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Therapeutic Effects of Ascorbic Acid on Hormonal and Histological Alteration Produced in The Reproductive System of Albino Rats Intoxicated by Herbicide Atrazine

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ABSTRACT
The aim of this study was to examine the cytotoxicity of the herbicide atrazine on the reproductive system. 48 male and 48 female albino rats were treated with atrazine daily for two different durations (15 and 30 days). Reproductive system toxicity was monitored by quantitative analysis of the serum Follicle-stimulating hormone (FSH), Luteinizing hormone (LH), Prolactin (PRL), Estrogen (E2), Progesterone (Prog) and Testosterone (Testo). On the other hand, the reproductive organs were collected for histopathology study. The study showed a significant elevation of estrogen, progesterone hormones with a significant decrease in testosterone hormone in male groups while in female groups there was a significant decrease in estrogen, progesterone & hormones with a significant increase in testosterone hormone. But there was no effect on PRL and LH hormones in both male and female groups intoxicated by ATZ, in comparison to the control groups. In addition to that, the Light microscopic examination of the seminiferous tubules (st) showed vacuolations within seminiferous tubules (V), degeneration of spermatozoa formation and hemorrhage (hg) in the interstitial tissue. These effects were increased by increasing the dose or the time of exposure. By using ascorbic acid in the treatment of those effects, we find a significant improvement and detoxification of the atrazine effects on both hormonal tests and histological sections. From our study results, we concluded that there is a potential contribution of herbicide mixtures in the etiology of somebody's diseases, while ascorbic acid has beneficial effects as it tends to dampen atrazine toxicity, in albino rats.

INTRODUCTION
Atrazine exposure interferes with normal meiosis, which affects spermatozoa production in male mice. Oxidative stress and disruptions in calcium homeostasis play an important role in the induction of immune-toxicity in mice by atrazine as depicted by (Gao S., 2016) The prime target of chlorinated atrazine on humans and mammals is the disruption of the endocrine system (Jin Y., 2014); (Kroon F.J., 2014); (Weber G.J., 2013). Secondly, it also induces oxidative stress by the formation of reactive oxygen species resulting in the reduced semen quality, sperm dysfunction and infertility of amphibians, rats and pigs (Gely-Pernot A., 2015); (Jestadi D.B., 2014); (Kniiewald J. J. M., 2000). Atrazine is known to cause hepatic
damage, as the liver is the primary organ for atrazine metabolism in mammals (Campos-Pereira F.D., 2012); (Gojmerac T., 1989). Atrazine is also responsible for cardiotoxicity in mice by disruption of ionic balance (Lin J. L. H., 2016a) (Lin J. L. H., 2016b). A considerable number of antioxidants as ascorbic acid and vitamin E have been tested in lab animals to minimize the clastogenicity induced by drugs (Antunes L.M., 1998), (Siddique YH, 2005). Antioxidant vitamins are able to inactivate highly reactive molecules, such as free radicals, which are generated during various biochemical processes in the cells (Costa W.F., 2006).

Atrazine (6-chloro-N-ethyl-N‘-(1-methylethyl)-1,3,5-triazine-2,4-diamine) is a synthetic triazine herbicide used to control grassy and broadleaf weeds in sugarcane, wheat, conifers, sorghum, nuts and corn crops (Iriel A., 2014); (Kumar A., 2016); (Zhao X., 2017). It is the second most widely consumed pesticide in the world with annual consumption of about 70,000–90,000 tons (Kumar V., 2013); (Cheng M., 2016). Because of its long half-life of 41–231 days (Karlsson A.S. W. L., 2016), low adsorption in soils and moderate aqueous solubility, it has a tremendous potential to contaminate not only agricultural areas but also ground and surface water with the highest concentration up to 30 µg/L (Cerejeira M.J., 2003); (Schwab A.P., 2006); (Kumar V., 2013). It was banned in several countries like Italy (Huang H., 2009), Denmark (Glesnner N., 2014), Finland and Germany (Vonberg D., 2014). Atrazine has been classified as an endocrine-disrupting pesticide by the US Environmental Protection Agency (Morales-Pérez A.A., 2016). The International Agency for Research on Cancer (IARC) has categorized atrazine in the list of carcinogenic pesticides (Mahler B.J., 2017)

Exposure to atrazine affects both germ cells as reduced motility and sperm counts in male rats (Victor-Costa A.B., 2010), (Pogrmic K., 2009) Atrazine supposedly increases aromatase enzyme activity via inhibition of phosphor-di-esterase, which increases the aromatization of testosterone to estrogen (Hayes T.B., 2006); (Cooper R.L., 2007). According to (Gely-Pernot A., 2015), atrazine exposure interferes with normal meiosis, which affects spermatozoa production in male mice. ATZ also induces oxidative stress by the formation of reactive oxygen species resulting in the reduced semen quality, sperm dysfunction and infertility of amphibians, rats and pigs (Kumar V., 2013); (Huang H., 2009); (Glesnner N., 2014). Atrazine is known to cause hepatic damage, as the liver is the primary organ for atrazine metabolism in mammals (Vonberg D., 2014); (Morales-Pérez A.A., 2016). Atrazine is also responsible for cardiotoxicity in mice by disruption of ionic balance (Mahler B.J., 2017) (Victor-Costa A.B., 2010). A considerable number of antioxidants as ascorbic acid and vitamin E have been tested in lab animals to minimize the clastogenicity induced by drugs (Pogrmic K., 2009), (Hayes T.B., 2006). Antioxidant vitamins are able to inactivate highly reactive molecules, such as free radicals, which are generated during various biochemical processes in the cells (Cooper R.L., 2007).

### MATERIALS AND METHODS

#### Animals:

All animals in this study were conducted in accordance with the criteria of the investigations and Ethics Committee of the Community Laws governing the use of experimental animals. The animals used in this study were 48 male and 48 female Albino rats, each weighing about 90 ± 10g and 9±1 Weeks old (obtained from Faculty farming and housed in the animal house in a room maintained at 22 ±, 2°C with a 14: 10 hours light: dark respectively schedule (lights on at 0500 h: lights out at 1900 h). Food and water were provided ad libitum. In animal house; faculty of Science Al Azhar University. In accordance
with the guidelines of the ethical committee for research on laboratory animals (National Research Council., 1996)

**Chemicals and Reagent:**

Atrazine (organo-chlorine herbicide) with 80% purity is the chemical material used for the toxicity tests. Atrazine (C8H14ClN5; 6-chloro-4-N-ethyl-2-N-propan-2-yl-1, 3, 5-triazine-2,4-diamine) is a commonly used chemical in farms in Egypt and worldwide for controlling weeds. The toxicity was induced by oral gavage tube (150 & 300 mg/kg) daily for (15 & 30) respectively consecutive days. Ascorbic acid (Vit C) from the International Company for Scientific and Medical Supplies at Cairo, Egypt, was evaluated for its antioxidant effect in a dose of (200 mg /Kg) for 15 and 30 days.

**Experimental Design:**

The animals, male and female albino rats, were randomly divided into 6 equal groups and labeled as groups 1, 2, 3, 4, 5 & 6 and each group contains 8 rats. Rats received all treatments daily via oral gavage tube along the period of the experiment. Atrazine was given in two doses: low dose (L) = 150 mg/kg and high dose (H) = 300 mg/kg, while ascorbic acid was given in a dose of (200 mg/Kg).

Group (1a) contains the control male rats, Group (1b) contains the control female rats, Group (2a) contains male rats were given Vit-C (200 mg/Kg) only, Group (2b) contains female rats given Vit-C (200 mg/Kg) only, Group (3a) contains male rats given atrazine in low dose (L), Group (3b) contains female rats given atrazine in low dose (L), Group (4a) contains male rats given atrazine in high dose (H), Group (4b) contains female rats given atrazine in high dose (H), Group (5a) contains male rats given atrazine in low dose (L) and Vit-C, Group (5b) contains female rats given atrazine in low dose (L) and Vit-C, Group (6a) contains male rats were given atrazine in high dose (H) and Vit-C, Group (6b) contains female rats given atrazine in high dose (H) and Vit-C. The animals were observed daily for signs of atrazine toxicity during the period of the experiment.

**Sample Collection:**

The animals were sacrificed after the treatments. Whole blood was taken on a vacutainer to separate the serum two separate times; firstly after two weeks from half animals and finally after four weeks from the rest of the animals and the following tests was performed: (E2, PRG, Testosterone, PRL, FSH and TSH). Then a section was performed in gonads to examine the changes in their structure.

**Biochemical Assay:**

Estimation of serum hormone level: coagulated blood samples were centrifuged for 15 min at 4000 rpm. Sera were separated for assessment of testosterone concentration by using ELISA kits. TSH was determined according to the method described by (Beck, 1986) and (Caldwell, 1985). Testosterone level was determined according to the method described by (Kim, 2012). PRL was investigated according to the method described by (Cooper R. e., 2000). LH was determined according to the method described by (Cooper R. J., 1999). Prog was determined according to the method described by (Cooper R.L., 1996). E2 was investigated according to the method described by (Connor, 1996).

**Histological Examination:**

H&E staining (Bancroft JD, 2008) and Immune-histo-chemical staining for detection of Bcl-2: the primary antibody used was mouse monoclonal Bcl-2 oncoprotein (N1587; Dako Corporation, Glostrup, Denmark). Bcl-2 was indicated by brown cytoplasmic staining. An immunohistochemical study was conducted using the avidin-biotin-peroxidase method. Paraffin sections were deparaffinized, rehydrated in descending grades of alcohol, and incubated overnight with the primary monoclonal antibody. Sections were rinsed three times with PBS, then incubated for 1 h with peroxidase-conjugated secondary antibody, and washed three times with PBS. The reaction was developed with 0.05% di-amino-benzidine
(Dakopatts, Glostrup, Denmark) as the substrate for peroxidase, and finally, the slides were counterstained with Mayer's hematoxylin. Negative control slides were prepared by replacing the primary antiserum with PBS. A tonsil slide was used as a positive control for Bcl-2 (Kiernan, 2008).

**Statistical Analysis:**

The statistical package for social sciences SPSS/PC computer program (version 19) was used for statistical analysis of the results. Data were analyzed using one-way analysis of variance (ANOVA). The data were expressed as mean ± S.E. Differences were considered statistically significant at (P < 0.05).

### RESULTS

**Biochemical Examination:** data found in (Table 1)

A non-significant effect of different doses (low and high) of atrazine on PRL hormone in male and female rats

A non-significant effect of different doses (low and high) of atrazine on LH hormone in male and female rats while there is a significant decrease in FSH level in male and female rats exposed to a low dose of atrazine on both 15 & 30 days periods and showed more decrease in female rats exposed to a high dose of atrazine.

A significant decrease (p < 0.05) in both estrogen and progesterone hormones in female rats exposed to a low dose of atrazine on both 15 & 30 days periods and showed more decrease in female rats exposed to a high dose of atrazine, while in male groups (table3) showed a significant increase (p > 0.05) in both estrogen and progesterone hormones when exposed to a low dose of atrazine on both 15 & 30 days periods and more increase in hormones level in males exposed to a high dose of atrazine.

On the other hand, data found in Table 1 showed a significant increase in testosterone hormone level in female groups which exposed to a low dose of atrazine on both 15 & 30dayes periods and more increase in hormone level in female groups which exposed to a high dose of atrazine on both 15 & 30dayes periods. While data showed a significant decrease in hormone level in male groups exposed to a low dose of atrazine on both 15 & 30 days periods and more decrease in groups exposed to the high dose on both 15 & 30 days periods.

As regards, ascorbic acid effect, used as an antidote to atrazine in this study, (Tables 1, 2, 3 & 4) showed a significant decrease of atrazine toxicity in female & male rates but it doesn’t eliminate the effect of atrazine completely. Yet with prolonged administration of ascorbic acid, the antidote effect became more prominent and atrazine toxicity became less obvious.

**Histological Examination:**

It showed gonads of males (Plate 1) (400x) in their normal structure in control groups (Fig. A) and groups exposed to ascorbic acid only (Fig. B), so we considered them as control groups. In male rats treated with a low dose of atrazine the photomicrograph of testicular tissue. Examination showed vacuolations within seminiferous tubules (V), degeneration of spermatozoa and hemorrhage in interstitial tissue (hg) in the group which treated for 15 days (Fig C.). While in the group treated for 30 days, the hemorrhage increased and atrophies appeared. (Fig. D).

In male rats treated with a high dose of atrazine the photomicrograph of testicular tissue: Examination showed degeneration of seminiferous tubule cells, wide gaps between neighboring cells and loss of spermatozoa & spermatids (arrowhead). Note the degeneration of sertoli cells (arrow) in the group treated for 15 days (Fig. E), while the group treated for 30 days showed degeneration of seminiferous tubule cells, abnormal spermatogenesis, loss of seminiferous tubule sheath, wide gaps between neighboring cells and interstitial tissues showed edema, hemorrhage and vacuolations (IT), (Fig. F).
### Table 1: Serum E2, PROG, and Testo, level (U/L) in adult female albino rats subjected to different treatment conditions for 15 and 30 days.

|        | Female | Control | Vit C | ATZ (L) | ATZ (H) | ATZ (L) + VitC | ATZ (H) + VitC |
|--------|--------|---------|-------|---------|---------|---------------|---------------|
|        | E2     |         |       |         |         |               |               |
| 15 days| 6.0±0.4a| 6.0±0.2a | 5.5±1.3a,b | 4.5±0.5a,b | 5.9±0.7a | 5.0±0.2a,b |               |
| 30 days| 5.9±0.4a | 5.9±0.3a | 5.0±0.6a,b | 4.0±0.2b | 5.4±0.6a,b | 4.5±0.4a,b |               |
|        | Prog.  |         |       |         |         |               |               |
| 15 days| 17.8±2.2a | 15.9±0.9a,b | 11.2±0.6b,c,e | 6.5±0.4c,d | 8.2±0.2c,d | 16.9±4.0a |               |
| 30 days| 16.8±0.4a | 18.3±1.3a | 9.4±0.9c | 3.8±0.6d | 9.8±0.2c | 15.4±0.5a,e |               |
|        | Testo  |         |       |         |         |               |               |
| 15 days| 6.2±0.6a | 5.9±0.7a,b | 3.6±0.3c,e | 3.1±0.4e | 4.0±0.6e | 3.1±0.5e |               |
| 30 days| 4.6±0.1b | 5.6±0.4a,b,d | 4.0±0.4c,e | 5.2±0.2a,b | 4.3±0.4c,d | 5.4±0.5a,b |               |

Each value represented means of 4 records ± S.E.

ab,cd means comparison between all groups which the groups have the same letter mean there is no significance ± difference and which have different letter mean there is a significance ± change.

### Table 2: Serum FSH, LH and PRL level (U/L) in adult female albino rats subjected to different treatment conditions for 15 and 30 days.

|        | Female | Control | Vit C | ATZ (L) | ATZ (H) | ATZ (L) + VitC | ATZ (H) + VitC |
|--------|--------|---------|-------|---------|---------|---------------|---------------|
|        | FSH    |         |       |         |         |               |               |
| 15 days| 20.6±1.8a | 26.6±0.9bce | 20.6±0.2a,f | 19.0±0.7f | 23.7±0.5a,e | 23.3±0.5a |               |
| 30 days| 23.0±0.3a | 28.2±3.1c | 19.3±1.0d | 20.5±1.2a,f | 23.9±1.0a,e | 22.0±1.0a |               |
|        | LH     |         |       |         |         |               |               |
| 15 days| 7.0±0.7a,c | 9.0±0.5abc | 8.7±0.1abce | 8.0±0.1abce | 6.8±0.6c | 9.2±0.5a,b |               |
| 30 days| 7.1±0.3a | 9.2±0.1a,b | 7.8±0.6abc | 8.5±0.1abce | 9.8±0.2b | 6.5±0.9c |               |
|        | PRL    |         |       |         |         |               |               |
| 15 days| 316.0±39.1abc | 389.3±15.6b | 356.0±14.1abe | 272.0±4.3edg | 216.5±6.9d | 338.5±15.0abcg |               |
| 30 days| 302.0±9.9a,c | 390.0±2.2b | 368.0±3.4abe | 335.0±3.8abg | 344.5±3.2abcg | 288.0±45.3adeg |               |

Each value represented means of 4 records ± S.E.

ab,cd means comparison between all groups which the groups have the same letter mean there is no significance ± difference and which have different letter mean there is a significance ± change.

### Table 3: Serum E2, PROG, and Testo, level (U/L) in adult male albino rats subjected to different treatment conditions for 15 and 30 days.

|        | Male | Control | Vit C | ATZ (L) | ATZ (H) | ATZ (L) + VitC | ATZ (H) + VitC |
|--------|------|---------|-------|---------|---------|---------------|---------------|
|        | E2   |         |       |         |         |               |               |
| 15 days| 4.10±0.5a | 4.18±1.1a | 8.05±0.3b,c | 10.0±0.7cdef | 7.02±0.3e | 9.55±0.5d,g |               |
| 30 days| 4.13±0.4a | 4.13±0.5a | 9.00±0.1b,d | 11.2±0.2cdef | 8.50±0.3bg | 10.5±0.2f,g |               |
|        | Prog. |         |       |         |         |               |               |
| 15 days| 0.47±0.1a | 0.78±0.1a | 1.50±0.2b | 5.9±0.4d | 6.8±0.1a | 0.1±0.1a |               |
| 30 days| 0.39±0.1a | 0.71±0.1a | 2.70±0.4c | 6.0±0.4d | 0.70±0.1a | 0.3±0.1a |               |
|        | Testo |         |       |         |         |               |               |
| 15 days| 42.8±1.7a | 43.8±1.0a | 19.5±3.4b | 5.4±0.6d,e | 26.8±3.6f | 9.1±1.0g,e |               |
| 30 days| 39.3±2.6a | 41.8±2.6a | 13.0±1.6c,g | 4.1±0.6d,e | 15.1±1.5b,c | 5.9±0.6e |               |

Each value represented means of 4 records ± S.E.

ab,cd means comparison between all groups which have the same letter mean there is no significance ± difference and which have different letter mean there is a significance ± change.

### Table 4: Serum FSH, LH and PRL, level (U/L) in adult male albino rats subjected to different treatment conditions for 15 and 30 days.

|        | Male | Control | Vit C | ATZ (L) | ATZ (H) | ATZ (L) + VitC | ATZ (H) + VitC |
|--------|------|---------|-------|---------|---------|---------------|---------------|
|        | FSH  |         |       |         |         |               |               |
| 15 days| 25.3±0.5bce | 27.9±1.0be | 26.6±3.5b,e | 20.6±1.0aced | 24.3±0.2abc | 24.5±0.1bde |               |
| 30 days| 27.4±2.1b,e | 28.5±0.7b | 20.9±0.9aced | 19.0±1.9e | 29.0±4.5e | 23.1±0.8a,b |               |
|        | LH    |         |       |         |         |               |               |
| 15 days| 5.7±0.1a,b | 7.1±0.6b,e | 8.1±0.1c,d | 7.6±0.2e | 8.1±0.4c,d | 8.2±0.1c,d |               |
| 30 days| 5.8±1.0a,b | 7.1±0.5abc | 8.4±0.2c | 7.1±0.5abc | 9.2±0.3d | 7.8±0.1c,d |               |
|        | PRL   |         |       |         |         |               |               |
| 15 days| 328.0±10.7a | 347.0±14.3a | 356.0±4.1a | 364.0±24.3a | 315.0±46.1a | 362.0±20.0a |               |
| 30 days| 326.0±22.3a | 359.0±18.9a | 366.3±5.5a | 372.0±42.9a | 325.3±21.7a | 344.0±2.1a |               |

Each value represented means of 4 records ± S.E.

ab,cd means comparison between all groups which have the same letter mean there is no significance ± difference and which have different letter mean there is a significance ± change.

Ascorbic acid as an antidote to atrazine effect in male groups was examined by (400x) in this study:

In male rats treated with a low dose of Atrazine in combination with ascorbic acid: showed necrosis (N) in the seminiferous tubules, deformation in the spermatocytes (arrowhead), with vacuolation (V) and hemorrhage (hg) in interstitial cells in the group
treated for 15 days (Fig. G). While the group treated for 30 days showed normal seminiferous tubule architecture with normal appearance of seminiferous tubule sheath (arrowhead), spermatogonia (SG), spermatocytes (SC), spermatids (ST) and spermatozoa (SP) (Fig. H).

In male rats treated with a high dose of Atrazine in combination with ascorbic acid: showed congestion of blood in the entire seminiferous tubule (arrowhead) and increase in vacuolation (V) and deformation of spermatogenesis. Note atrophy and hemorrhage in interstitial tissue in the group treated for 15 days (Fig. I). While the group treated for 30 days showed recovery of spermatogenesis and well-developed seminiferous tubule sheath (Fig. J).

Histological examination of female gonads (Plate 2) showed normal structure in control groups by using (400x) showed pre-ovulatory follicle with mature oocyte with normal nucleus (N) surrounded by granulosa cells (GC) with normal zona pellucida (zp). (Fig. A). And groups exposed to ascorbic acid only showed normal ovarian structure like that of the control group (Fig. B), so we considered them as control groups.

In female rats treated with a low dose of atrazine the photomicrograph of the ovarian section: showed reduced Grafian follicle (GF) number and size, deformed oocyte (Oo) with degenerated nucleus (N) and vacuolated (V) zona granuloza in the group treated for 15 days (Fig. C). While the group treated for 30 days showed atretic follicles (AF) with vacuolated (V) zona granuloza and blood congestion (cg) (Fig. D).

In female rats treated with a high dose of atrazine the photomicrograph of ovarian section: showed atretic follicles (AF) with vacuolation (V) and blood vessel congestion (cg) in corpus luteum in the group treated for 15 days (Fig. E). While the group treated for 30 days showed Grafian follicle (GF) with mature oocyte (Oo) with a degenerative nucleus, well-formed zona pelucida (double arrowheads) and deformed zona granuloza (arrowhead). Note vacuolated corpus luteum (arrows) and hemorrhage in interstitial cells (hg) (Fig. F).

**Ascorbic Acid as An Antidote to Atrazine Effect in Female Groups in This Study:**

In female rats treated with a low dose of Atrazine in combination with ascorbic acid: showed some recovery of the follicular tissue; Oocyte (Oo) within Grafian follicle (GF) and recovery of zona granuloza in the group treated for 15 days (Fig. G). While the group treated for 30 days showed atretic oocytes within vacuolated follicles (V) and hemorrhage in the interstitial tissue (hg). (Fig.H).

In female rats treated with a high dose of Atrazine in combination with ascorbic acid: showed atretic follicles (AF) with vacuolated (V) zona granuloza, Corpus luteum (CL) showing degenerative luteal cells with vacuolations in the group treated for 15 days (Fig. I). While the group treated for 30 days showed atretic follicles (AF) with vacuolated (V) zona granuloza, lymphocytes infiltrations (LI) and blood vessel congestion (cg) (Fig. J).
Plate 1: Photomicrograph of testicular tissue of male albino rat 400X subjected to different treatment condition.

(Fig. A) control
(Fig. C) low ATZ after 15 days
(Fig. E) high ATZ after 15 days
(Fig. G) low ATZ & Vit C after 15 days
(Fig. I) high ATZ & Vit C after 15 days

(Fig. B) Vit C
(Fig. D) low ATZ after 30 days
(Fig. F) high ATZ after 30 days
(Fig. H) low ATZ & Vit C after 30 days
(Fig. J) high ATZ & Vit C after 30 days
Plate 2: Photomicrograph of ovary of female albino rate 400X subjected to different treatment condition.
(Fig. A) control  
(Fig. C) low ATZ after 15 days  
(Fig. E) high ATZ after 15 days  
(Fig. G) low ATZ & Vit C after 15 days  
(Fig. I) high ATZ & Vit C after 15 days  
(Fig. B) Vit C  
(Fig. D) low ATZ after 30 days  
(Fig. F) high ATZ after 30 days  
(Fig. H) low ATZ & Vit C after 30 days  
(Fig. J) high ATZ & Vit C after 30 days
DISCUSSION

The results showed that after treatment with both low and high doses of atrazine during 15 & 30 days periods, there is a significant decrease in FSH level in male rates, similar to (Mokhtari, 2010) and inconsistent with (Yang, 2014), also showed a significant decrease in FSH level in female rates similar to (Bohn T., 2011) but our results are inconsistent with (Bohn T., 2011) in results of LH level where he found a decrease in LH level and our results didn't show any difference in the hormone level with a control group.

Results showed a significant decrease in both Estrogen and progesterone level on treatment with both low and high doses of atrazine in female groups during 15 & 30 days periods, although this result is inconsistent with (Taketa Y., 2011) who found that ATR induces luteal cell Hypertrophy and increases progesterone production, and this caused by newly formed corpora lutea in female rats, while in male groups Our results showed a significant increase in both Estrogen and progesterone level on treatment with both low and high doses of atrazine during 15 & 30 days periods as (Hayes T. B., 2011) who studied the evidence for atrazine as an endocrine disruptor that demasculinizes and feminizes the gonads of male vertebrates.

Results showed a significant decrease in testosterone level on both low and high doses of atrazine male groups during 15- & 30-days periods, similar to (Kniewald, 1979); (Babic-Gojmerac, 1989) & (Yang, 2014). And this is caused by the reduction of the number of testosterone receptors in the prostate gland by ATZ exposure (Kniewald et al., 1995).

The study showed that no significant effect of ATR on the level of prolactin level in both male and female groups, and this is inconsistent with the results of previous studies (Stoker T. E., 1999). That may be explained that previous studies were done on the period of lactation when the physiological level of prolactin is normally high because of active mammary glands which became more active on ATR use.

Effect of Ascorbic Acid:

The study showed that using ascorbic acid caused improvement in the level of (E2, PROG, TESTO, FSH and LH), although didn’t reach the normal value, in all groups treated with atrazine via capturing the free radicals released from herbicide reaction with these hormones, as similar to (Lin J L. H., 2016a), (Lin J L. H., 2016b), (Antunes LM, 1998), (Siddique YH, 2005) and (Costa WF, 2006).

REFERENCES

A. Donna, e. a. (1989). “Triazine Herbicides and Ovarian Epithelial Neo plasms,”. Scandinavian Journal of Work and Environmental Health, 15, 47-53.

Ahrens, W. (1994). Herbicide Handbook (7th Ed ed.). Champaign, IL: Weed Science Society of America.

Allran, J. a. (2001). Effects of atrazine on embryos, larvae, and adults of anuran amphibians. Journal of Environmental Toxicology and Chemistry, 20(4), 769–775.

Antunes LM, T. C. (1998). Effects of high doses of vitamins C and E against doxorubicin-induced chromosomal damage in Wistar rat bone marrow cells. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 419(1-3), 137–143.

Babic-Gojmerac. (1989). Testosterone metabolism in neuroendocrine organs in male rats under atrazine and deethylatrazine influence. Journal of steroid biochemistry, 33(1), 141-146.

Bagchi D, B. M. (1995). In vitro and in vivo generation of reactive oxygen species, DNA damage and lactate dehydrogenase leakage by selected pesticides. Toxicology, 104, 129–140.
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Bancroft JD, G. M. (2008). Theory and practice of histological techniques. In Churchill Livingstone. (6th ed ed.). New York, London, Philadelphia.

Battaglin, W. A. (2000). Occurrence of sulfonylurea, sulfonamide, imidazolinone, and other herbicides in rivers, reservoirs and ground water in the Midwestern United States 1998. Science of the Total Environment, 248(2-3), pp. 123-133. Retrieved from (2000). Occurrence of sulfonylurea, sulfonamide, imidazolinone, and other herbicides in rivers, reservoirs and ground water in the Midwestern United States, Science of the Total Envi.

Beck, J. R. (1986). Laboratory decision science applied to chemometrics: strategic testing of thyroid function. Clinical chemistry, 32(9), 1707-1713.

Berger, S. a. (1976). Fund. Clin. Chem., N. W. Tietz (ed.), W. B. Saunders Co., 14, 824-848.

Blaustein, A. R. (2002). Complexity in conservation: lessons from the global decline of amphibian populations. Ecology letters, 5(4), 597-608.

Bohn T, C. E. (2011). Determination of atrazine and degradation products in Luxembourgish drinking water: origin and fate of potential endocrine-disrupting pesticides. Food Additives & Contaminants: Part A, 28(8), 1041-1054. doi:10.1080/19440049.2011.580012

Budavarí, S. (1996). The Merck Index (12th Edition ed.). Whitehouse Station, NJ, Merck & Co.

Burtis A., e. a. (1999). Tietz Textbook of Clinical Chemistry (3rd ed AACC ed.).

Cai, y. S. (2003). Antioxidant activity of betalains from plants of the Amaranthaceae. Journal of agricultural and food chemistry, 51(8), 2288-2294.

Caldwell, G. G. (1985). A new strategy for thyroid function testing. The Lancet, 325(8438), 1117-1119.

Campero, M., O. F. (2007). Ecological relevance and sensitivity depending on the exposure time for two biomarkers. Environmental Toxicology: An International Journal, 22(6), pp. 572-581.

Campos-Pereira FD, O. C.-Z.-M.-A. (2012). Early cytotoxic and genotoxic effects of atrazine on Wistar rat liver: a morphological, immunohistochemical, biochemical, and molecular study Ecotoxicology and environmental safety, 78, 170–177.

CancerWeb. (1995-1998). The on-line medical dictionary.

Cerejeira MJ, V. P.-F. (2003). Pesticides in Portuguese surface and ground waters. Water Research, 37(5), 1055–1063.

Cheng M, Z. G. (2016). Degradation of atrazine by a novel Fenton-like process and assessment the influence on the treated soil. J Hazard Materials, 312, 184–191.

Chung, T.-S. (1996). A review of microporous composite polymeric membrane technology for air-separation. Polymers and Polymer Composites, 4 (4): 269-282. ScholarBank@NUS Repository.

Coady, D. G. (2004). Targeting of transfers in developing countries: Review of lessons and experience. Retrieved from The World Bank.

Connor, K. e. (1996). Failure of chloro-s-triazine- derived compounds to induce receptor-mediated responses in vivo and in vitro. Toxicological Sciences, 30, pp. 93-101.

Cooper R.L., e. a. (1996). Effect of atrazine on ovarian function in the rat. Reproductive Toxicology, 10(4), 257-264.

Cooper RL, L. S. (2007). Atrazine and reproductive function: mode and mechanism of action studies. Birth Defects Research Part B: Developmental and Reproductive Toxicology, 80(2), 98–112.

Cooper, R. e. (2000). Atrazine disrupts the hypothalamic control of pituitary-ovarian function. Toxicological sciences, 53(2), 53, pp. 297-307.

Cooper, R. J. (1999). Neuroendocrine and reproductive effects of contemporary-use
Therapeutic Effects of Ascorbic Acid on Hormonal and Histological Alteration Produced in The Reproductive System of Albino Rats

pesticides. *Toxicology and Industrial Health, 15*(1-2),26-36.

Costa Silva, R. G. (2010). Molecularly imprinted silica as a selective SPE sorbent for triazine herbicides. *Journal of separation science, 33*(9),1319–1324.

Costa WF, N. J. (2006). Protective effects of a mixture of antioxidant vitamins and minerals on the genotoxicity of doxorubicin in somatic cells of Drosophila melanogaster. *Environmental and molecular mutagenesis, 47*(1), 18-24.

Devaki, S. J. (2017). Vitamin C: source, function, sensing and analysis. Intech Open. Retrieved from http://dx.doi.org/10.5772/intechopen.70162

Diana, S. R. (2000). Effects of atrazine on amphibian growth and survival in artificial aquatic communities. *Environmental Toxicology and Chemistry, 19*(12), 2961-2967.

Drevenkar V, F. S. (2004). Levels of atrazine and simazine in waters in the rural and urban areas of North-West Croatia. 84, 207-16. doi:10.1080/0306731031000149679

Ellenhorn, M. J., & Barceloux, D. G. (1988). Medical toxicology: diagnosis and treatment of human poisoning. *ELSEVIER, NEW YORK, NY(USA). 1988.*

Ellenhorn, M. S. (1997). *Ellenhorn’s Medical Toxicology: Diagnosis and Treatment of Human Poisoning.* (2nd ed ed.). Williams and Wilkins a Waverly company.

EPA. (1999). California Environmental Protection Agency—Department of Pesticide Regulation: USEPA/OPP Pesticide Product Database Query. Retrieved from Environmental Protection Agency: [http://www.cdpr.ca.gov/docs/epa/m2.htm](http://www.cdpr.ca.gov/docs/epa/m2.htm)

EPA., U. (2000). Report to Congress. Washington, D.C. Retrieved from U.S. EPA. Endocrine Disruptor Screening Program: www.epa.gov/scipoly/oscpendo/index.htm, p. 4.

Eubanks, M. (1997). Hormones and health. *Environment. Health Perspective, 105,* pp. 482-487.

Ezrin, C. (1978). The Thyroid, SC Werner and SH Ingbar. *Harper and Row, Hagerstown MD,* 9, 174-178.

Found, W. E. (1998). "Investigations of Endocrine Disruption in Aquatic Systems Associated with the National Water Quality Assessment (NAWQA) Program.

Gao S, W. Z. (2016). Oral exposure to atrazine induces oxidative stress and calcium homeostasis disruption in spleen of mice. Oxid Med Cellular Longevity.

Gardner, D. F. (1979). Serum thyroglobulin in normal subjects and patients with hyperthyroidism due to graves’disease: effects of T3, iodide, 131 I and antithyroid drugs. *Clinical Endocrinology, 11*(6), 585-594.

Gely-Pernot A, H. C. (2015). The epigenetic processes of meiosis in male mice are broadly affected by the widely used herbicide atrazine. Retrieved from doi: 10.6084/m9.figshare.c.3623711

Giddings, K. S. (2004). Human CD59 is a receptor for the cholesterol-dependent cytolysin intermedilysin. *Nature structural & molecular biology, 11*(12), 1173-1178.

Glæsner, N., Bælum, J., Strobel, B. W., & Jacobsen, C. S. (2014). Ageing of atrazine in manure amended soils assessed by bioavailability to Pseudomonas sp. strain ADP. *Biodegradation, 25*(2), 217-225.

Gojmerac T, K. (1989). Atrazine biodegradation in rats a model for mammalian metabolism. * Bulletin of environmental contamination and toxicology,43*(2), 199–206.

Gojmerac, T. e. (1995). Serum biochemical and histopathological changes related to the hepatic function in pigs following atrazine treatment *Journal of applied toxicology, 15*(3),233-236.

Halliwell B, G. J. (1986). Oxygen free radicals and iron in relation to biology and medicine: Some problems and concepts. *Arch Biochem Biophys, 246,* 501–514.

Hanioka, N. e. (1999). In vitro metabolism of simazine, atrazine, and propazine by hepatic cytochrome P450 enzymes of rat, mouse and guinea pig, and oestrogenic activity of
chlorotriazines and their main metabolites. *Xenobiotica*, 29, pp. 1213-1226.

Hayes, T., Haston, K., Tsui, M., Hoang, A., Haeffele, C., & Vonk, A. (2003). Atrazine-induced hermaphroditism at 0.1 ppb in American leopard frogs (Rana pipiens): laboratory and field evidence. *Environmental health perspectives*, 111(4), 568-575.

Hayes TB, S. A. (2006). Characterization of atrazine-induced gonadal malformations in African clawed frogs (Xenopus laevis) and comparisons with effects of an androgen antagonist (cyproterone acetate) and exogenous estrogen (17beta-estradiol). *Environmental health perspectives*, 114(Suppl 1),134–141.

Hayes, T. B. (2002). Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. *Proceedings of the National Academy of Science*, 99(8), 5476-5480.

Hayes, T. B. (2011). Demasculinization and feminization of male gonads by atrazine: consistent effects across vertebrate classes. *The Journal of steroid biochemistry and molecular biology*, 127(1-2), 64-73.

Hoar, S. e. (1986). “Agricultural herbicide uses and risk of lymphoma and soft-tissue sarcoma. *Journal of American Medical Association*, 256(9), 1141-1147.

Hooghe, R. S.-P. (2000). Effects of selected herbicides on cytokine production in vitro. *Life Sciences*, 66(26) 2519-2525.

Huang H, Z. S. (2009). Influence of Glomus etunicatum/Zea mays mycorrhiza on atrazine degradation, soil phosphatase and dehydrogenase activities, and soil microbial community structure. *Soil Biology and Biochemistry*, 41, 14353-68. doi:10.3390/ijms160714353

IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. (1991). *Occupational exposures in insecticide application, and some pesticides* (Vol. 53). World Health Organization.

Inoue-Choi M, W. P. (2016). Atrazine in public water supplies and risk of ovarian cancer among postmenopausal women in the Iowa Women’s Health Study. *Occupational and environmental medicine*, 73(9), 582-587. doi:10.1136/oemed-2016-103575

Interim Reregistration Eligibility Decision for Atrazine”, 0062 (January 2003).

Jestadi DB, P. A. (2014). Effects of atrazine on reproductive health of nondiabetic and diabetic male rats. *International scholarly research notices*, 13, 1–7.

Jin Y, W. L. (2014). Exposure of mice to atrazine and its metabolite di-amino-chlorotriazine elicits oxidative stress and endocrine disruption. *Environmental toxicology and pharmacology*, 37(2), 782–790.

Karlsson AS, W. L. (2016). Field scale boscalid residues and dissipation half-life estimation in a sandy soil. *Chemosphere*, 145, 163–173.

Kiernan, J. (2008). Histological and histochemical methods: theory and practice. In Cold Spring Harbor Laboratory Press. (4th ed. ed.). Butterworth-Heinemann, Oxoford, UK.

Kim, S. K. (2012). A low level of serum total testosterone is independently associated with nonalcoholic fatty liver disease. *BMC gastroenterology*, 12(1), 69.

Kimbrough, R. A. (1996). Pesticidesin streams draining agricultural and urban areas in
Colorado. *Environmental science & technology*, 30(3), 908-916.

Kniewald et al. (1995). Effect of s-triazine compounds on testosterone metabolism in the rat prostate. *Journal of Applied Toxicology, 15*(3), 215-218.

Kniewald J., E. (1987). Indirect influence of straizines on rat gonadotropic mechanism at early post-natal period. *Journal of steroid biochemistry*, 27(4-6), 10095-1100.

Kniewald J., J. M. (2000). Disorders of male reproductive tract under the influence of atrazine. *Journal of Applied Toxicology: An International Journal, 20*(1), 61–68.

Kniewald J., E. A. (1995). Effect of s-triazine compounds on testosterone metabolism in the rat prostate. *Journal of Applied Toxicology, 15*(3), 215-218.

Kniewald, J., Mildner, P., & Kniewald, Z. (1979). Effects of s-triazine herbicides on hormone-receptor complex formation, 5α-reductase and 3α-hydroxysteroid dehydrogenase activity at the anterior pituitary level. In *Hormonal Steroids* (pp. 833-838). Pergamon.

Kornilovskaya, I. (1994). Thyroid mast cell heterogeneity in rat functional properties in response to the herbicide atrazine in rat. *European Journal of Endocrinology 130* (Suppl. 2), 129. (Abstract.)

Kroon FJ, H. S. (2014). Effects of atrazine on endocrinology and physiology in juvenile barramundi, *Lates calcarifer* (Bloch). *Environmental toxicology and chemistry, 33*(7), 1607–1614.

Kumar A., S. N. (2016). Atrazine and its metabolites degradation in mineral salts medium and soil using an enrichment culture. *Environmental monitoring and assessment, 188*(3), 1–12.

Kumar V., U. N. (2013). Thin-layer chromatography: comparative estimation of soil’s atrazine. *Current World Environment, 8*(3), 469–472.

LeBaron HM, M. J. (2008). The Triazine Herbicides 50 Years Revolutionizing Agriculture (1st ed ed.). Oxford: Elsevie.

Lide, D. (1997). *CRC Handbook of Chemistry and Physics* (78th Ed ed.). Boca Raton, FL, CRC Press.

Lin J., L. H. (2016a). A novel mechanism underlies atrazine toxicity in quails (Coturnix coturnix): triggering ionic disorder via disruption of ATPases. *Oncotarget, 7*(51), 83880.

Lin J., L. H. (2016b). The chemopreventive potential of lycopene against atrazine-induced cardiotoxicity modulation of ionic homeostasis. *Scientific reports, 6*, 24855.

Louis., K. A. (1984). Tryglycerides. *ClinChemThe C.V. Mosby Co. St Toronto. Princeton and Lipids, 437*, pp. 1194-1206.

Mahler BJ, V. M. (2017). Similarities and differences in occurrence and temporal fluctuations in glyphosate and atrazine in small Midwestern streams (USA) during the 2013 growing season. *Science of the Total Environment, 579*, 149-158.

Mokhtari, M. S. (2010). The Effects of Atrazine on Levels of Pituitary–testis Hormones in Adult Male Rat. *Egyptian Academic Journal of Biological Sciences, B. Zoology, 2*(2), 53-60.

Morales-Pérez AA, A. C.-Z. (2016). Removal of atrazine from water using an iron photo catalyst supported on activated carbon. *Adsorption, 22*(1), 49–58.

Mudiam, M. K. (2012). Studies on urban drinking water quality in a tropical zone. *Environmental Monitoring and Assessment, 184*(1), 461-469.

National Research Council Staff, e. a. (1999). Sustaining marine fisheries. . Haworth Press.

National Research Council. Institute of Laboratory Animal Resources. (1996). Guide for the care and use of laboratory animals.". National Academy of Sciences, Washington: DC.

Novartis. (1999). Crop Protection Products[http://www.cp.novartis.com/d_frame.htm].
Abdel salam Youssef et al.

Retrieved from Occupational Safety and Health Administration (1999) OSHA Chemical Sampling Information: [http://www.osha-slc.gov:80/OCIS/toc_chemsamp.html]

NRAAVC. (1997). Review summary on NRA Review Atrazine, Canberra. Retrieved from National Registration Authority for Agricultural and Veterinary Chemicals (NRA).

NTP. (1997). NTP Chemical Repository Data Sheet: Atrazine, Research. Retrieved from National Toxicology Program.

Nwani C.D., L. W. (2010). Toxicity of the herbicide atrazine: effects on lipid peroxidation and activities of antioxidant enzymes in the freshwater fish Channa punctatus (Bloch). *International journal of environmental research and public health*, 7(8), 3298-3312.

Osburn, S. (2001). Research Report: Do Pesticide Cause Lymphoma. *Foundation of America*.

OSU. (1996). Extoxnet (Extension Toxicology Network/Pesticide Information Profiles): Atrazine. Retrieved from Oregon State University: [http://ace.ace.orst.edu/info/extoxnet/pips/atrazine.htm]

Pierce, J. G. (1971). Eli Lilly Lecture: The subunits of pituitary thyrotropin—their relationship to other glycoprotein hormones. *Endocrinology*, 89(6), 1331-1344.

Pogrmic K, F. S. (2009). Atrazine oral exposure of peripubertal male rats downregulates steroidogenesis gene expression in Leydig cells. *Toxicological Sciences*, 111(1), 189-197.

Porter, W. J. (1999). “Endocrine, immune and behav ioral effects of aldicarb (carbam ate), atrazine (triazine) and nitrate (fertilizer) mixtures at groundwater concentrations”. *Toxicology and Industrial Health*, 15, 133-150.

Pucarević M, Š. R. (2002). Atrazine in groundwater of Vojvodina Province. *Water Research*, 36(20), 5120-6. doi:10.1016/S0043-1354(02)00245-2

Ralston-Hooper, K. H.-A. (2009). Acute and chronic toxicity of atrazine and its metabolites deethylatrazine and deisopropylatrazine on aquatic organisms. *Ecotoxicology*, 18(7), pp. 899-905.

Ross, M. K. (2009). Disposition of the Herbicide 2-Chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine (Atrazine) and Its Major Metabolites in Mice: A Liquid Chromatography/Mass Spectrometry Analysis of Urine, Plasma, and Tissue Levels. *Drug Metabolism and Disposition*, 37, pp. 776-786.

Sanderson, J. e. (2000). 2-chloro-s-triazine herbicides induce aromatase (CYP19) activity in H295R human adrenocortical carcinoma cells: A novel mechanism for estrogenicity? *Toxicological Sciences*, 54(1), 121-127.

Santa Maria, C. e. (1986). Subacute atrazine treatment effects on rat renal functions. *Bulletin of environmental contamination and toxicology*, 36(1),325-331.

Santa Maria, C. J.-C. (1987). Hepatotoxicity induced by the herbicide atrazine in the rat. *J. Journal of applied toxicology*, 7(6), 373-378.

Sass JB, C. A. (2006). European Union Bans Atrazine, while the United States negotiates continued use. *International journal of occupational and environmental health*, 12(3), 260-267.

Schwab AP, S. P. (2006). Persistence of atrazine and alachlor in ground water aquifers and soil. *Water, Air, & Soil Pollution*, 171(1–4), 203–235.

SCP. (2003). Atrazine Valuable Production Tool for Farmers. Retrieved from Syngenta Crop Protection: [http://www.syngentacropprotectionus.com/prod/herbicide/atrazine/index.asp](http://www.syngentacropprotectionus.com/prod/herbicide/atrazine/index.asp? follow “Atrazine’s Valueto Agriculture” hyperlink; then follow “Maximizing Economic Returns” hyperlink).

Sharma Y, B. S. (2005). Dimethoate-induced effects on antioxidant status of liver and brain of rats following subchronic exposure. *Toxicology*, 215, 173-181.
Siddique YH, B. T. (2005). Antigenotoxic effects of ascorbic acid against megestrol acetate-induced genotoxicity in mice. *Human & experimental toxicology, 24*(3), 121–127.

Simic, B. e. (1991). Reversibility of the inhibitory effect of atrazine and lindane on cytosol 5α-dihydrotestosterone receptor complex formation in rat prostate. *Bulletin of Environmental Contamination and Toxicology, 46*(1), 92-99.

Snyder, P. J. (1972). Thyrrotropin response to thyrotropin releasing hormone in normal females over forty. *The Journal of Clinical Endocrinology & Metabolism, 34*(6), 1096-1098.

Solomon, J. (1996). Applicability statement for IP mobility support.

Soos, M. &. (1982). Characterization of monoclonal antibodies directed against human thyroid stimulating hormone. *Journal of immunological methods*, 51(1), 57-68.

SRL. (1980). The infrared spectra atlas of monomers and polymers. Sadtler Research Laboratories.

Stevens, J. T. (1991). Herbicides. In *Handbook of Pesticide Toxicology*. New York: Hayes, W. J., Jr. and Laws, E. R., Jr., Eds. Academic Press, NY.

Stevens, J. T. (1991). Herbicides. In *Handbook of Pesticide Toxicology*. New York, NY: Hayes, W. J., Jr. and Laws, E. R., Jr., Eds. Academic Press.

Stevens, J. T. (1991). Herbicides. In *Handbook of Pesticide Toxicology*. Academic Press, 8-4.

Stoker, T. C. (1999). Maternal exposure to atrazine during lactation suppresses suckling-induced prolactin release and results in prostatitis in the adult offspring. *Toxicological sciences: an official journal of the Society of Toxicology, 52*(1), 68-79.

Stoker, T. E. (1999). Maternal exposure to atrazine during lactation suppresses suckling-induced prolactin release and results in prostatitis in the adult offspring. *Toxicological sciences: an official journal of the Society of Toxicology, 52*(1), 68-79.

Taketa Y, Y. M. (2011). Differential stimulation pathways of progesterone secretion from newly formed corpora lutea in rats treated with ethylene glycol monomethyl ether, sulpiride, or atrazine. *Toxicological Sciences, 121*(2), 267-278.

Tietz N W., e. a. (1995). Clinical Guide to Laboratory Tests. *Clinical Chemistry, 41*(10), pp. 1548-1548.

Tomlin, C. (1994). The Pesticide Manual Thornton Heath (10th Ed ed.). British Crop Protection Council/Cambridge: The Royal Society of Chemistry.

Tran, D. e. (1996). The inhibition of estrogen receptor-mediated responses by chlorostriazine-derived compounds is dependent on estradiol concentration in yeast. *Biochem. Biochemical and biophysical research communications, 227*(1), 140-146

Tyson, J. E. (1973). Factors influencing the secretion of human prolactin and growth hormone in menstrual and gestational women. *American Journal of Obstetrics and Gynecology, 116*(3), 377-387.

Victor-Costa AB, B. S. (2010). Changes in testicular morphology and steroidogenesis in adult rats exposed to Atrazine. *Reproductive toxicology, 29*(3), 323–331.

Vom Saal, F. S. (2006). Large effects from small exposures. II. The importance of positive controls in low-dose research on bisphenol A. *Environmental research, 100*(1), pp. 50-76.

Vonberg D. V. J. (2014). 20 years of long-term atrazine monitoring in a shallow aquifer in western Germany. *Water research, 50*, 294–306.

Vos, J. a. (1983.). Immuno-toxicity of pesticides. In Hayes, A.W., R.C. Schnell, and T.S. Miya. (eds.) Developments in the science and practice of toxicology. Proceedings of the 3rd International Congress on Toxicology. San Diego, CA., USA: Amsterdam, The Netherlands: Elsevier Scientific Publishers.

Wada, H. G. (1982). Enzyme immunoassay of the glycoprotein tropic hormones--
choriogonadotropin, lutropin, thyrotropin—with solid-phase monoclonal antibody for the alpha-subunit and enzyme-coupled monoclonal antibody specific for the beta-subunit. *Clinical chemistry*, 28(9), 1862-1866.

Waring, C. P., & Moore, A. (1996). *Environmental Atrazine: Physiological Effects on Atlantic Salmon Salmo Salar*. In *International Congress on the Biology of Fishes, San Francisco*.

Weber GJ, S. M. (2013). Transcriptome alterations following developmental atrazine exposure in zebrafish are associated with disruption of neuroendocrine and reproductive system function, cell cycle, and carcinogenesis. *Toxicological sciences* 132(2), 458–466.

Wetzel LT, L. L. (1994). Chronic effects of atrazine on estrus and mammary tumor formation in female Sprague-Dawley and Fischer 344 rats. *Journal of Toxicology and Environmental Health, Part A Current Issues*, 43(2), 169-182. doi: 10.1080/15287399409531913

Worthing C.R., W. S. (1987). The Pesticide Manual: A World Compendium (8th Ed ed.). Thornton Heath, British Crop Protection Council.

Yang, S. O. (2014). Toxic effects of atrazine on reproductive system of male rats. *Biomedical and Environmental Sciences*, 27(4), 281-288.

OSU. (1996, june). *Atrazine” Pesticide Information Profile, Extension Toxicology Network*. Retrieved from Oregon State University: http://ace.orst.edu/info/extoxnet/pips/atriazine.htm

Kiesecker, J. M. (2002, july 23). “Synergism between trematode infection and pesticide exposure: A link to amphibian limb deformities in nature?”. *Proceedings of the National Academy of Sciences*, 99(15), 9900-9904.

Hayes, T. H. (2003, april). “Atrazine-Induced Hermaphroditism at 0.1 ppb in American Leopard Frogs (Rana pipiens) Laboratory and Field Evidence. *Environmental Health Perspectives*, 111(4).

A.Trebst. (2008, Dec). The mode of action of triazine herbicides in plants. *Research Gate*, 50, pp. 101-110.

Hayes, T. H. (2003). Atrazine-induced hermaphroditism at 0.1 ppb in American leopard frogs (Rana pipiens): laboratory and field evidence. *Environmental Health Perspectives*, 111(4), 568-575.

Purcell M, N. J.-R. (2001). Interactions of atrazine and 2, 4-D with human serum albumin studied by gel and capillary electrophoresis and FTIR spectroscopy. *Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology*, 1548(1), 1548, 129–138.