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Impact of Food Toxicity on Blood Estrogen Levels of Female Albino Rat (*Rattus norvegicus domestica*)

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Abstract: Stress is a complex and challenging physiological as well as an ethological response of body which is triggered by stressors. Internal stressors include physiological, behavioral and hormonal changes etc. while external stressors include environmental changes, chemicals, air pollutants, food toxicants, contaminated food and water etc. To check the food toxicity in female rats, we used malathion as a toxicant. The experiment was divided into two phases, Phase I-- the experimental group was kept under acute stress and Phase II-- withdrawal of acute stress from experimental group for recovery. Blood samples were collected. The level of estrogen hormone in blood was observed by ELISA. The results showed no effect of short-term malathion on estrogen levels of rat.

Keywords: Reproductive health, Estrogen, Food toxicity, Malathion, Stress, Acute stress

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Introduction

Stress is a complex and challenging physiological as well as ethological response of the body which is triggered by stressors (Wheaton, 1994). Stress can be positive or negative depending on the stressors. Positive stress is called eustress while negative stress is called distress (Bienertova-Vasku *et al.*, 2020). Excessive exposure of stressors can cause adverse effect on mental and physiological health (Dar *et al.*, 2019). The stressors are natural part of life (Metreveli *et al.*, 2022). Stressors can be caused by internal or external stimuli (Chrousos, 2009). Internal stressors include psychological, behavioral, hormonal changes etc. (Stress - Anxiety UK, 2021). External stressors include environmental changes, chemicals, air pollutants, food toxicants, contaminated food and water etc. (Rasheed *et al.*, 2018; Manisalidis *et al.*, 2020). A study based on the transactional model of stress emphasized the interaction of organisms with their environment and how organisms perceive and appraise stressors depending upon their coping abilities

(Biggs *et al.*, 2017). Depending on the time period of exposure, stress can be divided into two categories-- acute stress and chronic stress (Hammen *et al.*, 2009). Acute stress is a short-term reactive response of a body towards the stressors (Epel *et al.*, 2018). It activates fight or flight response of the body which lead to increase in heart rate, heightened alertness, and release of stress hormones by increasing level of stress hormone (Karuppaiah *et al.*, 2023). Chronic stress is a response against the exposure of prolonged or ongoing stressors (Epel *et al.*, 2018). For example, exposure of any chemical or toxicants for a long period of time can cause many physiological and psychological changes in an individual (James *et al.*, 2023).

Prolonged exposure to stress hormones involves mental health issues and shows adverse effect on the brain structures at any stage of life including prenatal period, infancy, childhood adolescence, adulthood or aging (Lupien *et al.*, 2009).

The stressors can cause minor or major changes in organisms which is the root cause of several health issues and to understand the effect many studies have been conducted, including chemical stressors like pesticides, drugs, chemicals used food additives/preservatives etc. (Onyeaka *et al.*, 2023; Dinca *et al.*, 2023).

Several pesticides such as malathion, λ-cyhalothrin, chlorpyrifos and heavy metals were found in samples of rice brown Super Kernel Basmati and Super Basmati varieties irrigated by tube well irrigation method (Hamid *et al.*, 2020).

Mostly the workers who worked in agriculture field have more risk to get exposed with these chemicals directly or indirectly specially to pesticides. This may cause adverse health issues such as diabetes, fertility disorders, neurological malady, cancer and respiratory disorders (De Cock *et al.*, 1994; Rani *et al.*, 2020).

There are various studies which interpreted that pesticide exposure can cause disruption in reproductive system including gonadal hormones in females (Farr *et al.*, 2004; Bretveld *et al.*, 2006).The study based on relation between high level of pesticides and reproductive hormone in women interpreted that fertility of human can be adversely affected by the pesticide including organophosphate, pyrethroids and triazines and organochlorine such as DDT (dichlorodiphenyl-trichloroethane), HCB (hexachlorobenzene) etc. (Jain *et al.*, 2023).

The pesticides not only effect adversely on the human health but also affect the production of grains or crops (Alengebawy *et al.*, 2021). Considering the serious consequences of above mentioned effect of pesticides on the organisms and grains in agricultural field this study aimed to check the acute effect of toxic food treated by pesticide namely malathion on estrogen hormone of female albino rats.

Materials and Methods

The present study focuses on understanding the effect of stress on reproductive health by using female albino rats (*Rattus norvegicus domestica*). The rats are used as model organism for various studies and so the results obtained can be used to understand the same issue in mammals especially humans.

The presence of pesticides in food that is consumed daily is a necessary evil. Of all these varied pesticides we have used Malathion as a representative for our study. The study is based on *in vivo* model and use of animals under study was under ethical approval.

The rats were acclimatized under laboratory conditions for 2 weeks . Total 24 rats were used for the setting up the experiment (12 rats for each phase). They were almost similar in weight and age. The rats were housed in spacious and well-ventilated cages, measuring 20 x 18 inches. Rats were fed food and water *ad libitum*. They were kept in natural environmental conditions during the whole experiment.

To conduct the study, rats were divided into two groups namely control and experimental. There were 12 rats in each group. Entire

experimental study was conducted into two phases-

Phase I: Experimental group were kept under acute stress.

Phase II: Withdrawal of acute stress from experimental group for recovery.

Phase I:

Duration of phase I was 72 h. In this phase the control rats were fed with normal food including clarified butter, wheat grains, leafy greens, carrots, and water *ad libitum*.

On the other hand the rats in experimental group were fed with the same food but their clarified butter (10 ml) was mixed with 4.71 ml Malathion (in liquid form, Malathion 50% EC insecticide purity, below the lethal concentration value of malathion for rats which is 5400 mg or 5.4 ml) (Malathion Technical Fact sheet).

After the completion of 72 h (3 days) the blood samples were collected from both groups (control group and experimental group) to check effect of the acute stress of food toxicity by using pesticide (malathion) on the level of estrogen hormone in blood.

Phase II:

Duration of phase II was 144 h. In this phase the rats in a control group were fed with normal food including clarified butter, wheat grains, leafy greens, carrots, and water *ad libitum*.

On other hand the rats in experimental group were given food similar to the malathion exposed group in Phase I (i.e. clarified butter (10 ml) mixed with 4.71 ml malathion) for 72 h. Thereafter the rats were given malathion free food for next 72 h.

Following the termination of 144 h of Phase II the blood samples were collected from both groups (control group and experimental group) to check effect of the acute stress of food toxicity by using pesticide (malathion) on the level of estrogen hormone in blood. Phase II also shows the effects of withdrawl of malathion on the estrogen levels in blood.

The blood samples were collected from both the groups from infraorbital region in heparinized eppendorf tubes. The blood samples were tested to estimate estrogen levels using ELISA.

The results obtained were tested by One-way analysis of variance (ANOVA) and t-test was used for comparison between the levels of gonadal hormones. $P < 0.05$ was considered as significant level.

Results and Discussion

The estrogen levels remain unaffected ($p > 0.05$) in both phases-- Phase I and Phase II (Table 1, Figs. 1-4). The difference between two independent groups of Phase I was analyzed by t-test. Same statistical method used to obtain difference between the two independent groups of Phase II.

To check the significance level between two Phases (Phase I and Phase II) ANOVA were applied. The obtained result was not significant ($p > 0.05$) which simply means that short time exposure of malathion does not provoke any significant impact on the estrogen level.

The study concerns the effect of food toxicants on the estrogen (a reproductive hormone) in female albino rats. We found no significant difference in Phase I and Phase II. The study on Low doses of malathion impair ovarian, uterine, and follicular integrity by altering oxidative profile and gene expression of rats exposed during the peripubertal period supports our findings as they observed that the level of estradiol and pubertal onset were unaffected (Erthal-Michelato *et al*., 2024).

Food Toxicity is one of the serious matters of concern as every organism including human depends on food to survive in this world. For fulfilling the requirement of the population nowadays usage of chemicals are normal in agricultural field (Nicolopoulou-Stamati *et al*., 2016). These chemicals include food packaging materials, drugs given to animals, pesticide residues (National Academies Press US., 1983). Nowadays usage of pesticides is very common

Table 1: Estrogen level of control and experimental groups of Phase I and Phase II

S. No.	Control Group Phase I	Experimental Group Phase I	Control Group Phase II	Experimental Group Phase II
R1	5.83	6.91	5.83	7.01
R2	6.72	7.31	6.72	7.05
R3	7.19	7.7	7.19	7.63
R4	5.97	7.5	5.97	6.51
R5	6.06	4	6.06	6.7
R6	6.89	5.78	6.89	6.6

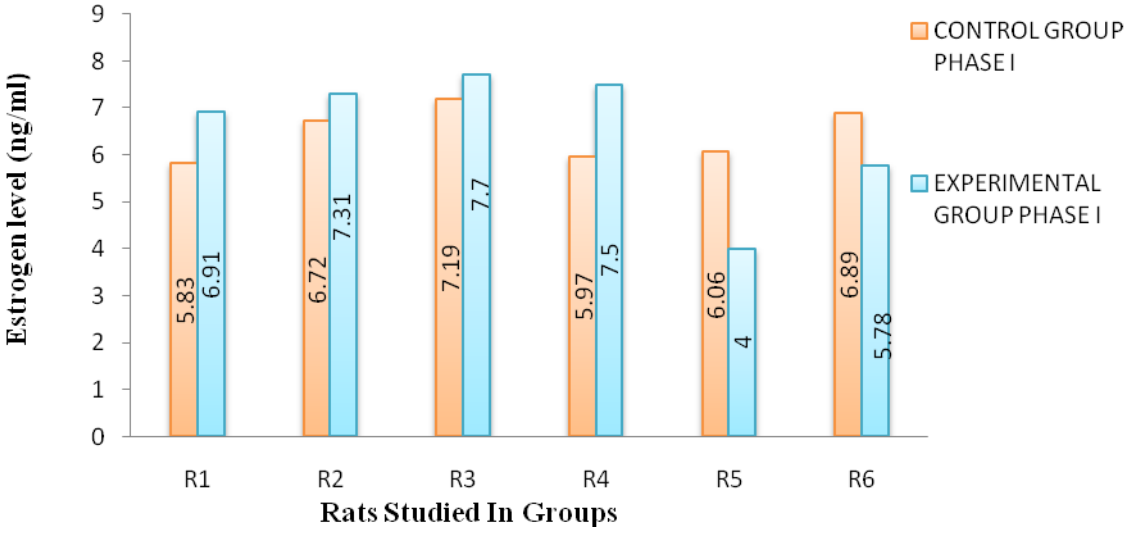

Fig. 1: Estrogen level observed between control and experimental groups of Phase I.

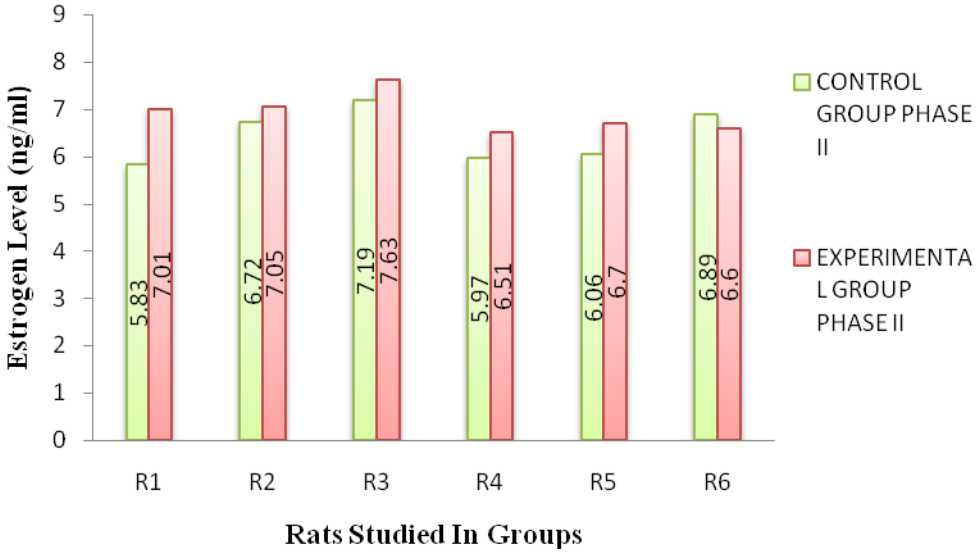

Fig. 2: Estrogen level observed between control and experimental groups of Phase II.

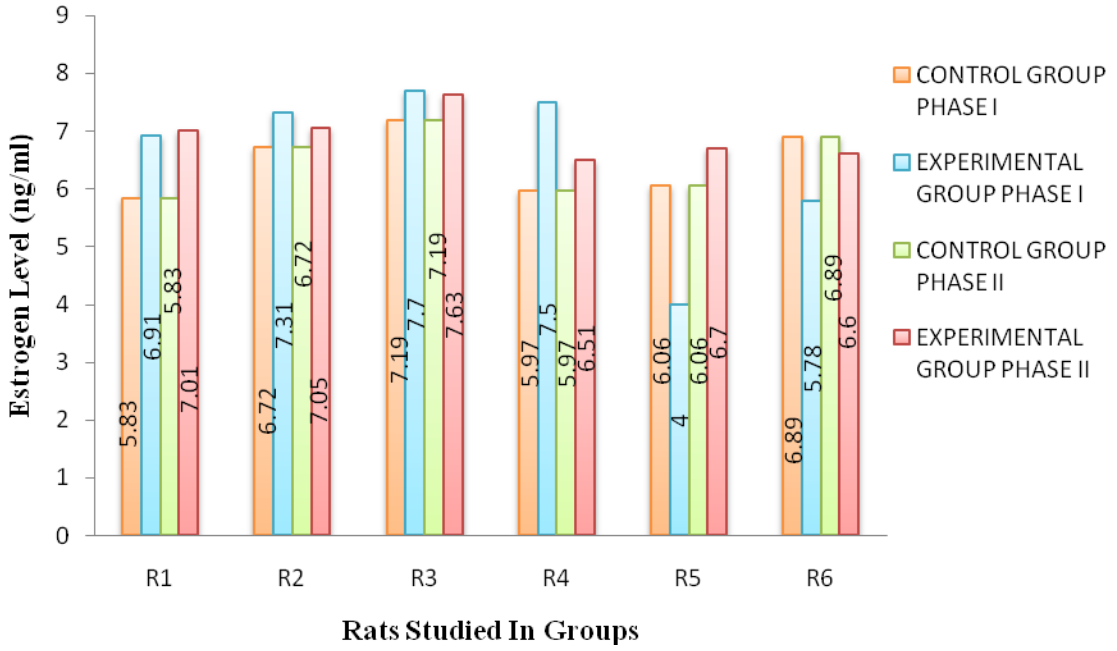

Fig. 3: Comparison of estrogen level observed in control and experimental groups of Phase I and Phase II. P-value: 0.72998 (ns= P>0.05) obtained by one way-ANOVA.

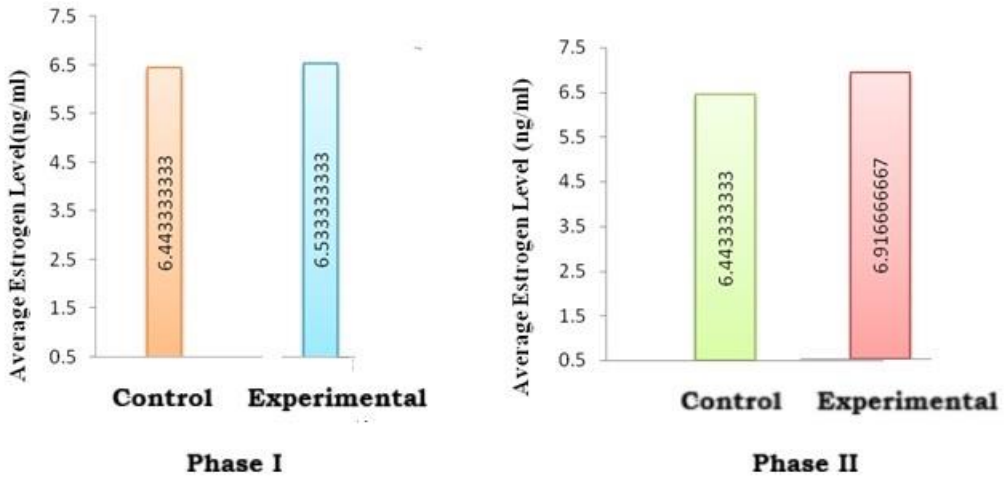

Fig. 4: Average estrogen level difference observed in control and experimental groups of Phase I and Phase II. P > 0.05 for Phase I and Phase II, analyzed by t-test two sample assuming unequal variance.

everywhere some easily reachable or most commonly used category of pesticide is organophosphate (Narayan *et al*., 2013).

The studies suggest that commercial malathion, an organophosphate contains alkylating activities with several impurities (Imamura and Talcott, 1985). These are potent inactivators of malathion carboxyl esterases (Mallipudi *et al*., 1980) and by this it potentiate its toxicity (Pellegrini and Santi, 1972; Umetsu *et al*., 1981).

Scientists have interpreted in various studies the effect of malathion on some hormone levels and tissues secreting these hormones in rats and

found no difference in the analysis of testosterone, T3, T4 and estradiol 17-beta while aldosterone and serum cortisol were decreased in some rats. No histopathological changes were observed in ovaries and thyroid gland in any group (Ozmen and Akay, 1993). Further study done by Prakash *et al.* (1992) interpreted that malathion exposed group for 48 h did not show any significant changes in the plasma FSH or E2 concentrations. Sub-chronic dose of malathion shows adverse effect on the male reproductive system such as it can cause histopathological changes in testis and disrupt the level of sex hormones. Organophosphate did not show estrogenic potential in a study done by Chen *et al.* (2002), this provides support to the observations of the present study.

Ethical Statement

The authors confirm that all ethical issues have been dealt with the research ethics guidelines provided by the Department of Zoology, Dayanand Girls PG College, Kanpur, Uttar Pradesh, India

Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

References

Alengebawy A, Abdelkhalek ST, Qureshi SR and Wang M. (2021) Heavy metals and pesticides toxicity in agricultural soil and plants: ecological risks and human health implications. Toxics *9*(3): 42.

Badr AM. (2020) Organophosphate toxicity: updates of malathion potential toxic effects in mammals and potential treatments. Environ Sci Pollut Res. 27(21): 26036-26057.

Bienertova-Vasku J, Lenart P and Scheringer M. (2020) Eustress and distress: Neither good nor bad, but rather the same?. BioEssays 42(7): 1900238.

Biggs A, Brough P and Drummond S. (2017) Lazarus and Folkman's psychological stress and coping theory. In: The Handbook of Stress and Health: A Guide to Research and Practice, (eds.) Cary L. C. and James C.Q., Wiley. https://doi.org/10.1002/9781118993811.ch21

Bretveld RW, Thomas CM, Scheepers PT, Zielhuis GA and Roeleveld N. (2006) Pesticide exposure: the hormonal function of the female reproductive system disrupted?. Reproductive Biol Endocrinol. 4: 30.

Chen H, Xiao J, Hu G, Zhou J, Xiao H and Wang X. (2002) Estrogenicity of organophosphorus and pyrethroid pesticides. J Toxicol Environ Hlth Part A 65(19): 1419-1435.

Chrousos GP. (2009) Stress and disorders of the stress system. Nature Rev Endocrinol. 5(7): 374-381.

Dar T, Radfar A, Abohashem S, Pitman RK, Tawakol A and Osborne MT. (2019) Psychosocial stress and cardiovascular disease. Curr Treatment Options Cardiovascular Med. 21(5): 23.

De Cock J, Westveer K, Heederik D, Velde ET and Van Kooij R. (1994) Time to pregnancy and occupational exposure to pesticides in fruit growers in The Netherlands. Occupational Environ Med. 51(10): 693-699.

Dickerson SS and Kemeny ME. (2004) Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research. Psychological Bull. 130(3): 355-391.

Dinca V, Docea AO, Drocas AI, Nikolouzakis TK, Stivaktakis PD, Nikitovic D, Golokhvast KS, Hernandez AF, Calina D and Tsatsakis A. (2023) A mixture of 13 pesticides, contaminants, and food additives below individual NOAELs produces histopathological and organ weight changes in rats. Arch Toxicol. 97(5): 1285-1298.

Epel ES, Crosswell AD, Mayer SE, Prather AA, Slavich GM, Puterman E and Mendes WB. (2018) More than a feeling: A unified view of stress measurement for population science. Front Neuroendocrinol. 49: 146-169.

Erthal-Michelato RP, Frigoli GF, de Aquino AM, Punhagui-Umbelino APF, Scarano WR and Fernandes GSA. (2024) Low doses of malathion impair ovarian, uterine, and follicular integrity by altering oxidative profile and gene expression of rats exposed during the peripubertal period. Environ Sci Pollut Res Int. 31(14): 21721-21736.

Farr SL, Cooper GS, Cai J, Savitz DA and Sandler DP. (2004) Pesticide use and menstrual cycle characteristics among premenopausal women in the agricultural health study. American J Epidemiol. 160(12) 1194-1204.

Flehi-Slim I, Boughattas S, Belaïd-Nouira Y, Sakly A, Neffati F, Najjar MF, Haouas Z and Ben Cheikh H. (2016) Toxicological effects of 30-day intake of

malathion on the male reproductive system of Wistar rats. J Food Quality Hazards Control 3: 152-156.

Hamid A, Yaqub G, Ayub M and Naeem M. (2020) Determination of malathion, chlorpyrifos, λ-cyhalothrin and arsenic in rice. Food Sci Technol. 41(2): 461-466.

Hammen C, Kim EY, Eberhart NK and Brennan PA. (2009) Chronic and acute stress and the prediction of major depression in women. Depression Anxiety 26(8): 718-723.

Imamura T and Talcott RE. (1985) Mutagenic and alkylating activities of organophosphate impurities of commercial malathion. Mutation Research/ Genetic Toxicol. 155(1-2): 1-6.

Jain D, Verma RK, Sharma V, Kaur A, Rai AR, Kumari P, Nagar V, Sankhla MS and Parihar K. (2023) Associations between high levels pesticide and adverse reproductive outcomes in females: A comprehensive review. Materials Today Proc. 95: 50–60.

James AA and OShaughnessy KL. (2023) Environmental chemical exposures and mental health outcomes in children: a narrative review of recent literature. Front Toxicol. 5: 1290119.

Karuppaiah G, Lee M, Bhansali S and Manickam P. (2023) Electrochemical sensors for cortisol detection: Principles, designs, fabrication, and characterisation. Biosensors Bioelectronics 239: 115600.

Lupien SJ, McEwen BS, Gunnar MR and Heim C. (2009) Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nature Rev Neurosci. 10(6): 434-445.

Malathion Technical Fact sheet. (n.d.-b). https://npic.orst.edu/factsheets/archive/malatech.html#:~:text=Malathion%20is%20a%20colorless%20to,%2D%20or%20garlic%2Dlike%20odor.&text=Vapor%20pressure2%2C3%2C4,be%20estimated%20or%20derived%20experimentally.

Mallipudi NM, Talcott RE, Ketterman A and Fukuto TR. (1980) Properties and inhibition of rat malathion carboxylesterases. J Toxicol Environ Hlth. 6(3): 585-596.

Manisalidis I, Stavropoulou E, Stavropoulos A and Bezirtzoglou E. (2020) Environmental and health impacts of air pollution: A review. Front Public Health 8: 14.

Metreveli L and Japaridze K. (2022) Stress as an integral part of our life. European Scientific J. 9: 812.

Narayan S, Liew Z, Paul K, Lee PC, Sinsheimer JS, Bronstein JM and Ritz B. (2013) Household organophosphorus pesticide use and Parkinson's disease. Int J Epidemiology 42(5): 1476-1485.

National Academies Press (US). (1983). Food additives, contaminants, carcinogens, and mutagens. In: Diet, Nutrition, and Cancer: Directions for Research. https://www.ncbi.nlm.nih.gov/books/NBK216714/

Nicolopoulou-Stamati P, Maipas S, Kotampasi C, Stamatis P and Hens L. (2016) Chemical pesticides and human health: The urgent need for a new concept in agriculture. Front Public Health 4: 148.

Onyeaka H, Ghosh S, Obileke K, Miri T, Odeyemi OA, Nwaiwu O and Tamasiga P. (2023) Preventing chemical contaminants in food: Challenges and prospects for safe and sustainable food production. Food Control 155: 110040.

Ozmen G and Akay MT. (1993) The effects of malathion on some hormone levels and tissues secreting these hormones in rats. Vet Hum Toxicol. 35(1): 22-24.

Pellegrini G and Santi R. (1972) Potentiation of toxicity of organophosphorus compounds containing carboxylic ester functions toward warm-blooded animals by some organophosphorus impurities. J Agricult Food Chem. 20(5): 944-950.

Prakash N, Narayana K, Murthy GS, Moudgal NR and Honnegowda (1992) The effect of malathion, an organophosphate, on the plasma FSH, 17 beta-estradiol and progesterone concentrations and acetylcholinesterase activity and conception in dairy cattle. Vet Hum Toxicol. 34(2): 116-119.

Rani L, Thapa K, Kanojia N, Sharma N, Singh S, Grewal AS, Srivastav AL and Kaushal J. (2020) An extensive review on the consequences of chemical pesticides on human health and environment. J Cleaner Production 283: 124657.

Rasheed T, Bilal M, Nabeel F, Adeel M and Iqbal HM. (2018) Environmentally-related contaminants of high concern: Potential sources and analytical modalities for detection, quantification, and treatment. Environ Int. 122 : 52-66.

Stress - Anxiety UK. (2021) https://www.anxietyuk.org.uk/anxiety-ype/stress/#:~:text=Internal%20stressors%20are%20the%20sources,low%20self%20esteem%20and%20apprehensions.

Umetsu N, Mallipudi NM, Toia RF, March RB and Fukuto TR. (1981) Toxicological properties of phosphorothioate and related esters present as impurities in technical organophosphorus insecticides. J Toxicol Environ Hlth. 7(3-4): 481-497.

Wheaton B. (1994) Sampling the Stress Universe. In: Stress and Mental Health. The Springer Series on Stress and Coping, (eds.) Avison W.R. and Gotlib I.H., Springer, Boston, MA.