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Mutagenic, Teratogenic and Biochemical Effects of Ethephon on Pregnant Mice and Their Fetuses

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Abstract: Ethephon (ETF) (2- chloroethylphosphonic acid) is an organophosphorus pesticide. Pesticides are used to control pests. They are selective toxicants in the form and manner used. The mutagenic, teratogenic and biochemical effects of organophosphorus pesticide ethephon on mouse dams and fetuses were investigated. ETF was administered to Swiss albino pregnant mice with three doses level (50, 100 and 150 mg/ kg bw/ day) for 3 weeks. An increase in structural chromosomal aberrations was observed in both mouse dams and fetuses, especially with the high dose of ETF in the form of chromatid breaks and gaps, centromeric attenuation, endomitosis, end to end fusion and fragments, as well a decreased in mitotic activity as compared to the control. ETF was found to reduce DNA and RNA concentrations in all tissues tested (brain, liver and kidney) of the dams and fetuses. Similar results were observed for the protein content, cholinesterase enzyme activity, hemoglobin and body weight of dams and fetuses. However, the level of creatinine and urea (for kidney function) and GOT and GPT (for liver function), cholesterol and LDH was increased. In conclusion, ETF could be harmful and has mutagenic, teratogenic and biochemical effects on mouse dams and fetuses. Producers and consumers should become – more conscious about the toxic effects of these pesticides.

Key words: Ethephon · Mutagenicity · Teratogenicity · Biochemical parameters · Mice

INTRODUCTION

Ethephon (ETF) (2-chloroethylphosphonic acid) is organophosphorus pesticide (OPP)and also used as a plant growth regulator, its use varies with plant species, chemical concentration and time of application [1]. OPP are used in agriculture. They are known as an important class of environmental carcinogenesis and mutagenesis [2]. Thus, occupational exposure of agriculture and industrial workers to pesticides possess several serious problems including mutagenic effects. OPP which is the most important group of pesticides are known to react with DNA generally as alkylating agents and/or carcinogenesis. Some organophosphorus compounds were proved to be effective mutagens in a variety of organisms [3].

Several studies have revealed that at low doses, OPP not only act as genotoxic agents, but also affect several other teratogenic and biochemical pathways [4].

Ethephon was also found to inhibits the activity of plasma cholinesterase (ChE) in humans, dogs, rats and mice [5,6]. Mouse plasma cholinesterase (ChE) *in vitro* and *in vivo* are more sensitive to ETF than any other esterases. All mouse liver esterases observed are less sensitive than plasma ChE to ETF *in vitro* and *in vivo*. Thus, ChE inhibition continues to be the most sensitive marker of ETF exposure [7, 8]. The mechanism of pesticide action is it act primarily on four nerve targets, i.e.; acetylcholinesterase, the voltage-gated chloride channel, the acetylcholine receptor and the γ- aminobutyric acid receptor, systems which are present in animals [9].

A mammalian model, Swiss Albino mice, was used to evaluate the mutagenicity of ETF by detecting its capacity to inhibit enzymes (mainly ChE), measuring change of plasma protein content and determination of DNA and RNA concentration [10-12].

Therefore, the present study was undertaken to investigate the mutagenic, teratogenic and biochemical effects of organophosphorus pesticide, ETF on mouse dams and their fetuses by detecting the chromosomal aberrations induction, the level of DNA, RNA and protein in different tissues, kidney and liver function and measuring the levels of cholinesterase and other biochemical parameters.

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MATERIALS AND METHODS

Chemical Material: Ethephon (ETF) (2-chloroethylphosphonic acid) CAS no.16672-87-0, was obtained from Sigma Chemical Company, St Louis, MO, USA and used in this study.

Animals and Housing: Eighty adult virgin Swiss albino female mice, 8-12 weeks old and weighting between 25-30 g were obtained from the Department of Animal House colony of the National Research Centre, were used in this study. The animals were divided into two main groups, the first group was used for cytogenetic analysis and the second group was used for biochemical analysis. Animals in each mean group were divided into four subgroups, each group included 10 animals (40 pregnant female mice for each mean group). *Cytogenetic analysis* was done from bone marrow cells of pregnant dams and fetal liver cells (5 fetuses for each mother). *Biochemical part:* blood samples with anticoagulant heparin and different tissues (brain, liver and kidney) of dams and (brain, liver) for each fetus of treated mother were collected and subjected for biochemical analysis.

Animal Groups:

Group I : 10 pregnant females were used as standard or untreated female mice served as control.

Group II : 10 pregnant females were orally administrated low dose of ETF for three weeks (50 mg/kg bw/day, in water).

Group III : 10 pregnant females were orally administrated mid-dose of ETF for three weeks (100 mg / kg bw/day, in water).

Group IV : 10 pregnant females were orally administrated with high dose of ETF for three weeks (150 mg/kg bw/day, in water). The administrated doses was calculated according to IPCS [13].

Cytogenetical Examination: Pregnant female mice were sacrificed at the end of day 20th of gestation and chromosomes were prepared from bone marrow cells of the dams according to Yosida *et al*. [14]. In order to study the genotoxic effects on fetal liver cells, the method described by Romagnano *et al*. [15] was used.

Scoring of Slides: To obtain the frequencies of chromosomal aberrations in bone marrow cells, 60 metaphases for each animal were examined, with a total of 600 cells per each dose and the control. For the fetal cells, chromosomal aberrations of liver cells per each group were examined for a total of 2500 cells per group.

Biochemical Studies:

- Determination of nucleic acids: Total DNA and RNA contents were determined according to Peares [16] in liver, kidney and brain of pregnant females and liver and brain of the fetuses.
- Determination of total protein: Protein was determined according to the method of Peters [17], using protein kits at wave length of 545 nm using spectrophotometer in different tissues (brain, liver and kidney) of dams and liver and brain of the fetuses.
- Determination of urea and creatinine concentration: Determination of urea concentration in plasma was carried out according to Henry [18], using spectrophotometer at wave length 600 nm. Determination of creatinine was carried out according to Young *et al*. [19] using spectrophotometer at wave length 492nm (490-570).
- Determination of Glutamic – oxaloacetic transaminase (GOT or AST) and Glutamic–pyruvic transaminase (GPT or ALT) activity: AST and ALT (GOT and GPT) was determined according to the method of Reitman and Frankel [20] using kits at wave length 490-520 nm.
- Determination of cholinesterase activity: Determination of cholinesterase activity in plasma and brain was carried out according to Jakobs *et al*. [21] using spectrophotometer at wave length 405 (400-440 nm).
- Determination of cholesterol, triglycerides, glucose, LDH and hemoglobin: Cholesterol, triglycerides, glucose, LDH (lactate dehydrogenase activity) and hemoglobin were determined using chemical kits obtained from bio Mérieux (bio Merieux sa, F-69280 Marcy l'Etoile, France).

Statistical Analysis: The data were analyzed using the statistical package SPSS for windows (release 11.0). Numerical values were reported as mean±SE of the sample size. P value $\leq$ 0.05 was considered as statistically significant.

RESULTS

Effect Of Ethephon On Chromosomal Aberrations Frequency In Bone Marrow Cells Of Pregnant Female Mice: Data shown in table 1 revealed an increase in all types of structural chromosomal aberrations (chromatid breaks and gaps, centromeric attenuations, endomitosis, deletions, End to end and fragments) and

Table 1: Effect of Ethephon on chromosomal aberrations frequency in bone marrow cells of pregnant mice

			Structural aberration (mean±S.E)							Total excluding gap	Mitotic index	
Groups	No of animals	No ofcells	Break	Centromeric attenuation	Endo-mitosis	Deletions	End to end	Fragments	Gaps	Mean ±S.E	No of cells	Mean ±SF
Control	10	600	0.9±0.331	1±0.222	0.6±0.233	0.4±0.3	0.3±0.161	0.5±1.76	1.3±0.274	6±1.011	10000	420±5.9
Low dose	10	600	1.4±0.233	1.7±0.274	1±0.272	0.9±0.292	0.4±0.23	0.8±0.211	1.7±0.274	10.3±1.53*	10000	416.4±5.39
Mid- dose	10	600	1.6±0.23	2±0.272*	1.1±0.189	1±0.272	1±0.22	1±0.314	1.8±0.263	12.83±1.405**	10000	329.7±9.54**
High dose	10	600	1.9±0.331*	2.7±0.473**	1.5±0.283*	1.2±0.306**	1.1±0.189**	1.6±0.23**	2.2±0.439	16.67±1.94**	10000	324.8±10.22**

* P (significant at ≤ 0.05), **P (significant at ≤ 0.01).

Table 2: Effect of Ethephon on chromosomal aberrations frequency in fetal liver cells

			Structural aberration (mean±S.E)							Total excluding gap	Mitotic index	
Groups	No of animals	No ofcells	Break	Centromeric attenuation	Endo-mitosis	Deletions	End to end	Fragments	Gaps	Mean ±S.E	No of cells	Mean ±SF
Control	25	1250	0.96±0.227	1.23±0.26	0.36±0.128	0.2±0.01	0.12±0.088	0.12±0.12	1±0.173	12.5±2.406	50000	277.027±3.57
Low dose	25	1250	1.48±0.25	1.8±0.283	0.68±0.150	0.76±0.166*	0.36±0.098	0.52±0.201	1.96±0.22*	23.33±2.867*	50000	276.26±3.211
Mid- dose	25	1250	2.2±0.258**	2.86±0.293**	1.04±0.158*	1.24±0.26**	0.92±0.191**	1.23±0.26**	2.84±0.335**	38.8±3.57***	50000	273.24±3.635
High dose	25	1250	2.76±0.353**	3.24±0.312**	1.32±0.256*	1.56±0.239***	1.24±0.26***	1.48±0.259***	3.56±0.432***	48.3±4.238***	50000	270.78±3.59

* P (significant at ≤ 0.05), **P (significant at ≤ 0.01).

Table 3: Effect of Ethephon on total DNA and RNA in different tissues (mg g^{-1} tissue) of pregnant female mice and their fetuses

	Dams						Fetuses			
	Brain		Liver		Kidney		Liver		Brain	
Groups	DNA	RNA	DNA	RNA	DNA	RNA	DNA	RNA	DNA	RNA
Control	0.424±0.005	0.300±0.005	0.443±0.008	0.261±0.008	0.349±0.007	0.252±0.005	0.277±0.008	0.209±0.004	0.232±0.006	0.202±0.003
Low dose	0.413±0.004	0.245±0.004**	0.417±0.005*	0.235±0.005*	0.316±0.006**	0.228±0.006*	0.257±0.006*	0.188±0.014	0.217±0.005	0.186±0.004
Mid- dose	0.348±0.01***	0.212±0.005***	0.392±0.004**	0.201±0.009**	0.303±0.003***	0.198±0.006**	0.227±0.006**	0.169±0.014*	0.185±0.006	0.167±0.006
High dose	0.310±0.006***	0.199±0.003***	0.352±0.009***	0.190±0.004***	0.288±0.006***	0.171±0.005**	0.211±0.05***	0.150±0.007**	0.157±0.008	0.147±0.605

*P(significant at ≤ 0.05), **P (significant at ≤ 0.01)

a decrease in mitotic index as compared to control. Whereas, the high dose treatment induced high significant (P<0.01)increase in all types of structural chromosomal aberrations (except for deletions and gaps) and a decrease in mitotic index compared to the control.

Effect Of ETF On Chromosomal Aberrations In Fetal Liver Cells: Table 2 shows an increase in all types of structural chromosomal aberrations and a decrease in mitotic index as compared to the control. Meanwhile, that increase was highly significant (P<0.01), especially with the mid-and high-doses of ETF treatment as compared to the control.

Effect of ETF on DNA and RNA Content In Different Tissues of Mice Dams and Fetuses:

Changes in DNA Content: Table 3 shows that the effect of low dose of ETF on the total content of dams brain and embryos brain DNA was not significant, whereas the mid- and high- doses of ETF had high significant (P<0.01) reduction on DNA content of dams brain liver and kidney and fetal liver cells, but that effect was not significant in fetal brain cells compared to control group.

Changes in RNA Content: Statistical analysis of total RNA content is presented in table 3.There were a significant (P<0.05) decrease in the mean values of RNA content in treated mice dams (liver, brain and kidney) as compared to the control. The RNA content in fetal liver cells was also reduced significantly (P<0.05) with the mid- and high-dose treatment, but that is not occurred in fetal brain as compared to the control.

Changes in Total Protein: The effect of ETF on total protein content in different tissues of mouse dams and fetuses was shown in table 4. The reduction in total protein level in the treated mice was correlated with dose increase (dose dependent), where it was high significant decrease in total protein content with all doses for mouse dams brain (except for the low dose), liver and kidney and fetal brain and liver compared to the control.

Effect of ETF On Kidney And Liver Function: The effect of ETF on kidney and liver function was appeared as creatinine (mg/dL) and urea (g/dL) and GOT (U/ml) and GPT (U/ml) levels in pregnant mice presented in table 5, where the results revealed that the mid- and high- dose treatment increased significantly the creatinine and urea levels compared to the control.

Table 4: Effect of ETF on total protein (g dL^{-1}) in different tissues of pregnant female mice and their fetuses (mean ±SE)

	Mouse dams			Fetuses	
Groups	Brain	Liver	Kidney	Brain	Liver
Control	7.878±0.354	6.967±0.117	6.037±0.193	4.92±0.157	4.436±0.137
Low dose	7.126±0.208	5.972±0.168**	5.837±0.177	3.57±0.127**	3.427±0.774**
Mid- dose	6.859±0.21*	5.363±0.111***	5.097±0.124**	3.129±0.061***	3.073±0.193***
High dose	6.342±0.224**	5.016±0.155***	4.581±0.743***	2.878±0.142***	2.485±0.112***

Table 5:. Effect of Ethephon on creatinine, urea, GOT and GPT in pregnant female mice (mean ±SE)

Groups	Creatinine (mg/dL)	Urea (g/dL)	GOT (AST) (U/ ml)	GPT (ALT) (U/ml)
Control	0.586±0.009	18.214±0.161	50.97±0.916	60.6±2.115
Low dose	0.603±0.025	18.684±0.187	51.2±1.538	61.7±1.813
Mid- dose	0.634±0.029*	19.008±0.228*	53.1±1.567	62.8±1.609
High dose	0.689±0.011***	19.556±0.293***	55.28±1.922	63.2±1.892

Table 6: Effect of Ethephon on Cholinesterase enzyme in dams plasma and in dams and fetal brain tissues (mean±SE)

		Cholinesterase (U/mg)	
Groups	Cholinesterase (U/ml) Dams plasma	Dams brain tissues	Fetal brain tissues
Control	7.625±0.196	6.891±0.113	4.143±0.081
Low dose	7.240±0.126	6.813±0.134	3.729±0.123
Mid- dose	5.663±0.143***	5.748±0.124***	3.566±0.176**
High dose	4.303±0.11***	4.27±0.11***	3.344±0.144***

Table 7: Effect of Ethephon on cholesterol, triglyceride, glucose, LDH and hemoglobin in pregnant dams (mean±SE)

Groups	Cholesterol (mg/dl)	Triglyceride (mg/dl)	Glucose (mg/dl)	LDH (U/L)	Hemoglobin (g/dl)
Control	166.59±2.196	58.206±0.906	79.828±0.537	252.071±4.239	15.921±0.142
Low dose	167.787±1.311	57.276±0.946	79.328±0.452	253.794±5.675	15.573±0.106
Mid- dose	169.457±1.877	56.278±1.628	79.176±0.323	254.594±2.348	15.171±0.109*
High dose	170.649±1.59	55.705±1.321	79.02±0.306	256.502±1.747	14.883±0.164**

Table 8: Effect of Ethephon on body weight of dams and fetuses (mean±SE)

Groups	Weight of dams (gm)	Weight of fetuses (gm)
Control	34.8±0.966	1.83±0.025
Low dose	29.2±1.215**	1.652 ±0.096
Mid- dose	23.73±0.113***	1.029±0.033*
High dose	21.97±0.097***	0.83±0.036***

Concerning the effect of ETF on liver function of mouse dams. Table 5 showed that there was increased in GOT (glutamate oxaloacetate transaminase) and GPT (glutamate pyruvate transaminase) levels as indicator of the liver function as compared to the control but that increment was not significant.

Effect of ETF on Cholinesterase Activity: The results for measuring the changes in cholinesterase activity of blood plasma after treating mice with ETF were detected in blood plasma of dams and in brain tissues of the dams and fetuses are shown in table 6. It was clear that the cholinesterase content is significantly ($P<0.001$) decreased when mid- and high-doses of ETF were used. The low dose also showed a decrease in the enzyme activity comparing to the control but this was not significant.

Effect of ETF on Cholesterol, Triglyceride, Glucose, LDH And Hemoglobin In Pregnant Dams: An increased in cholesterol (mg/dL) and LDH (U/L) levels was obtained with all doses of ETF treated groups. The levels of triglyceride and hemoglobin were reduced, especially with the mid-and high- doses of ETF treatment compared to the control. However, the level of glucose was not affected (Table 7).

Effect ETF on Body Weight Of Mouse Dams And Fetuses: It was noticed (Table 8) that ETF treatment of mice affected the body weight of both mouse dams and fetuses where the body weight reduced significantly in a dose dependent manner compared to the control mice in both dams and their fetuses.

DISCUSSION

It has been established that numerous environmental agents can induce genetic alteration in human and livestock cells. These alterations (mutations) are deleterious to normal cell function. There are several examples of exposure to genotoxic agents such as OPP. especially in agriculture and industry. Related studies and the knowledge about mutagenic, teratogenic and biochemical effects of ETF on higher animals are very limited, therefore, the present study was conducted to investigate the effects of ETF on these parameter in mouse dams and fetuses. The results of the present study presented in table 1 revealed that ETF treated mouse dams and fetuses showed a significant increase in structural chromosomal aberrations, especially with high dose treatment as compared with control group. That in agreement with Yu *et al*. [22] and Al- Twaty and Alakilli [23] who found an increase in structural chromosomal aberrations in ETF treated mice and attributed that effect due to mutagenicity or clastogenicity of ETF. The genotoxic effect of organophosphorus pesticide ETF could be attributed to many reasons such as the sensitivity of the treated animals itself, the way, time of application and the administrated dose of ETF.

Concerning the effect of ETF on DNA and RNA concentrations in treated mice, the significant decrease in nucleic acids content in brain, liver and kidney of mouse dams and brain and liver of treated fetuses were correlated to increasing doses of pesticide ETF. This coincide with Rahman *et al*. [2] and Al- Twaty [24] who observed DNA damage and reduction at all doses used of OPP in albino mice as compared with control.

Because cellular RNA synthesis is a DNA dependent process, thus, significant decrease in RNA was recoded after treatment with several insecticides due to inhibition of DNA dependent RNA polymerase [25]. In contrast, a study of Barfknecht *et al*. [26] informed that ETF has no genotoxic effect, when tested for unscheduled DNA synthesis in the rat hepatocyte system.

The reduction of total protein content in the liver and brain of mice dams and fetuses with all does used in the study coincide with those of Olson. and. Hinsdill [27] and El-Fiky *et al*. [28] who found a decrease in total protein in plasma protein in liver and brain of mice treated with OPP. The highly significant decreased of protein content of mice treated with ETF may due to the toxicity and mutagenicity of the pesticide.

Because ETF causes neurotoxin effects (cholinesterase inhibition), ACE determination in plasma has been used as a good method to evaluate exposure to OPP [29, 30].

In the present study, the gradual decrease in cholinesterase (ChE) activity in blood plasma and brain tissues of mouse dams and fetuses treated with ETF was observed. These results emphasize the positive correlation between mutagenic effect and toxicity as reported in mice by Haux *et al*. [7], who found that ChE inhibition continues to be the most sensitive marker of ETF exposure. The inhibition rate is generally related to ETF concentration and also due to that ETF acts as a phosphorylating agent in inhibiting ChE activity. Similarly, in the ETF treated rats, plasma and brain ChE activity were found to be significantly different from the controls at all dose level tested [31].

In addition, the short term effects of ETF were studied by EFSA [32] in two oral 28-day studies in the rat and mouse to investigate the ChE inhibition and concluded that the main effect of ETF is an inhibition of ChE activity in plasma and erythrocytes of female rats and mice with dose – related inhibition specially at the mid- and high- dose groups, are in agreement with our results. In contrast, in a chronic toxicity/ oncogencity study operated by EXTOXNET [33] using Swiss albino mice treated with ETF. The brain ChE activity was not different from control values at any dose level in treated males or females.

There is an increase of potency of ACh and decrease of maximum effect of Ach on rat ileum after incubating with ETF. These indicated that the ACh concentration increased in synaptic cleft by AChE inhibition because agent act as acetylcholinesterase inhibitors and also the contractions are decreased because agent bind directly to the receptors [8] which was in accordance with our findings whereas cholinesterase activity was significantly decreased in mice treated with ETF.

The effects of ETF on cholesterol, triglyceride, LDH, glucose and hemoglobin were investigated. The important findings of this study were an increase of cholesterol and LDH and a decrease in hemoglobin levels. That agreed with Yazar and Baydan [34] who found a significant increase in liver lipids with ETF treatment in female mice. That may due to the toxicity of the pesticide.

Although kidney shown as a target organ for ETF toxicity [31], usages of ETF caused liver and kidney toxicity in our study. Toxic effects on mouse dams kidney were more evident,especially with mid- and high – dose treatment which coincide with the finding of Yazar and Baydan [34], whereas the urea and creatinine levels increased significantly with mid- and high- dose treatment, however GOT and GPT increased in a non significant manner.

The reduced maternal and fetal body weight with ETF treatment in our study was also occurred in other studies. In the teratogenicity study in rabbits, reduced fetal body weight was observed at a dose level of ETF which caused severe maternal effects [32]. In rat, ETF caused body weight decrease at the highest dose levels [29], as well in mice [27]. That may be attributed to its teratogenic and pesticidal activity.

In conclusion, the results of the present study showed that organophosphorus pesticide ETF is harmful on mouse dams and fetuses whereas it caused mutagenic, teratogenic and biochemical alterations. It should be noted that it is impossible to forbid the utilization of these kinds of chemicals but producers and consumers should be conscious for the probable toxic effects of these pesticides, which should be important for the environment and public health.

REFERENCES

1. Kidd, H. and D.R. James, 1991. The Agrochemicals Handbook, 3rd Edn. Royal Society of Chemistry Information Services, Cambridge, UK, pp: 10-12.
2. Rahman, M.F., M. Mohboob, K. Danadevi, B. Saleha Banu and P. Grover, 2002. Assessment of genotoxic effects of chloropyriphos and acephate by the comet assay in mice leucocytes. Mutat. Res., 516: 139-147.
3. Waters, M.D., V.F. Simmon, A.D. Mitchell, T.A. Jorgenon and R. Valencia, 1980. An over view of short term tests for the mutagenic and carcinogenic potential of pesticides. J. Environ. Sci. Health, 15: 867-906.
4. Das, G.P., A.P. Shaik and K. Jamil, 2006. Estimation of apoptosis and necrosis caused by pesticides *in vitro* on human lymphocytes using DNA diffusion assay. Drug Chem. Toxicol., 29: 147-165.
5. Brock, A., 1991. Inter and intra individual variations in plasma cholinesterase activity and substance concentration in employees of an organphosphorus insecticide factory. Br. J. Indus. Med., 48: 556-562.
6. Haux, J.E., G.B. Quistad and J.E. Casida, 2000. Phosphobutyrylcholinesterase: Phosphorylatin of the esteratic site of butyrlcholinesterase by ethephon (2- choloroethyl phosphoric acid) dianon. Chem. Res. Toxicol., 13: 646-651.
7. Haux, J.E., O. Lockridge and J.E. Casida, 2002. Specificity of ethephon as a butyryleholinesterase inhibitor and phosphorylating agent. Chem. Res. Toxicol., 15: 1527-1533.
8. Cetinkaya, M.A. and E. Baydan, 2010. Investigation of *in vitro* effects of ethephon and chlorpyrifos, either alone or in combination, on rat intestinal muscle contraction. Interdise Toxicol., 3(1): 35-39.
9. Casida, J.E., 2009. Pest toxicology: The primary mechanisms of pesticide action. Chem. Res. Toxicol., 22(4): 609-619.
10. Shukla, Y. and P. Taneja, 2000. Mutagenic evaluation of deltamethrin using rodent dominant lethal assay. Mutat. Res., 467: 119-27.
11. Seiler, F., B. Rehn, S. Rehn and J. Bruchs, 2001. Significant differences in the cellular and molecular reactions of rat and hamster lung after quartz exposure: Toxicol. Lett., 199: 1-9.
12. Quistad, G.B. and J.E. Casida, 2004. Lysophospholipase inhibition by organophophorus toxicants. Toxicol. Appl. Pharmacol., 196: 319-326.
13. IPCS, 1993. International Programme on Chemical Safety 1993. Ethephon. Pesticide residue in food: 1993 evaluation Part II Toxicology.
14. Yosida, T.H., K. Truchiya and K. Moriwaki, 1971. Frequency of chromosome polymorphism of Rattus Rattus collected in Japan. Chromosoma, 33: 30-40.
15. Romagnano, A., A.W. King, C.L. Richer and M.A. Perrone, 1985. A direct technique for the preparation of chromosomes from carly enquine embryos. Can. J. Genet. Cytol., 27: 365-396.
16. Peares A.G.E., 1985. Histochemistry theoretical and Applied. Volume two: Analytical technology, Churchill livingston, 4th ed. Edinburgh, London, Melbourne and New York.
17. Peters, T., 1968. Protein colorimetric method. Clin. Chem., 14: 1149-1159.
18. Henry, J.B., 1974. Todd, Sanford, Davidsohn: Clinical Diagnosis and Measurement by laboratory Motheds, 16thed, W.B. Saunders and Co, Philadelphia, P.A, pp: 260.
19. Young, D.S., L.C. Pestaner and V. Gibberman, 1975. Effect of drugs on clinical laboratory tests. Clin. Chem., 21(5): ID-432 D.

20. Reitman, A. and S. Frankel, 1957. Colorimetric determination of Glutomic Pyruvic Transminase (GPT) and Glutamic Oxaloacetic Transaminase (GOT). Am. J. Clin. Path., 25: 56-59.
21. Jakobs, D.S., J.B. Kasten, W.R. Demmott and W.L. Wolfson. 1990. Laboratory Test Hand book, Lexi – Company and Williams & Wilkins Ed (2nd Edition- 1990).
22. Yu, W., Y. Gao, W. Zhao, X. Qi and J. Yang, 2006. Study on mutation of ethephon in mice. Clinese J. of Pesticide Sci., pp: 02.
23. Al- Twaty, N.H.A. and S.Y.M. Alakilli, 2008. Genotoxic effect of an organophosphorus pesticide Ethephon on somatic and germ cells of male mice. Biosci. Biotech. Res. Asia, 5(1): 1-8.
24. Al-Twaty, N.H.A., 2006. Mutagenic effects of ethephon on albino mice. J. Biological Sci., 6(6): 1041-1046.
25. De Hondt, H.A., A.M. Fahmy and S.A. Abd El-Baset, 1979. Chromosomal and biochemical studies on laboratory rats exposed to cannabis extract. Egypt. J. Gene. Cytol., 9: 217-231.
26. Barfknecht, T.R., R.W. Naismith and R.J. Mattheews, 1984. Rat hepatocyte primary culture / DNA repair test. Unpublished report No. PH311-UC- 002-84. from Farmason Research International, Inc.; Waverly, Pennsylvania 18471, USA. Submitted to WHO by Rhone – Poulenc, Secteur Agro, Lyon, France.
27. Olson, L.J. and R.D. Hinsdill, 1984. Influence of feeding chlorocholine chloride and glyphosine on selected immune parameters in deer mice, peromycscus manipulates. Toxisl., 30(2): 103-114.
28. El-Fiky, S.A., K.B. Abdel-Aziz and S.A. Abd El-Baset, 1992. Protective role of soybean against chromosomal and biochemical effects of carbaryal (Sevin) in male mice. J. Egypt. Soc. Toxicol., 9: 15-25.
29. US Environmental Protection Agency (US EPA). 1995. Prevention, pesticides and Toxic substances, Ethephon. EAP- 738- F-95-004. April, 1995.
30. Eduardo, B.O. and G.H. Patricio, 2003. Effect of a single dose of malathion on spermatogenesis in mice. Asian J. Androl., 5: 105-107.
31. US Environmental Protection Agency (US EPA). 1998. Guidance for the registration of pesticide products containing Ethephon as the active ingredient. Sept. 1998.
32. European Food Safety Authority (EFSA) Scientific Report. 2008. Conclusion on the peer review of ethephon, 174: 1-65.
33. Extension Toxicology Network (EXTOXNET). 1995. Pesticide Information Profile. Ethephon, Sept., 1995.
34. Yazar, S. and E. Baydan, 2008. The subchronic toxic effects of plant growth promoters in mice. Ankara Univ. Vet. Fak Derg., 55: 17-21.