

Dose- Response Analysis of Acephate – Induced Developmental Toxicity in Mice

By

Amina T. Farag , Mohamed I. Kamel^{*}, M. H. Eweidah^{}**

**Department of Pesticide Chemistry, Faculty of Agriculture,
Department of Community Medicine^{*}, and Department of Anatomy &
Embryology^{**} Faculty of Medicine, Alexandria University, Egypt.**

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ABSTRACT

Acephate, an organophosphate insecticide, is evaluated to realize whether or not statistically significant increased response rates existed for the percentage of a particular endpoint of developmental toxicity parameters. The study involved four groups of 10 pregnant dams, one group serving as a control (distilled water) and the others exposed to various levels of acephate (7, 14, 28 mg/kg/day), by gavage, on gestation days 6 through 15. The outcomes of each pregnancy were recorded, and fetuses were examined for a variety of developmental defects. The Kruskal Wallis ANOVA was used in this study to test for a dose-response. The analysis revealed an overall statistically significant differences between all treated groups. While a significant increase in response rate in the reduction of the maternal weight gain occurred in 7 and 14 mg/kg/day treated groups at day 6 of gestation, there was no significant effects at day 15 of gestation. At day 18 of gestation, a significant increase in the percentage of dose-response in maternal weight gain of the group treated with the highest dose of acephate 28 mg/kg/day was noted. No effects were found in the response rates of heart and placenta weights compared to the control. However, the response rates of maternal liver, kidneys, brain, and spleen weights were significantly affected among all the treated groups compared to the control. Maternal exposure to acephate during organogenesis significantly affected the

response rate of fetal weight, and induced an increase in response rates of external and skeletal malformations. On the basis of the present results acephate caused increase in response rates of developmental toxicity endpoints in all treated groups without dose-related pattern.

INTRODUCTION

Developmental toxicity studies in rodents play an important role in testing and regulating substances that might endanger developing fetuses (Lefkopoulou *et al.*, 1989). Acephate is the N-acetyl derivative of the insecticide methamidophos. Although methamidophos itself is highly toxic to mammals (LD_{50} mice =27, rat =21 mg/kg), the acetylation causes a dramatic decrease in the mammalian toxicity, the oral LD_{50} of acephate for mice and rats is 361 and 946 mg/kg, respectively (Salama *et al.*, 1992). Acephate is a systemic insecticide with moderate persistence and it is effective against sucking pests and chewing insects. Acephate proved to have a cytogenetic impact, liver neoplastic lesions, positive results of carcinogenicity testing in mammals, bone marrow chromosome aberrations, sperm-shape abnormality in mice and point mutations, mitotic recombination, and chromosomal malsegregation in *Saccharmyces cervisiae* (Perocco *et al.*, 1996, International Research and Development Corporation, 1982, Behera and Bhunya, 1989, Zimmermann *et al.*, 1984, Waters *et al.*, 1982, Garrett *et al.*, 1986). In addition, developmental toxicity data of acephate in mice have been documented in our laboratory (In press).

The statistical analysis of developmental toxicity data presents several challenging statistical problems. For the normal outcomes, such as weight or length, methods for assessing correlated data are readily available. Methods for correlated discrete outcomes are not widely available, although several approaches have been proposed recently (Goldstein 1986, Liang and Zeger 1986, Moore 1986, Williams 1982, Zeger and Liang 1986, and Lefkopoulou *et al.*, 1989). The present study is conducted to visualize whether or not statistically significant increased response rates existed for the percentage of a particular endpoint of developmental toxicity parameters.

MATERIAL AND METHODS

Test Material:

Acephate was obtained from the EPA (Environmental Protection Agency, R.T.P., N.C. USA). The purity of the test material was 98.00% (Lot: 127-125A).

Test Species and Husbandry :

Male and female ICR (CD-1) mice, approximately 10 weeks old, were obtained from the High Institute of Public Health, Alexandria University, Alexandria, Egypt. All mice were examined for health status and acclimated to the laboratory environment for 2 weeks prior to use. The animal room was designed to maintain temperature at 25°C, relative humidity at approximately 50% and a 12 hr light : 12 hr dark photoperiod. All animals were housed in stainless-steel cages and given standard diet and water *ad libitum* throughout the study. Adult virgin female mice were mated with adult males (one male /two females). The day that a vaginal plug found, was considered day 0 of gestation. Bred females were randomly assigned to treatment groups. Groups each of 10 bred mice were given acephate by gavage on days 6 through 15 of gestation at the dose levels of 0 (distilled water), 7, 14, 28 mg acephate /kg/day using distilled water as a vehicle.

Maternal Observations :

Animals were observed daily throughout the experimental period for signs of toxicity. Maternal weight gain was recorded on days 6, 15 and 18 of gestation. Weights of maternal organs (liver, brain, kidney, spleen, heart and placenta) were recorded at the time of Cesarean section on day 18 of gestation.

Fetal Observations :

Test animals were anaesthetized by diethyl ether on day 18 of gestation. The uterine horns were exteriorized through a midline abdominal incision. The following data were recorded : number of implantation sites, number of live and dead fetuses, number of resorption

sites, fetal sex ratio, fetal body weight, and gross external fetal alterations. The uteri of apparently nonpregnant mice were stained with a 10% sodium sulfide (Kopf *et al.*, 1964) and examined for evidence of implantation sites. Half of the fetuses were fixed in Bouin's solution and then were examined by the serial sectioning technique of Wilson (1965). The remained fetuses were preserved in 95% ethanol, eviscerated, subsequently cleared and stained with Alizarin Red S (Wilson, 1965) for detection of skeletal malformations.

Statistical Analysis :

Cut off points: The following cut off points were selected as endpoint (response): The overall response for each parameter was calculated for each test as response percent. The control values were used as standard for the endpoints of the treated groups (affected or not affected) either increased effects or decreased one.

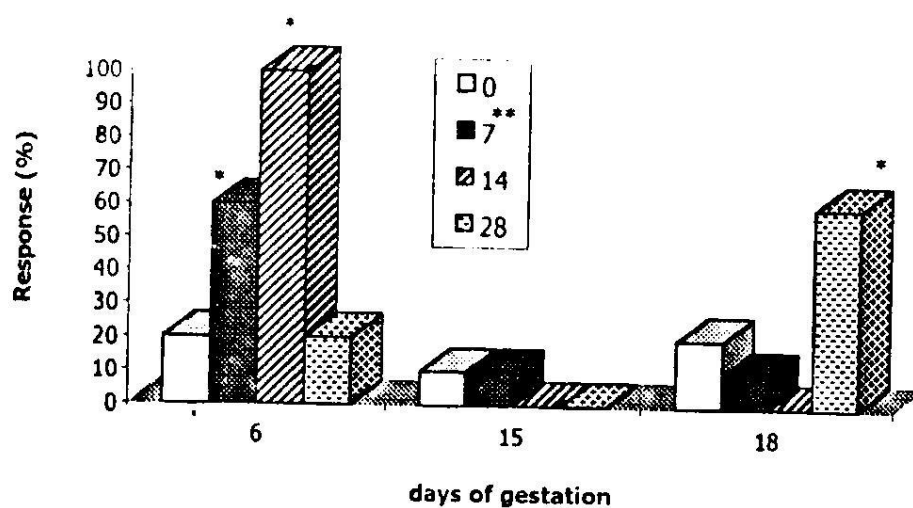
Due to the small number of animals used in each dose group (ten); non parametric statistical test (Kruskal-Wallis ANOVA) was utilized to study differences at the selected doses of acephate. The responses were ranked according to the values obtained for each parameter. The level of significance selected for this study was the 0.05 level (Norusis, 1992).

RESULTS AND DISCUSSION

Dose- Response of Maternal Weight Gain:

The present study is designed to reveal whether or not statistically increased response rate existed for the percentage change of status (affected/ not affected) of each developmental toxicity parameter of acephate in mice. These adverse effects had not been previously reported. Kruskal-Wallis test was employed to determine any statistically significant differences among the studied groups. A highly significant increase of response rate for decreased of maternal weight gain was noted in the 7 and 14 mg/kg/day treated groups at day 6 of gestation (Chi-square = 11.45). This increase was virtually 100% in 14 mg/kg/day treated group

compared to the control group and other treated groups (Chi-square = 9.98). At day 15 of gestation (the end of the period of organogenesis) no significant difference in response rate in any of the treated groups was observed.



* Significantly different from the control

** Dose of acephate in mg/kg/day

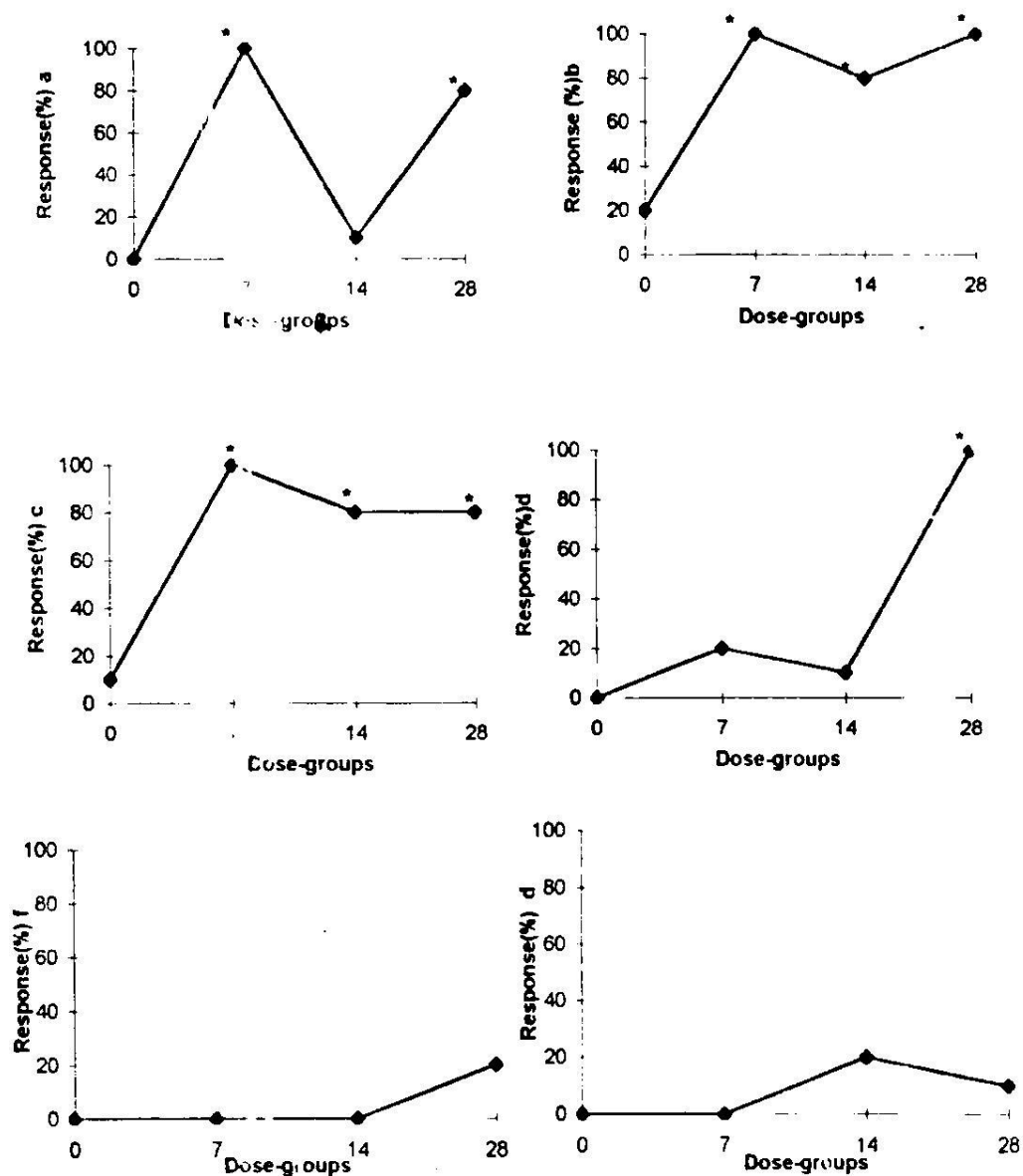
Fig (1): Acephate; analysis for maternal weight gain and dose - response

A significant increase in the percent of dose-response in decreased maternal weight gain of the group treated with the highest dose of acephate (28 mg/kg/day) was observed at day 18 of gestation (Chi-square = 10.63) (Fig.1). However, a possible explanation for these changes of weight gain response rates among the treated groups and at the different periods of gestation can be attributed to a multifactorial etiology which can be caused either by not eating or by metabolic effects of the administered insecticide (Chapin *et al.*, 1993). The aim is to predict how much of those changes of response rates might be due to the different rates and pathway of the metabolic effects of acephate among the different gestation periods. The design used in this study cannot explain the metabolic effects produced by the tested levels of acephate during the different periods of gestation. Nevertheless, these data will be useful in helping to separate acephate-related toxicities from those caused by maternal weight gain changes. Moreover, the increase of dose-response of the reduction of maternal body weight might attribute to the reduction of fetal body weight.

Dose- Response of Maternal Organ Weights:

The changes of response rates of organ weights are presented in Fig (2). No significant dose-related increase in response-rate appeared in the maternal organ weights in any of treated groups. While a clear increase in response rate was noted for liver, kidney, brain, and spleen weights (Chi-square = 9.57, 9.36, 10.31, 14.51, respectively), there was no significant variation in response rate of heart and placenta weights in any of treated groups (Chi-square = 11.93, 12.66, respectively). A highly statistically significant increases of response rates in liver were occurred in the 7 and 28 mg/kg/day treated groups and in all treated groups for kidneys and brain weights compared to the control. On the other hand, the response rate of spleen weight was 100% in the high dose (28 mg/kg/day). The present finding may indicate that there is a case of poor fit, the dose-response pattern was nonmonotonic : an increase in response rate of maternal organ weight was followed by a decrease in response rate as the doses were of acephate increased. Nonmonotonic dose-response patterns are problematic for any statistical approach (Allen, *et al.*, 1994).

Fig.2. Dose-Response Curve for Maternal Organs weights



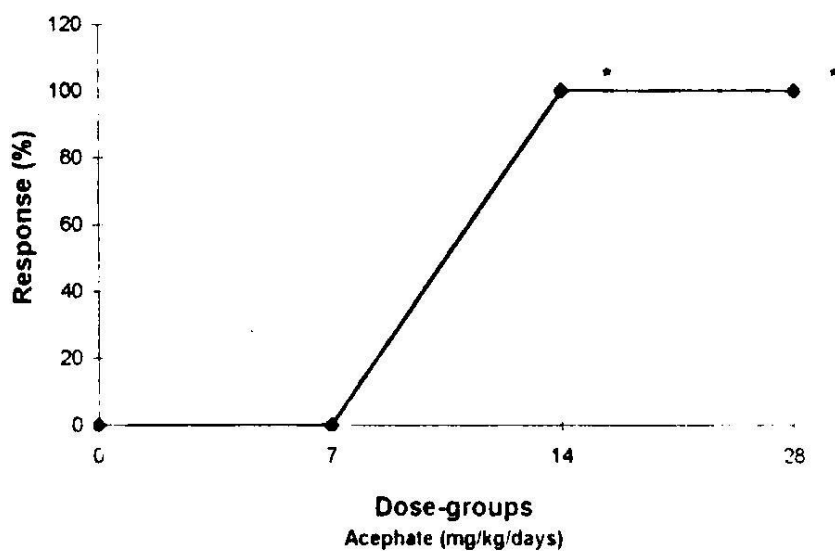
a,b,c,d,f,g = For liver, kidney, brain, spleen, heart, and placenta, respectively.

\$= Acephate (mg/kg/day).

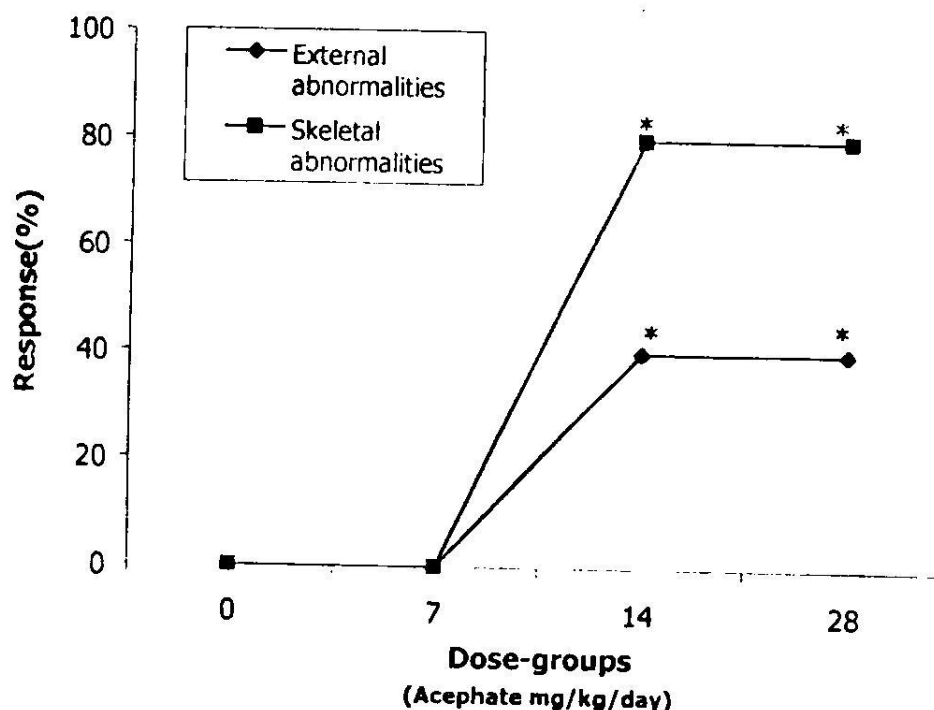
* = Significant different from the control.

However, an increase of the response rates of all organ weights were more severe in 28 mg/kg/day treated group. These increases of the effects may be made due to the increase of histological changes at this tested level of 28 mg/kg/day. Acephate caused severe hepatic injury, hypervascularity of the renal corpuscles, degenerative changes of white and red pulp in the spleen, heart injury, and cellular infiltration appeared in the fetal part of the placenta (unpublished data).

Fig.3. Dose-Reponse Curve for Fetal Body Weight.



* = Significant different from control group



* = Significantly different from control

Fig (4) : Dose – response curve for external and skeletal malformations

Fetal observations :

End point parameters for fetal body weight are given in Fig (3). No significant increase was observed in the response rate of live, dead, aborted, and resorbed fetuses in any treated groups. However, there was a highly statistically significant increases of response-rate of reduction of fetal body weight in the 14 or 28 mg/kg/day treated groups compared to the 7 mg/kg and the control groups .

Response rates for malformation are presented in Fig(4). Neither increase of response rate of visceral malformations nor cleft palate was observed in the fetuses of any of the treated groups. The results from individual analysis measured in each fetus revealed increase of response rate of external fetal malformations in the form of polydactyly, syndactyly, and short or long hindlimbs in the 14 and 28 mg/kg/day treated groups. Response rate of skeletal malformation as absent and abnormal distribution of phalanges, malformed metacarpals and metatarsal, abnormal size of tibiae and bent fibulae was encountered in 80% of fetuses at the same treated groups (14 and 28 mg/kg/day). The present finding may indicate that, there is a correlation between exposure to acephate at the tested levels and the rate response of limb deformities. A positive correlation between pesticides exposure and the prevalence of limb deformity was reported by Gordon and Shy, 1981, Kricker *et al.*, 1986, Mohar, 1987, White *et al.*, 1988.

The results of the present investigation of the Kruskal-Wallis test applied to developmental toxicity data of acephate verify that this test can be applied routinely to typical developmental toxicity data. In the present study, acephate caused increase in rates of developmental toxicity endpoints.

Although the main mode of action of acephate is attributed to the bioactivation on metabolic conversion mainly to methamidophos which acts as an acetylcholinesterase (AChE) inhibitor, yet the developmental effects can not be attributed directly to cholinesterase inhibition. Mahajna *et al.*, 1997 showed that a carboxyamidase activates acephate in mice which in turn undergoes inhibition by the hydrolysis product methamidophos; thus, the bioactivation when started is then immediately turned off. Furthermore, acephate is a pentavalent phosphorus ester. Its major reactions are phosphorylation and alkylation. Therefore, the phosphorylation reaction which is responsible for inhibition of acetylcholinesterase (Ando and Wakamatsu, 1982), will be simultaneous with the cellular DNA alkylation potential leading to mutagenic effects leading also to malformation. These present results are in accordance with the results reported by Garrett *et al.*, 1986 who reported that acephate was grouped as a positive mutagenic agent and with the recent findings

reported by Behera and Bhunya, 1989 and Perocco *et al.*, 1996 who concluded that acephate is a potential mutagen and carcinogen. Thus, the developmental toxicity observed in the present work may reflect the cytotoxic potential of acephate. Besides, the fact that placental transport plays an active role in the uptake of acephate or its metabolites, adds another risk during pregnant exposure (Salama *et al.*, 1992).

The toxic high dose of acephate tested in the present study (28 mg/ kg/ day) is expected to exist under real human exposure in the environment especially in those occupationally exposed. Thus, it can be concluded the possibility of an increases of response rates of endpoints parameters by acephate exposure.

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الملخص العربي

تقدير سمية الكشف لمبيد الأسفيت عن طريق تحليل
النسبة المئوية لمدى استجابة الفئران للجرعات

د. أمينة فرج ، أ.د. محمد كامل ، د. محمد عويضة

تم دراسة المبيد الفوسفوري لاسفيت على مدى استجابة الفئران للجرعات
المعطاة وتأثير ذلك على الكشف الجنيني. تم استخدام أربعة مجاميع من الفئران
(١٠ فئران حوامل / مجموعة).

المجموعة الأولى عبارة عن الكنترول (ماء مقطر) والمجاميع الأخرى
تم تعريضها لجرعات مختلفة من المبيد عن طريق الفم (٧، ١٤، ٢٨
مجم/كجم/يوم) خلال المدة من اليوم ٦-١٥ من الحمل. تم تحليل النتائج باستخدام
طريقة Kruskal-Wallis ANOVA لتقدير مدى الاستجابة للجرعات وأقل
المجاميع المعاملة المختلفة.

وقد أوضحت النتائج وجود استجابة معنوية عالية في الانخفاض
الحادث لوزن الأمهات على جميع الجرعات المستخدمة خاصة في نهاية فترة
الحمل (اليوم الثامن عشر). بينما لا توجد استجابة معنوية للجرعات على وزن
القلب والمشيمة، ازدادت الاستجابة للجرعات بالنسبة لوزن الكبد، المخ، الكلية،
الطحال مقارنة بالكنترول. هناك أيضاً استجابة عالية للجرعات في حدوث
تشوهات ظاهرية وفي الجهاز العظمي ووزن الأجنة.

وبناء على هذه النتائج يمكننا القول أن مبيد الأسفيت يحدث زيادة في
الاستجابة للجرعات المستخدمة مؤدياً إلى حدوث سمية في الكشف الجنيني وذلك
بدون وجود Dose-related pattern.