ANALELE UNIVERSITATII DIN ORADEA
FASCICULA: ECOTOXICOLOGIE, ZOOTEHNIE SI TEHNOLOGII DE INDUSTRIE ALIMENTARA, VOL. VII, ANUL 7, 2008

TOXICITY AND DISTRIBUTION OF 2-METHYL-4-CHLOROPHENOXY ACETIC ACID (MCPA) IN DEVELOPING CHICK EMBRYOS

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Abstract

The toxicity of methyl-4-chlorophenoxyacetic acid (MCPA) containing 28% MCPA as sodium-potassium salt and 72% of unknown ingredients, was tested on chick embryos. Sterile aqueous solutions of MCPA were injected into the air chamber at doses of 0, 1.5, 3.0, 6.0, 9.0, or 10.5 mg/egg on day 0 or on day 4 of incubation. The mortality rate for the embryos treated on day 0 of incubation was high in the first 5 days, low from 5-12 days and again increased by 15 days. The15-day LD_{50} was 4.4 mg/egg (95% C.I. 3.7 - 5.3 mg/egg). HPLC analysis of albumen and yolk showed that concentrations of MCPA in the albumen were detectable at 5 min, highest at 7 days and markedly diminished by 14 days of incubation; a significantly lower concentration of MCP A was found in the yolk throughout the incubation period, except at 14 days when the yolk concentration was 4 times higher than the albumen concentration. At 15 days of incubation, MCPA was evenly distributed in the tissues of the embryo. MCPA was more toxic to 4-day embryos; concentrations above 6.0 mg/egg were lethal to all embryos within the first week of incubation. The 15-day LD_{50} for treatment on day 4 of incubation was 2.8 mg/egg (95% C.I. 2.5-3.2 mg/egg). The liver was affected by treatment with MCPA, being green in treated embryos. However, histological examination revealed few changes in the liver parenchyma.

Key words: methyl-4-chlorophenoxyacetic acid (MCPA), chick embryons, toxicity

INTRODUCTION

2-Methyl-4-chlorophenoxyacetic acid (MCPA) is currently used in Italy and other countries as a herbicide for a variety of crops.

Acute toxicity studies in mammals indicate that MCP A is of moderate toxicity, its LD_{50} being 400 to over 1000 mg/kg as determined after different routes of administration and for different MCPA formulations. In tissue distribution studies in rats, the highest concentrations of MCPA were observed in liver, kidney, lung and blood. Little information is available on the toxicity of MCPA to birds. Later has been reported that the hatching rate was markedly lowered by an injection of 10 mg of MCPA into hen's eggs. Adverse effects of MCPA on the hatchability of eggs as well as on the viability of chicks were reported later . However, in neither of these studies was the LD_{50} value calculated. MCPA appears to be moderately toxic also to amphibians and fishes.

In the present study the toxic effects of a commercial formulation of MCP A on developing chick embryos were further investigated and its distribution in the egg and in the embryonic tissues at different times of

incubation was evaluated.

MATERIALS AND METHODS

A total of 506 fertile White Leghorn hen eggs were used in the present study. Erbitox E30, containing 28% of active ingredient as 2methyl-4-chlorophenoxyacetic acid sodium-potassium salt and 72% of unknown ingredients, was dissolved in distilled water and injecteR into the air chamber in single doses of 0, 1.5, 3.0, 6.0, 9.0, or 10.5 mg/egg on day 0 or 4 of incubation. The total volume injected was 0.1 ml/egg. All solutions were sterilized through a 0.45 µm Millex-Ha filter. Eggs were then incubated in a forced-draught incubator with automatic hourly rotation of eggs at a temperature of 37.8°C and at a relative humidity of about 85% for 15 days. The eggs were candled every 2 or 3 days to determine the number of dead embryos. At the end of the observation period, the LD_{50} values and their 95% confidence intervals were calculated by the pro bit method of Finney. The embryos were inspected for external and visceral malformations. Samples of embryonic tissues were fixed in Bouin's fluid for histological examination and paraffin-embedded sections were stained with hematoxylin-eosin. An additional group of 50 eggs was treated on day 0 of incubation with 10.5 mg Erbitox E30 in a volume of 0.1 ml/egg for high pressure liquid chromatography (HPLC) analysis of tissues. Tissue samples were weighed and homogenized in 0.1 M $NaHCO_3$ buffer (volume: tissue weight or volume 10:1) with an Ultra Turrax (Ika Werk) homogenizer. Then an equal volume of 60% methanol in NaH_2PO_4 acidified with H_3PO_4 to pH 3.7 was added to the homogenates. After centrifugation at 20 000 *9* for 20 min at O°C, the supernatant was collected and filtered through a Gilson Versapor filter (0.45 µm pore size) and chromatographed by an J ASCO HPLC apparatus equipped with a 3-heads Familic 300-S pump, a variable VL 614 sample loop injector and an Uvidec 100-V variable wavelength UV detector. Samples were eluted at room temperature from a Jasco CIS (250 mm x 4.6 mm J.D.) reversed-phase column at 1.0 ml min-1 with mobile phase containing 60% methanol in NaH~04 0.1 M acidified to pH 3.7. The analytic wavelength was 230 nm. The chromatogram was recorded on an Omniscribe Model B500 recorder (Houston Instrument), the peak heights and areas were determined with an integrator of a fraction collector. Concentrations of MCP A in the tissues were determined from a calibration curve prepared from the areas of appropriate standard concentrations. Because no extraction losses occurred, final concentrations were corrected only for dilution and expressed as µg/g or ml of fresh tissue. Differences were analyzed for statistical significance by Student's *t-test.*

RESULTS AND DISCUSSION

A single treatment with MCPA on day 0 of incubation was toxic to developing chick embyros at all the dose levels tested. From the mortality curves it appears that there are 3 distinct mortality rates. Between 3 and 5 days of incubation there was an increase in mortality at all concentrations, whereas after day 5 and until day 12 the mortality was low.

Toward the end of the experiment (day 15 of incubation) the mortality rate had again increased. From the mortality data the 15-day LD_{50} was 4.5 mg/ egg (95% C.I. 3.7 -5.3 mg/egg). MCPA began to be detectable in the albumen 5 min after inoculation (μg 10.3/tissue wt) and its concentration increased progressively during the following 7 days, at which time the highest levels of MCPA are reached in the albumen. This might be due to the relatively hydrophilic nature of the herbicide. There was also an increase in MCPA concentration in the yolk with a parallel increase in the whole embryo. Between 7 and 14 days of incubation the concentration of MCPA in the albumen fell dramatically, whereas in the yolk it increased further, probably because of the movement of part of albumen into the yolk sac that occurs in the chick embryo at about the thirteenth day of incubation. MCPA was distributed evenly in the embryo, without accumulation in any specific tissues. A point of interest is that the kinetics of MCPA concentration in the egg and in the embryonic tissues seem to agree well with the kinetics of mortality. The raise in mortality occurred practically simultaneously with the time at which MCPA levels increased in the yolk and hence MCPA became available to the embryo. A single treatment with MCPA on day 4 of incubation more severely affected development of chick embryos. Two days after treatment the mortality ranged from 10% (at 1.5 mg/egg) to above 50% (at 6.0 mg/egg) and increased further in the following days.

Toward the end of the experiment (day 15 of incubation) there was a moderate increase in the rate of mortality at the lower doses and a marked increase at 6.0 mg/egg, this concentration being lethal for all the embryos by 15 days of incubation. Doses above 6.0 mg/egg were highly toxic to embryos injected on day 4, resulting in 100% mortality early in the incubation period. All the embryos treated with 9.0 and 10.5 mg/egg died within 8 days of incubation. From the mortality data the 15-day LD_{50} for treatment on day 4 of incubation was 2.8 mg/egg (95% C.I. 2.5-3.2 mg/egg).

Only the liver was affected by treatment with MCPA. At gross examination it was green in chick embryos from groups treated either on day 0 or on day 4 of incubation. However, histological examination of the livers showed few changes, consisting of vacuolization of the hepatocytes and occasional bile thrombi. Frequently the gallbladder was empty, suggesting that there may be a disturbance in the efflux of bile from liver.

Toxic effects on liver were observed in MCPA-treated rats.

Other gross lesions, such as oedema and leg contracture, were seen occasionally and were viewed as unrelated to treatment. No pathological changes were seen in any other of the various organs examined histologically.

In summary, the chick embryo appears to be sensitive to the effects of MCPA and liver seems to be a target organ for MCPA toxicity. Indeed, though in this study no preferential accumulation of MCPA in liver has been found nor were significant changes detectable by routine histological examination, in other recent studies with chick embryo MCPA was found to interfere with the activity of the enzymes linked to cytochrome *P*-450 and with the activity of- grotathione S-transferase.

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