection of diflubenzuron in their tissues.

   c- To investigate the transplacental induction of tumor, fourty nine offspring were left with their mothers, then slaughtered after three months. Histopathological examinations were carried out in all organs.

3- Extraction, Detection and Analysis of Diflubenzuron in tissues: Extraction of diflubenzuron from different tissues was carried out by precipitation tissue protein by the classic stas otto process, the protein free residue was extracted in alkaline medium by chloroform which then separated and evaporated to dryness. The residue dissolved in a known volume of ethanol, then extraction in acid medium by chloroform. Detection and quantitative analysis were carried out by colour, tests, thin layer chromatography and ultraviolet spectrophotometric analysis using the automatic recording ultraviolt unicum sp. 1800. Detection of the compound in different organs and foetus was also confirmed by the infrared spectroscopy using infrared Unicum spectrophotometer Sp. 1100 using potassium bromide disc technique.

Statistical analysis of data were analysed according to Steel and Torrie method (1960).

RESULTS

The oral LD50 of diflubenzuron for Agoza strain female rats was found to be 4000 mg/kg. with 19/20 confidence limits of 3007 - 5320 mg/kg. using Lichtfield and WILCOXON method (1949).

1- TLC technique using system of benzene ethanol (4:1) and sprayed by dragendoff reagent showed that the RF value of the insecticide equal to 0.63.

2- The ultraviolt spectrophotometric analysis of diflubenzuron using automatic recordidng Unicum Sp. 1800 showed maximum absorbance at 262 um wave length.

3- The infrared spectrum showed three major bands for diflubenzuron 720, 1750 and 2115 cm.

4- Detection and estimation of the compound from different organs of adult female rats showed its distribution in G.I.T (stomach and intestine), liver, kidneys and brain. The total abundance is given in Table 1.

TABLE (1)

Total abundance of diflubenzuron in different organs of adult female rats.

Sample No.	Level of diflubenzuron (mg/gm of organ)			
	Stomach and intestine	Liver	Kidney	Brain
I	1.9	1.75	0.30	0.32
II	1.5	1.80	0.45	0.28
III	1.8	1.55	0.40	0.35
Mean ± S.E.	1.73±0.25	1.70±0.13	0.38±0.08	0.32±0.03

* each sample consisted of organs taken from four animals.

5- The insecticide was detected in the tissue of embryos obtained from mothers administered the compound at last stage of pregnancy using combind TLC and ultraviolt spectroscopy techniques and confirmed by infrared spectroscopy (Fig. 1).

Histopathological changes produced in Agoza strain female rats after oral administration of single dose of Dimilin (LD50) and slaughtered after 11 months post administration:

Stomach: The epithelial lining showed squamous cell papilloma in the fore stomach (Fig. 2) while the glandular portion was ulcerated and infiltrated with lymphocytes, and macrophages. The qasteric gland shwoed hyperplastic hyaline degeneration of the muscular layer and congestion of the subserosal blood vessels.

Intestine: The intestine showed catarrhal enteritis. The doudenal submucosa showed hyperplasia of Brunner's glands. The submucosa was infiltrated with macrophages and showed lymphocytic hyperplasia, the muscular coat presented hyaline degeneration.

Liver: Congestion and inflammatory cells, mainly lymphocytes, macrophages and few nutrophils were present around the portal and central veins and in portal tracts. Dissociation of the hepatic cells with focal areas of coagulative necrosis were present. Haemosiderin pigments could be seen inside the hypertrophic kupffer's cells and macrophages, also pleomorphism of the hepatic cells and hepatomegalocytes with large hyperchromatic nuclei and mitotic divisions were seen. Telangiectasis could be seen, necrotic areas were replaced by macrophage and lymphocytes cell infiltration and the number of bile ducts was increased (Fig. 3).

Spleen: Congestion with severe haemorrhage in white and red pulps were evident. Hyperplasia of Malpighian bodies could also be seen, other Malpighian bodies showed degeneration.

Lungs: The lungs showed bronchopneumonia and the peribronchial blood vessels surrounded with numerous fibroblasts. lymphocytes and macrophages. The pulmnary tissue showed some alveoli filled with serous exudate mixed with few erythrocytes and giant cells. Hyperplastic nodular aggregations of lymphocytes partially replaced the pulmonary tissue. Adjacent areas of collapse and emphysema were present. Carcinoma of lung tissue could be seen in the form of polygonal cell carcinoma inside the lumina of the alveoli and squamous carcinoma was present (Fig. 4, 5)

Heart: The myocardium showed acute non suppurative myocarditis.

Kidneys: Both kidneys exhibited acute interstitial non suppurative nephritis and focal areas of coagulative necrosis were evident in the renal parenchyma.

Brain: The meninges were congested and haemorrhagic, satellitosis, neuronophagia and nodular proliferation of glial cells were seen in the cerbral hemispheres. Degenerated purkinje cells were numerous and the chroid plexus was congested.

Histopathological changes in offspring three months old after prental administration of Dimiline:

Stomach: It showed catarrhal gastritis with submucosal oedema and focal lymphocytic aggregations. Haemorrhagic myositis was seen in the muscular coat, where some muscle fibres were hyalinized.

Intestine: Catarrhal enteritis was evident.

Liver: Proliferated fibroblasts were present around the congested central and portal veins. The hepatic cells showed hydropic degeneration hepatocellular carcinoma with clear cells was seen.

Spleen: Haemorrhage was seen inside the white pulp. The red pulp was highly congested. The malpighian bodies were partially depleted.

Lungs: The pulmonary tissue contained secondary hepatic cell carcinoma surrounded with haemorrhage (Fig. 6) in addition to dilatation of peribronchial blood vessels and surrounded with lymphocytes mixed with few neutrophils.

Heart: Some cardiac muscle fibres were hylainized. Fibroblasts, lymphocytes and macrophages were seen among the degenerated muscle fibres.

Kidneys: The renal parenchyma was congested, haemorrhagic and showed cloudy swelling, focal aggregation of round cells were present in the renal cortex. Some renal tubules specialy in the medulla showed hyaline casts and cystic dilatation. Proliferated fibroblasts were seen among the renal tubules.

Testes: They were congested and haemorrhagic, degnerative changes were seen in the germ cells. Spermatogenesis was almost completely stopped.

Brain: Meningeal and cerebral congestion and haemorrhage, were noticed, the cerebrum showed focal gliosis and neuronophagia.

The effect of Dimiline on the Embryos:

Stomach: Hyperplasia was present in the epithelial cells of the mucosa.

Intestine: Submucosal congestion and subserosal haemorhage were seen.

Liver: The central and portal veins were congested.

Lungs: Congestion of the peribronchial blood vessels and haemorrhage in the alveoli were evident.

Kidneys: Congestion and haemorrhage were seen in both renal cortex and medulla.

Brain: Congestion and haemorrhage were seen in the meninges especially around the spinal cord.

Skin: Congestion of the subcutanous blood vessels and haemorrhage among the muscle fibres.

Gravid Uterus and Placenta:

The uterine lamina propria was highly infiltrated with lymphocytes and macrophages. Some uterine glands were degenerated, vacular degeneration was seen in the myometrium.

The placenta showed circulatory disturbances in the form of congestion, haemorrhage and exudation in the communicating zone between placenta fetalis and placenta maternalis.

Degenerative decidual cellls and chorial trophoblast were seen. Leucocytic infiltration mainly with lymphocytes and macrophages in addition to some fibrin threads were present in the placenta fetalis (Fig. 7).

DISCUSSION

Our results showed that diflubenzuron is of extreemly low toxicity, LD50 4 gm/kgm. FERRELL (1977), also showed its low mammalian toxicity but its tumorogenic activity was not investigated before our experimental work. During our experimental studies the LD50 dose was used in order to obtain quick and best results since in general a typical dose response pattern seen after direct exposure to chemical carcinogens applied to transplacental exposure (EVERSON, 1980) i.e. the smaller the dose, the lower the tumor yield, and the larger the latent period. In addition to previous fact mentioned by EVERSON (1980) tumor sites and histopathological findings differed by dose category (SWENBERG et al., 1972, VESSELINOVITCH, 1973).

Since diflubenzuron can be exereted through milk (LEVI, 1978) it detection was carried out and proved from foetuses obtained either by cessarian section or taken just after delivery (not allowed to nurse by their mothers), otherwise destinction can not be made between prental and postnatal exposure. The presence of the compound in fetal tissue strongly proved that diflubenzuron reached through transplacental route and not through milk factor.

Placental disturbance, manifested by degenerative changes in decidual cells and chorional trophoblasts, in addition to leucocytic infiltration and presence of fibrin threads in placenta fetalis may play a part in the production of embryopathic effects of diflubenzuron in embryos. The presence of polygonal cell carcinoma and squamous cell carcinoma inside the lumen of the alveoli of lung tissues and squamous cell papilloma in the fore stomach in non pregnant mature animals after intragastric administration of diflubenzuron in LD50 dose and slaughtered after eleven month post administration strongly proved that diflubenzuron can be listed under chemical carcinogens. The absence of hepatic carcinoma in the previous group of animals and its presence in offspring exposed prenatally to diflubenzuron may be due to age differences. Our results confirm the previous work (HARD, 1979) regarding the effect of age at treatment on incidence and time of neoplasma induced in rat by a single dose of chemical carcinogens. Several other previous studies (KLEIN, 1966; TERRACINI and TESTA 1970 ; TERRACINI et al. 1976), indicate that newly born animal is more susceptible than its older counter parts to chemically induced neoplasia. Neonatis found to be more susceptible to the development of thymic lymphomas and liver cell tumors than were weaned counter parts (TOTH, 1968). Since the precious fact was applied to animals dosed postnatally but differ in age factor, the prental administration may differ from postnatal treatment. On the other side histopathological appearance of the liver of these group of animals (mature non pregnant) showed hepatocellular pleomorphism and the presence of hepatomegalocytes with large hyperchromatic nuclei and mitotic division in addition to increase in number of bile ducts may be primary stage of cancer formation.

The presence of secondary hepatocarcinoma in the offsprings is due to metastasis from liver carcinoma. The metastasis to lung tissues probably occured postnantally during the three months of life. The effect of diflubenzuron on male reproduction need further investigation, since in male offspring that exposed prenatally, spermatogenesis nearly completely stopped. Our results also indicated that diflubenzuron has a carcinogenic effects, but the question arises whether it act as proximate carcinogen or procarcinogen and its metabolite hydroxylated metabolites (N- 4 chlorophenyl amino carbonyl) -2,6 difluoro-3 hydroxybenzamide are responsible for its tumorgenic effect.

Distribution of diflubenzuron after oral LD50 was higher in stomach and intestine and liver nearly have the same amount followed by the kidney and brain. The previous result is of important toxicological significance since distribution throw light on organ chosen for analysis in toxicological cases.

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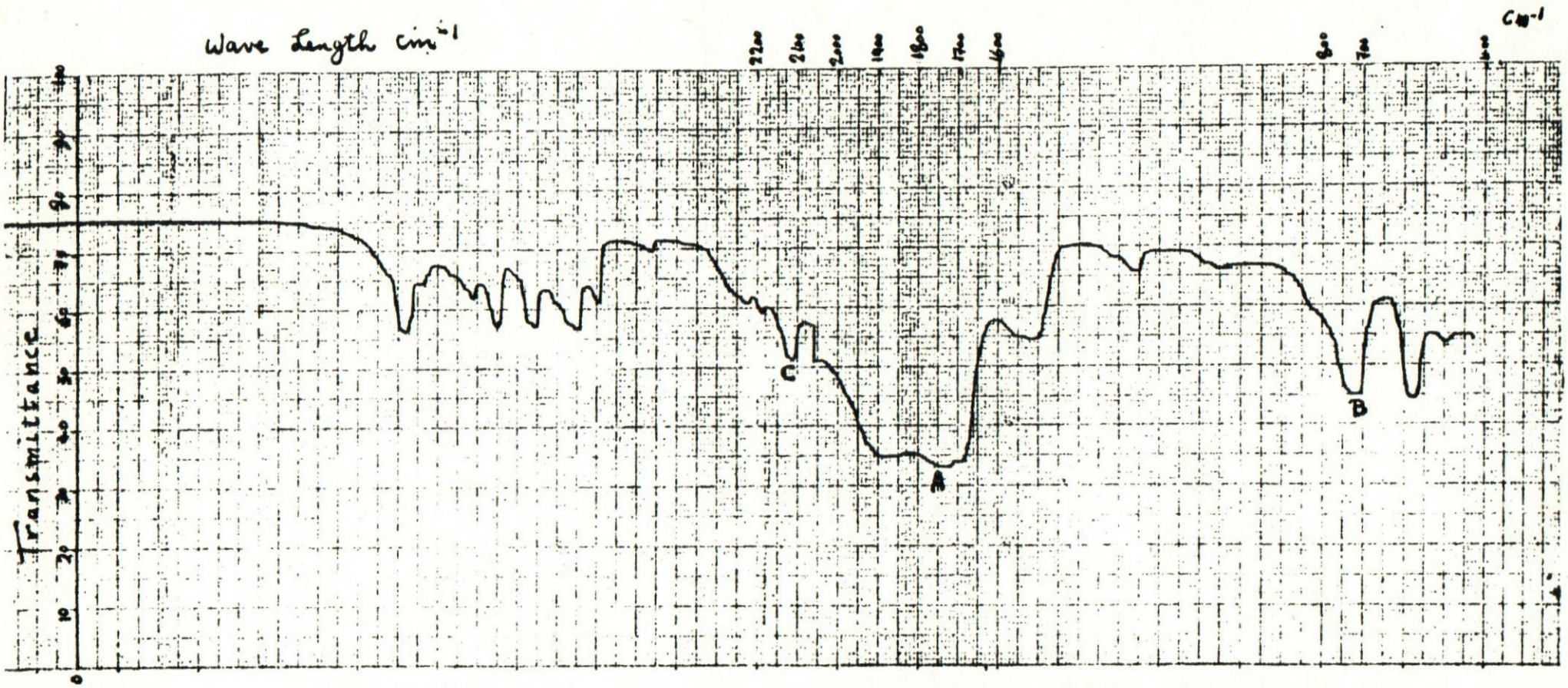

Fig. (1): Infra-red Spectrum (K Br) of Diflubenzuron.

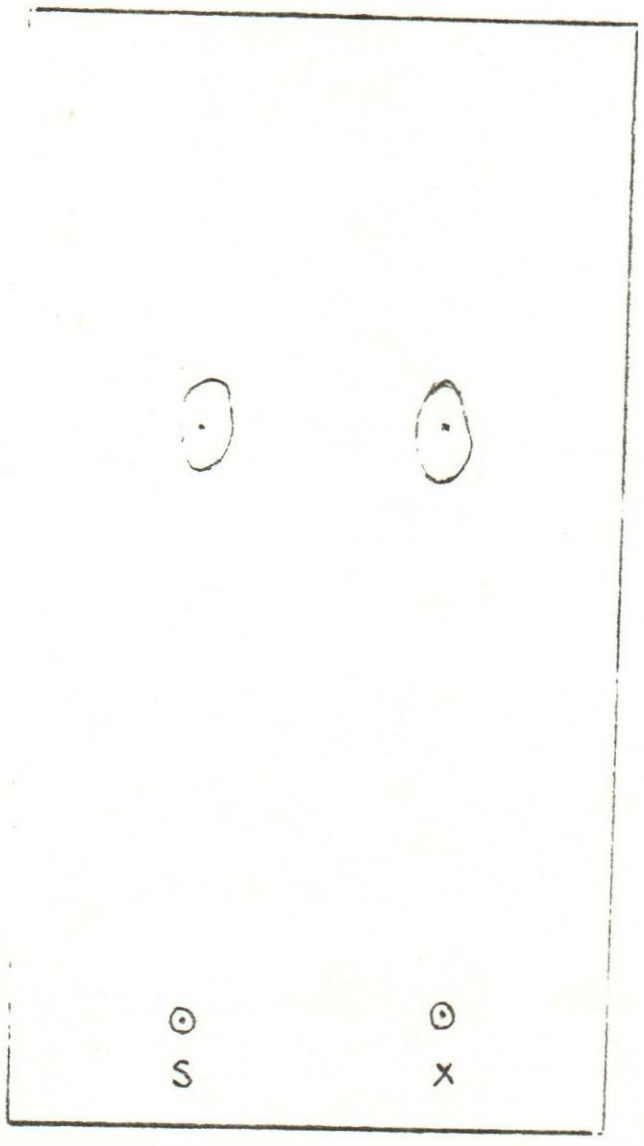

Fig. (1): TLC: of extracted Diflubensuron
S = Standard Dimiline.
X = Unknown Dimiline extracted.
System = benzene : ethanol. 4:1
Spray = Dragendorf reagent.
Absorbant = Silica gel G.

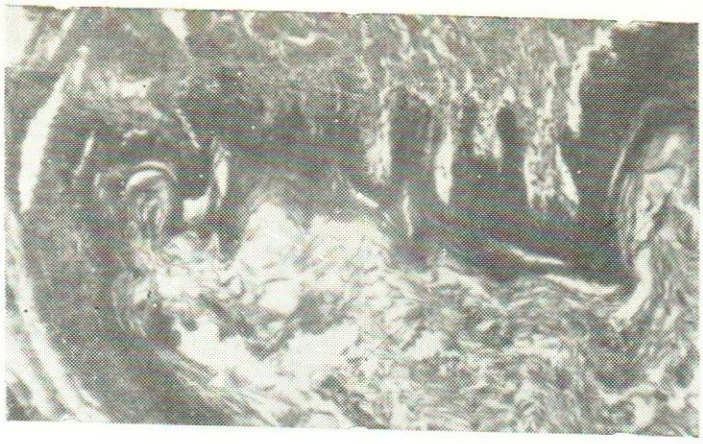

Fig. 2 : Stomach : Showing squamous cell papilloma of forestomach (H & E, x 100) Diflubenzuron (LD 50) 11 months post administration.

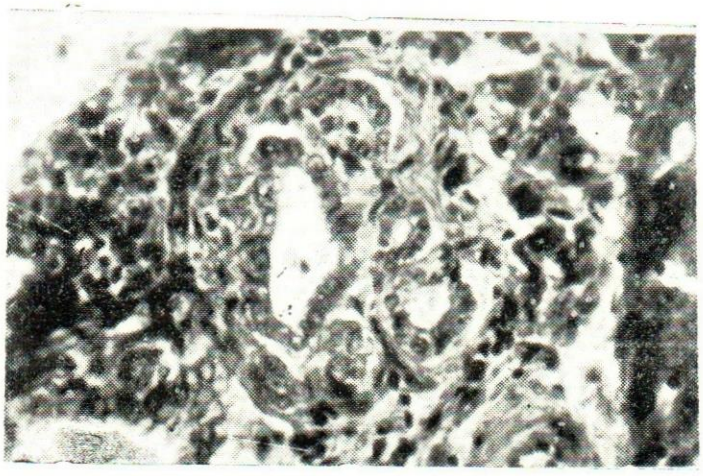

Fig. 3 : Liver : An increase in the numbers of bile du (H & E, x 400) Diflubenzuron (LD 50) 11 months post administration.

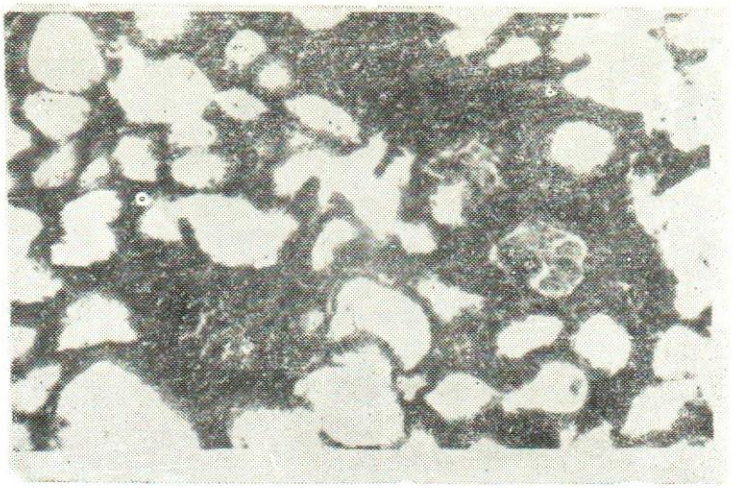

Fig. 4 : Lung : Showing polygonal cell carcinoma inside the luminae of the alveoli, focal areas of round cell aggregation, (H & E x 100) Diflubenzuron (LD 50), 11 months post administration.

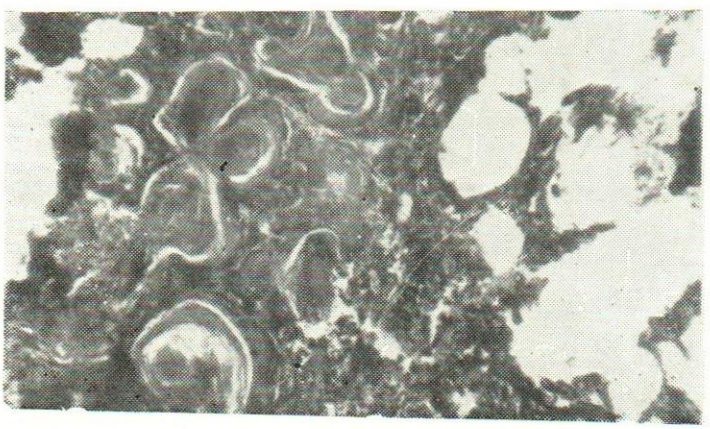

Fig. 5 : Lung : Squamous cell carcinoma (H & E, x 100) Diflubenzuron (LD 50), 11 months post administration.

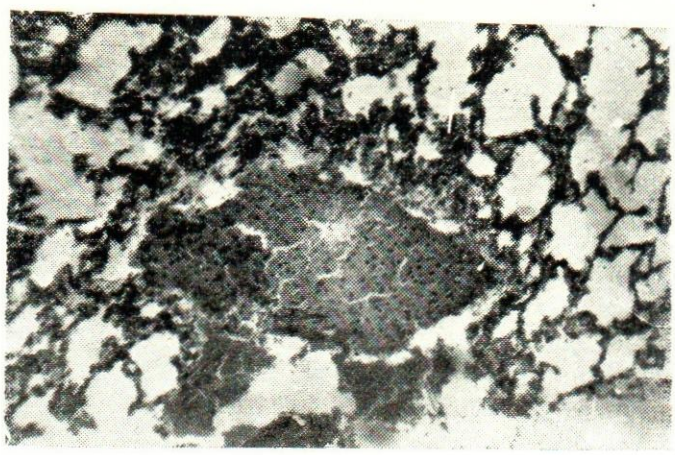

Fig. 6 : Lung : Secondary hepatic cell carcinoma in lung tissue with haemorrhage (H & E, x 100) Diflubenzuron (LD 50) offspring 3 months old.

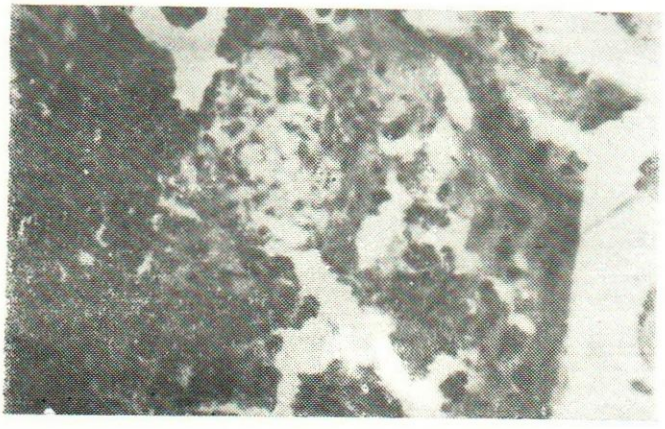

Fig. 7 : Placenta : Showing degeneration of trophoblast and decidual cells (H & E, x 100) Diflubenzuron (LD 50).