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GENOTOXICITY AND EMBRYOTOXICITY OF THE INSECTICIDE PRIMICID IN CHICK EMBRYO

By

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السمية الوراثية والجنينية للمبيد الحشري بريميسيد في جنين الدجاج

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عُولج بيض الدجاج المخصب بالمبيد الحشري بريميسيد ٥٠٪ أي سي بهدف فحص آثاره السامة على المادة الوراثية وعلى الأجنة، وفي الحالة الأولى تم حقن البريميسيد المذاب في الماء المقطر في الحجرة الهوائية عند التركيزات التالية : ٠,٢٥٪، ٠,٥٪، ١٪، ٢٪ و ٤٪، وبعد ثلاثة أيام من بدء الدراسة، وجد أن البريميسيد يزيد معدل التبادلات الشق صبغية في خلايا براعم الأطراف وخلايا المبار، غير أن هذه الزيادة لم تكن ذات دلالة إحصائية إلا عند المستوى الأعلى للمبيد (٤٪)، وفيما يتعلق بالأجنة، فقد غُمس البيض المخصب في المحاليل السابقة، لمدة ٣٠ ثانية، قبل وضعه في الحاضنة، ولم تلاحظ أية فروق إحصائية في أوزان الأجنة التي بقيت حية بعد انتهاء التجربة عند اليوم السادس عشر، هذا وقد أحدث المبيد الحشري زيادات في معدلات الوفاة متناسقة مع الزيادات في مستويات التعرض، كما أنه تسبب في ظهور بعض التشوهات الخلقية الخارجية في الأجنة، وبصورة عامة، لم تتضح تغيرات ذات معنى إحصائي إلا عند تعرض الأجنة للتركيزين الأعليين ٢٪ و٤٪، واللذان يعادلان ٥ و١٠ أضعاف ما يوصى به عند أستخدام البريميسيد في الحقل، على التوالي، وتمكن النتائج من إصدار توصية بأن تعرض الإنسان لهذا المبيد الحشري لمدة طويلة يمكن أن يؤدي إلى تغيرات وراثية وجينية في الخلايا البشرية.

Running Title : Toxicity of primicide in chick embryo

Keywords : Chick embryo; Embryotoxicityl Primicid; Sister-chromatid exchange.

ABSTRACT

Growing chick embryos were treated with primicid to examine the cytogenetic and embryotoxic effects of this insecticide. For the cytogenetic assay, eggs were injected through the air chamber with primicid at a concentration of 0.25, 0.50, 1.0, 2.0 and 4.0% aqueous solutions. A single exposure of 3-day embroys was found to increae the incidence of sister-chromatid exchanges (SCE) in cells from allantois and limb buds. However, significant SCE values were observed only at 4.0% primicid (1.88 ± 0.21 SCE per cell) as compared to the baseline SCE level in the control (0.95 ± 0.11). In respect to the embryotoxicity, the eggs were immersed, for 30 seconds, in the above solutions, before incubation. No significant differences were obtained in weight between different treatment groups. Furthermore, primicid resulted in dose-related increase in mortality rate and induced external malformations. In general, these variations were not significant except at the highest two exposure doses (2.0% and 4.0%), which correspond to 5 and 10 times the recommended application level. These result suggest that long-term developmental and genetic alterations may occur following human exposure to primicid.

INTRODUCTION

Pesticides are an important class of toxic environmental pollutants, due to their widespread application and because man can be extensively exposed to them directly or indirectly. Some pesticides have dangerous effects on cellular structures and herreditary material, as demonstrated by several short-term tests in exposed organisms [1-7]. In addition, pesticides residues in food and water may have the ability to induce heritable changes that can lead to cancer in human populations [8,9].

Primicid is a broad spectrum insecticide containing the active gradient pirimophos-ethyl. It is widely and efficiently used for long-lasting control of many soil pests and destroys insect strains resistant to other insecticides [10,11]. However, prominent degradation of neurosecretory granules was observed in the earthworm (*Aporrectodea caliginosa and Allolobophora caliginosa*) kept in primicid contaminated soil [12.13]. In mammals, primicid also has been found to have damaging effects in rat cells [14]. As far as known, no attention, has been given in the literature regarding primicid genotoxicity and embryotoxicity of avian embryos. In this study, developing chick embryo was employed to exmaine the DNA-damaging effects of primicid, expressed as sister-chromatid exchanges (SCE) or as gross morphological abnormalities. The chick ebyryo is a conveninet model that has been used as an alternative to mammalian system for monitoring the cytogenetic damage and predicting the health caused by environmental pollutants [5,15]. Moreover, the results are not affected by the presence by utero-environmental factors and possible maternal influence found in mammals and may be in other vertebrate animals.

MATERIALS AND METHODS

Insecticide

Technical grade primicid 50% EC [2(diethylamino-6-methyl-4-pyrimidinyl diethyl phosphorothioate] manufactured by Ferhurst Haslemere (Surrey, England) was a gift from agricultural materials Co. Ltd (Doha, Qatar).

EXPERIMENTAL PROCEDURES

Genotoxicity

Fertilized eggs of domestic fowl; *Gallus domesticus* were obtained from a local hatchery and randomly divided into five groups (5 eggs, each). Suspensions of primicid were made in distilled water to get the following concentrations: 0.25%, 0.50%, 1.0%, 2.0% and 4.0%. In each group, an egg was injected with 0.05 ml suspension at zero time. The injection was made horizontally through the air chamber using a 1 ml tuberculin syringe and a 1 inch 26-gauge needle. The injection hole was sealed with melted paraffin wax. A sixth control group was injected with 0.05 ml sterile distilled water. Mitomycin C (mmc, The United States Biochemical Corporation, USA) at a concentration of 0.05 μg/egg, injected at the beginning of the incubation period, served as a positive control. The above experiment was repeated three times. The eggs were incubated at 37.5 ± 0.5°C and a relative humidity of 78 ± 2.0%.

Testing for SCE was carried out as described before [15,16]. Briefly, 100 μl (150 μg) bromodeoxyuridine/egg was injected at 72 h of incubation. After 25h, 20 μl of 0.5% colchicine solution was injected into each egg, and the embryos were removed 1 h later. Chromosome spreads were prepared from the allantois and limb buds according to a solid tissues techniqu [15]. For the differential chromatid staining, the fluorescence plus Giemsa procedure was followed [17]. When possible, 10-15 cells from each embryo, were screened for SCE in the first 5 pairs of macrochromosomes. These chromosomes are known to contain about 50% of the nucelar Dan per cell. The SCE data were analysed statistically by the *Student's t-test.*

EMBRYOTOXICITY

In nature, avian eggs are usually exposed to pollutants externally through the shell, therefore, the experimental protocol of Arias [16] was used in the study of chick embryo development. Six groups (20 fertile eggs, each) were randomly selected. The eggs of the first five groups were immersed individually, for 30 sec, in the previous concerntrations of primicid solutions, respectively. The sixth group of eggs were treated with distilled water for the same period and served as a control group. After that, the eggs were dried on filter paper and incubated under the above indicated conditions. Three repeats of this experiment were made. Occasionally, the eggs were rotated. They were candled every three days to determine the survival of the embryos, and the infetile eggs as well as dead embryos were discarded. On day 16 incubation, the eggs were opened and alive as well as dead embryos were scored to find the LD_{50} values. The 16-day embryos were weighed and examined morphologically to determine the abnormalities. The 95% confidence intervals

for the calculated LD_{50} values were estimated by the probit method of Finney [18].

RESULTS

The results of SCE investigation are shown in Table 1. The mean SCE frequency for the negative control reached 0.95 ± 0.11 SCE per cell. No significant differences were observed between the mean SCE rate in the control and those in the primicid-treated embryos up to 2.0% level. A significant ($P \leq 0.05$) two-fold increase (1.88 ± 0.21 SCE/cell) in SCE values was calculated only when embryos were exposed to 4.0% primicid.

Generally, the percentage of surviving embryos at different stages progressively decreased, when eggs were exposed to increasing concentrations of primicid (Table 2, Figure 1). In the earlier stages, most of the dead embryos were resorbed or highly resorbed. On day 16, the mortality rate markedly increased. The mortality data indicated that 16-day LD_{50} was 1.93 (95% C.I. 1.53 – 2.33). Furthermore, the mean weight of living embryos at day 16 slightly decreased. These decreases were not significant as compared with that of the control. In the early stages of chick development, primicid caused hydrocephaly, while in later stages, the most prominent malformations were observed in one or both of the hindlimbs and in the beak (Table 2). In the hindlimbs, the tarsus, metatarsus and the digits appeared shorter, thinner and/or curled. The lower beak was usually shorter than the upper. In addition, haemorrhagic spots in the hindbrain and abdomen were observed in few cases. Some abnormalities were also encountered in the control embryos, but their incidence was much lower than in the experimental groups.

DISCUSSION

The present study represents as attempt to evaluate the genotoxic and the embryotoxic effects of the insecticide (primicid). These effects are considered among the most serious of the possible side-effects of pesticides. It has been suggested that the potency for inducing SCE in the developing avian embryos correlates with the true mutagenic potential, DNA inhibition, and to certain extent with carcinogenic activity [19]. unfortunately, we could ot put the present data into perspective of the existing literature because published data on primicid are limited and some are not available. However, our results concerning the action of primicid are comparable to Hoffman data, when crude oil was applied to the avian egg, cited by Bloom [15]. We observed higher mortality rates in the experimental groups with primicid than that in control (Table 2). In addition, the embryotoxicity data do not indicate specific effects beyond generalized toxicity. Similar findings were reported by other workers [20] using the fungicide maneb in chick embryos. However, it should be recalled that the highest two concentrations used in this study were 5- and 10-fold of that recommended for application. Search for possible differences in the activity between the commercial formulation of primicid, used in the present study, and the pure

Table 1

Sister-chromatid exchange frequency in chick embryo treated with primicid[a]

Concentration (%)	Number of cells scored	SCE per cell (Mean±S.E.M)
0	190	0.95 ± 0.11
0	180	1.05 ± 0.20
0.50	160	1.18 ± 0.13
1.00	170	1.13 ± 0.16
2.00	130	1.25 ± 0.18
4.00	105	1.88* ± 0.21
0.05 µg/egg mitomycin C	80	3.66* ± 0.67

(a) Eggs were injected with test solution at zero time.

* Statistically greater than control value (t.test at $P \leq 0.5$)

Table 2

Incidence of mortality and abnormalities in chick embryo derived from primicid-treated eggs. The eggs were dipped for 30 sec in the test solution before incubation.

Treatment (% pricimid)	Number of eggs	%of dead embryos at day					% of abnormalities observed in 16-day embryos					16-day average
		4	7	10	13	16	Beak	Hindlimbs	Neck	Head	Viceral hernia	
0	54	0	3.70	5.56	7.41	0	7.84	0	0	0	18.3	
0.25	57	3.51	3.51	5.26	8.77	17.54	3.85	13.46	15.3	0	0	18.6
0.50	56	0	5.36	7.14	16.70	26.79	6.52	15.22	9	0	2.17	17.5
1.00	58	0	3.45	6.90	17.24	32.76	0	12.50	0	6.25	0	17.9
2.00	56	0	7.14	14.29	16.07	51.79	14.89	34.04	0	0	0	16.8
4.00	54	0	7.41	13.00	22.22	59.26	23.81	30.95	4.26	7.14	4.76	16.9
								0				

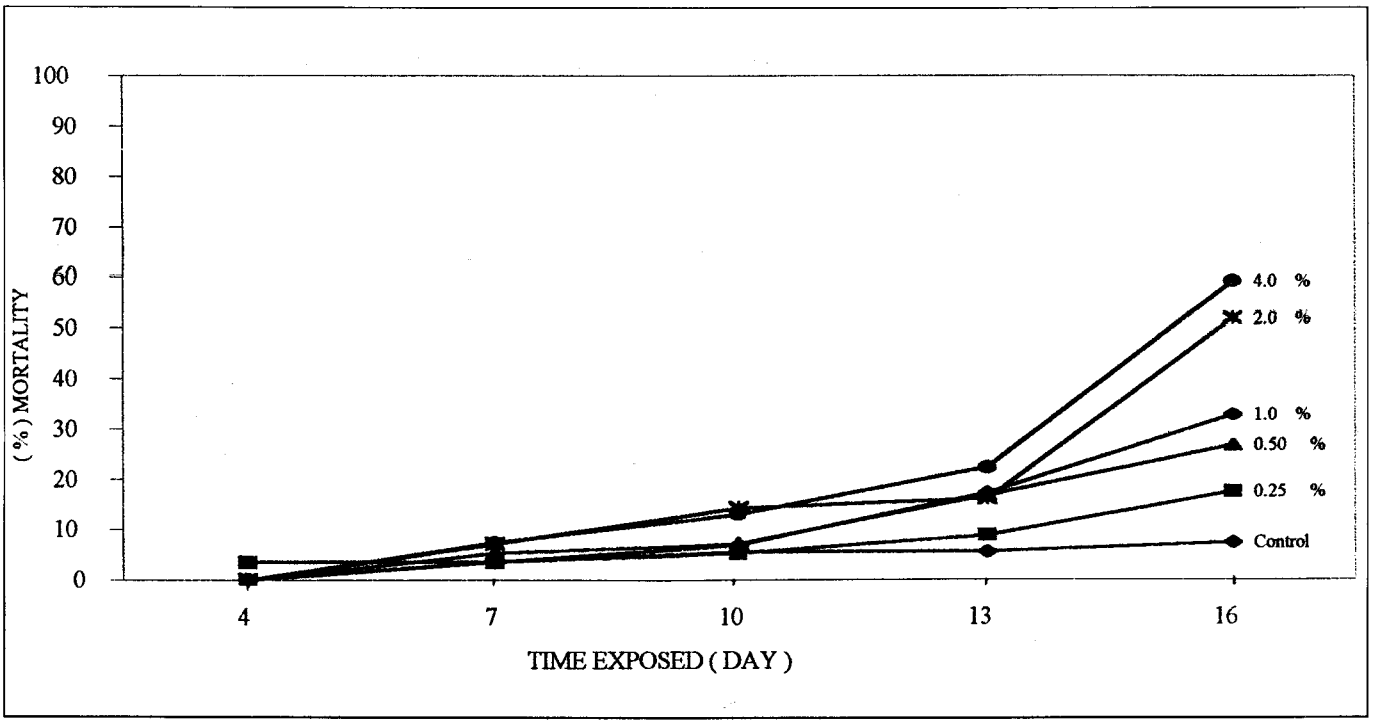

Fig. 1 : Effects of primicid on mortality of chick embryo after treatment on day zero of incubation.

compound deserved another investigation.

The mode of action of primicid is unclear, but it may exerts its effects possibly by modifying celluar protein metabolism, as has been shown previously in fungicides [21]. The action of primicid may further be affected by the rate of clearance of the compound from the physiological system. In this context, Bloom [15, 19] reported the chick embryos as early as 3-4 days of incubation appear to posses metabolic machinery capable of converting chemicals to toxic and mutant forms. Still other mechanisms can be proposed; the first is the interference of primicid with calcium and phosphorous absorption from the egg yolk, while the second could be related to the hormonal alterations. These nutrional influences were indicated in a different unrelated study [22] on chick embryo. Another point of interest, which needs to be further examined, is to find the correlations between the kinetics of the primicid concentration in in the egg and in the embryonic tissues with the kinetics of toxicity.

Finally, although it is difficult to extrapolate, due to principal differences in exposure and kinetics, taken togther, the present findings suggest that primicid may also cause health hazards to human populations. In general, pesticides exposure can present a health risk to humans, especially to production workers, formulators and other applicators. The likelihood of exposure, the dose received and the rout of entry to the body vary depending on the region, the type of crop and the use of protective measures. For these reasons, extending our experiment on primicid using other genetic bioassay systems would no doubt aid in verification of the mechanism of action and in understanding potential health effects.

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