

## Investigation of Ecotoxicological Effects of 2,4-D Acid Herbicide on the Ecosystem

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**Abstract:** 2,4-D Acid (Dichlorophenoxy acetic acid) is a herbicide which is commonly used for agricultural protection in our country. However, the scope of environmental and ecological problems that 2,4-D acid may cause is not known in detail. The ecotoxic studies showed that the usage of 2,4-D causes soft tissue sarcomas in human beings, increase in neoplastic variations in organs such as stomach, pancreas, lung, skin and urinary bladder, decrease in the number of sperms in males and increase in the risk prostate cancer. Moreover, the studies showed that 2,4-D is directly or indirectly effective on all organisms present in aquatic environment such as birds, insects, fish and algae by mixing into the air, soil and water and has some toxic effects on ecosystem depending on this. For this reason, it's very important to raise awareness of public about the unawareness usage of 2,4-D resulting in ecologic risk factors. It seems as an obligatory to raise awareness of public about the usage of this pesticide and to bring new arrangements about its usage in our country.

**Key words:** 2,4-D Acid • Herbicide • Living things and environment • Ecotoxicologic effect

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### INTRODUCTION

2,4-D (Dichlorophenoxy acetic acid) which was used as a regulatory chemical for the growth of plant at the beginning was developed by Zimmerman and Hithoock in 1942 and was used as a herbicide by Hammer and Tukes in 1944 [1]. 2,4-Dichlorophenoxy acetic acid (2,4-D) which is in the chlorophenoxyacetic acid group herbicides is used in different areas for different aims [2]. The amine salt or ester of 2,4-D is used in the areas such as meadows and agricultural areas against broad-leaved weeds. Its esters are generally used in pinewood plantation and cutting areas and in the regions where non-evergreen trees are present [3]. The granular form of 2,4-D, on the other hand, is used in irrigation, channels and lake areas such as pools. Low dosages of 2,4-D (20-40 mg/lt) is used for the aim of dropping immature fruits, increasing the color of red varieties of potatoes or increasing the number of middle size tubers. It is also known about these pesticides that they increase the storage period of citrus fruits [3, 4].

Codex Alimentarius Commission of United Nations Food and Agriculture Organization (FAO) and World

Health Organization (WHO) has limited the maximum level of 2,4-D in some food materials. According to this, the following 2,4-D residual levels are permitted; 0.5 mg/kg in barley, wheat and rye, 2 mg/kg in citrus fruits, 0.05 mg/kg in meat, milk and egg, 0.5 mg/kg in herbs. These amounts are determined in Germany and in other European countries as follows: 0.1 mg/kg in wheat and other cereals, 0.2 mg/kg in fresh fruits and vegetables [5-7]. Daily intake for 2,4-D is determined as 300 µg/kg/day by World Health Organization [8, 9].

It was also stated that the usage of 2,4-D acid among in agriculture sector of USA was (28-33 million pound) in the fifth order among other pesticides [10]. Although the usage of this pesticide is prohibited or some restrictions are imposed in Sweden and in some European countries, there is no prohibition or restriction in our country. Although it was determined that the usage of these pesticides for the aim of agriculture has no negative ecologic effects on living things present and environment, the number of studies showing the toxic effects of these pesticides on immune system and different tissues is increasing day by day [11].

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Table 1: Herbicides that are commonly used in Turkey and their consumption rates [12]

Herbicide	Consumption rates of herbicides by years (%)			
	1999	2000	2001	2002
2,4-D	45,28	44,34	47,49	33,62
Trifluralin	27,02	27,86	20,21	24,60
Molinate	7,10	6,44	3,69	3,50
Propanil	4,54	3,62	-	-
Glyphosate isopropylamin	4,10	6,94	9,08	7,57
Chloridazon	-	-	5,38	-
Metalochlor	-	-	-	5,10
TOTAL	88,04	89,20	85,85	74,39

Table 2: Physical and chemical properties of 2,4-D [22]

Molecular formula	$C_8H_6O_3Cl_2$
Molecular weight	221.04 g/mol
Solubility in water	900 mg/L
Melting point	138°C
Boiling point	160°C
Vapor pressure	0 mm Hg (20°C) 1.4x10 <sup>-7</sup> mm Hg (25°C)
pKa	2.3

It's a known fact that the rate of 2,4-D acid, which is one of the most commonly used herbicides in our country, in herbicide consumption by years is approximately 42.68% (Table 1) [12]. Since 2,4-D is cheaper than other pesticides, this caused this pesticide to be commonly used. Moreover, there is a risk for contamination of 2,4-D with dioxins which can be formed during synthesis. It was also suggested that dioxins which mostly occur after burning of pesticides have toxic effects and cause neoplastic variations [13, 14]. For this reason, most of the countries set out a condition that the pesticides including 2,4-D which will be consumed for the aim of agriculture should be decontaminated from dioxins [15], however, there is no such an obligation in our country.

Therefore, it seems as it's possible for preparates with 2,4-D which are consumed for agriculture to be contaminated with dioxins [12]. Unaware usage of agricultural pesticides causes some environmental problems and the residuals of these materials also cause environmental pollution and threaten every kind of living things [16]. Possible environmental effects caused by 2,4-D which is commonly used in our country for different targets are still not known. For this reason, it was aimed in this study to search possible environmental and ecologic problems caused by 2,4-D.

## MATERIALS AND METHODS

It was aimed to carry out experimental studies with 2,4-D and to investigate the information in literature in order to analyze possible environmental and ecologic problems that might occur depending on the usage of 2,4-D.

### Physical, Chemical and Biological Properties of 2,4-D:

The molecular formula of 2,4-D which is in the group of chlorinated phenoxy acid is  $C_8H_6Cl_2O_3$  and its structural formula is given in Figure 1. 2,4-D acid is a pesticide having phenolic aroma which is present as white-yellow flakes, powder, crystalline or as solid state under normal conditions [17]. Phenoxy herbicides are commercially present as amine, ester or derivatives of their salts and its active matter is its free acid form [18]. Physical and chemical properties of 2,4-D are given in Table 2. 2,4-D acid which is commonly used for broad-leaved herbs is taken in by roots and leaves and is transported to the upper parts by floems [19]. It stays in soil for 2 to 4 weeks after application [20].

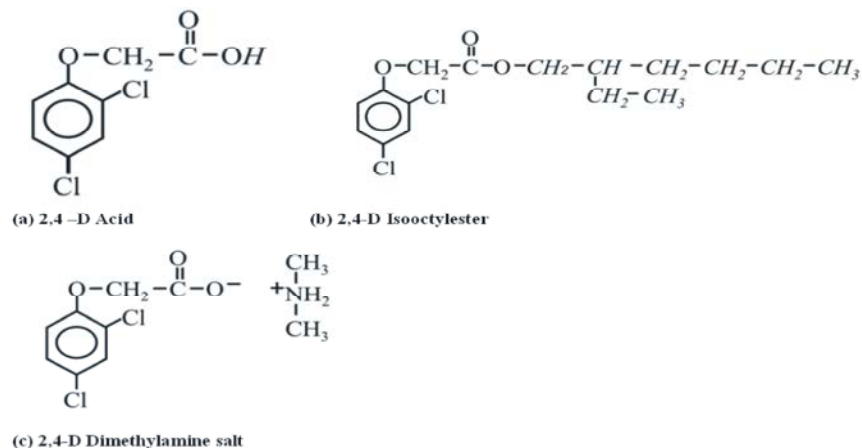


Fig. 1: Chemical structures of 2,4-D(a), 2,4-D Isooctylester (b) and 2,4-D Dimethylamine salt (c) [21]

**Absorption, distribution and excretion of 2,4-D:** 2,4-D is well absorbed by gastrointestinal system and its intake by lungs is low. Its intake through the skin, on the other hand, is at the minimum level [23]. 2,4-D accumulation was determined in blood, liver, kidney and lungs of pigs and spleens of rats in which 1 mg/kg 2,4-D was applied for six to eight hours. It was found that the accumulation of the material in muscles and brain was low. At the end of 24-hour period, no pesticide residual was found in these tissues. It was determined that 2,4-D acid was directly transferred to placenta in rats and pigs and 20% 2,4-D material was found in uterus, placenta, fetus and amniotic fluids of rats. Similarly, 2,4-D acid and its derivatives were determined at low levels in the eggs of chickens [24, 25]. It was observed that 2,4-D acid had its maximum concentration in the plasma of most of living things within 8 hours after its oral intake [26]. When 2,4-D acid is taken by human beings as 5 mg/kg body weight, it was determined that this pesticide was absorbed well. It was found that it reached its maximum level in the plasma within 4-8 hours [27, 28] whereas 17% of single dosage of the herbicide in pregnant mammals passed to the embryo through placenta [29].

The solubility of 2,4-D acid in water is very high and for this reason has a rapid distribution through tissues. It is also known that the intake and distribution of 2,4-D acid by aquatic living things is very high. In a study based on four rivers in China, it was observed that 80% of 2,4-D acid present in the medium was still there after 56 days [30]. Although 2,4-D was present at 61 ppm and higher values in water where it was applied directly, its concentration was found lower than 1 ppm in lakes and spring water. According to U.S. EPA criteria, 0.1 ppm levels in drinking water was accepted as permissible limit in USA [31, 19]. 2,4-D was determined in blood, liver, kidney and lungs of pigs and spleens of rats in which 1 mg/kg 2,4-D was applied even after 4-8 hours. It was found that this rate in muscles and brain was lower than it was in other tissues [25]. There is a close relationship between the concentration of 2,4-D in blood together with tissues and its binding properties to the proteins in *in vitro* mediums [32].

The excretion of 2,4-D occurs by excretory system through urine in all species. The average half-life of this pesticide in human body is 13-39 hours and it's known that it increases the alkalinity of the medium during excretion period. The half-life extends depending on the dosage intake [33-35]. It was informed that all 2,4-D was excreted completely from the human body through urine after 5 days following its intake [36]. Although loss of consciousness can be

experienced depending on its intake at high dosages, the person recovers consciousness within 48-96 hours following the excretion of this toxic material through urine [33, 34, 37].

#### **Possible Ecologic Effects**

**Its Ecotoxic Effects on Human Beings:** It was observed that 2,4-Ds have some biochemical parameter variations and oxidant activity and cause a decrease in the level of glutathione and superoxide dismutase in blood as a result of treating human erythrocyte with 250 and 500 ppm 2,4-D [38]. It was also determined that 2,4-D caused number and structure variations in erythrocyte, leucocyte and red marrow cells and caused amount variations in hemoglobin [39].

It was informed that soft tissue sarcomas occur in human beings who are exposed to chronic 2,4-D acid and the rate of becoming cancerous in organs such as stomach, pancreas, lung, skin and urinary bladder is very high [40,41,42,43,44]. Vineis *et al.* [45] determined that the occurrence of soft tissue sarcomas in female workers who work in rice planting where 2,4-D group pesticides were used was higher than the normal. In another study, 60 Hodgkin patients, 109 non-Hodgkin patients and 338 control group selected from general population were compared and it was reported that the people who were exposed to 2,4-D had higher risk to get cancerous [46]. In a research of Zahm *et al.* [47], it was concluded that the rate of non-Hodgkin lymphoma which is a type of lymphoma was higher than normal populations. Coggon *et al.* [48] suggested that the occurrence risk of various cancerous in workers who were exposed to 2,4-D group herbicides partially increased. In epidemiological researches of Lynge [49] which were carried out on workers working for more than ten years in two 2,4-D including pesticide producing factories in Denmark, it was concluded that the rate of malignant lymphoma in these populations was higher than normal populations. An increase was observed in coronary ectasia of farmers who applied pesticides in Australia. It's a known fact that 2,4-D (dichlorophenoxy acetic acid) is an acetylcholine esterase inhibitor. In another study, it was determined that 2,4-D caused a decrease in the secretion of testosterone in living things whereas it increased the secretion of oestrogen from testicular tissue and secretions of progesterone and prolactin and caused abnormalities in menstrual cycle. Moreover, it was observed that the number of sperms decreased and abnormalities occurred in their shapes in male pesticide sprayers [50]. In another study, on the other hand, the risk of prostate cancerous increased in workers who applied these herbicides [51].

When the pesticides belonging to 2,4-D group are taken orally or through skin, they have slight toxic effect and it was shown that the oral LD<sub>50</sub> value for human beings is 400-2000 mg/kg body weight/day whereas LD<sub>50</sub> dosage in the case of absorbing through skin is 2000 mg/kg body weight/day [52]. It is also known that the children living in rural areas are exposed to this herbicide in agricultural areas and it was suggested that they are affected more than adults since their skins are softer and moist [53]. It was determined that if 2,4-D group was taken orally by human beings, its digestion was very rapid, 73% of the amount taken in was voided through urine in 48 hours after application [54] and its absorption through skin was around 5.8% [55]. In rats, on the other hand, it was determined that the level of 2,4-D was maximum in blood after three hours following its oral intake and it was maximum in kidney, liver, spleen and lung after six hours [56]. When exposed to 2,4-D as a result of respiration or absorption through skin, gastrointestinal and peripheral neuromuscular symptoms were reported. Symptoms such as nausea, vomiting, anorexia, muscle weakness, pain and cramps in muscles, malaise, stomachache, tachycardia were observed [57].

For this reason, 2,4-D group pesticides are accepted as standard poison in some countries and although not all of the formulations have toxic effect, it was suggested that at least a part of them and their compounds have toxic effect. Dioxin (2,3,7, 8-TCDD) which is known to be present in these pesticides and which cause neoplastic variations in pancreas is at a level of 0.7 µg/kg in 2,4-D group pesticides. The acceptable level by WHO, on the other hand, is 10 µg/kg [58].

In a research carried out by Alexander *et al.* [59], 2,4-D acid amount was searched for the farmer family members who were living in the farms in Minnesota and South Carolina and exposed to pesticides including 2,4-D acid and systematic dosage assumption was performed. Urine samples were taken at 24-hour intervals from family members living in the farm 1 day before and 3 days after 2,4-D application. Median urine 2,4-D concentrations were found as 2.1 and 73.1 µg/L for the executers, 1.5 and 2.9 µg/L for the children and 1.2 µg/L for their spouses after 2,4-D application. Geometric mean systemic dosage (microgram per kg body weight) was found as 2.46 µg/L for executers, 0.8 µg/L for their spouses, 0.32 µg/L for children between 4-11 years old, 0.12 µg/L for children older than 12 years and 0.22 µg/L for all children. The reference dosage (RfD) determined by U.S. EPA [60] is 0.01 mg/kg/day or 10 µg/kg/day. These values show that the usage of 2,4-D pesticide affects the people who are living around the area where it is used.

Conjugates that can be hydrolyzed by the acid were found in the urine of some workers who were exposed to 2,4-D esters [61]. In the urine sample analysis of 28 people who were exposed to 2,4-D group herbicides during production and disinfection in Turkey, 2,4-D was determined between 0.06 and 9.51 ppm [62].

In a study performed by Nishioka *et al.* [9], 2,4-D residuals were measured in the samples taken from the inner surfaces (in the rooms, on the table, window borders) of 11 inhabitable and 2 empty houses in a week before and after 2,4-D application to the grass. As a result of measurements, 2,4-D was determined as 2.5-10 µg particle size in the house. After 2,4-D application, it was determined that children might take 2,4-D by nutrition as 0,2-30 µg/day from the top of the table and 1-10 µg/day from the rooms. It was indicated that this rate might increase 10 folds after first application.

Depending on the usage of herbicides produced from 2,4-D and phenoxy acetic acid, abnormalities in central nervous system, circulation/respiration, urogenital or muscle and skeleton were observed more than normal in the people living in West Minnesota [63]. Recently, the studies carried out in Croatia showed that there was an increase in chromosome abnormalities and in the frequencies of brother chromatid variation of workers who were exposed to compounds formed by mixing atrazine, malathion, cyanazine and 2,4-dichlorophenoxy acetic acid [64, 65,66]. In another study [67], brain neoplasm was observed more than normal in people who were working in production, formulation or packaging of 2,4-D.

**Its Ecotoxic Effects on the Animals:** It was stated that more than 60% of herbicides including 2,4-D and MCPA (4-chloro-2-methylphenoxyacetic acid) used in agriculture in America has a potential to endmange endocrine and/or reproduction system of animals [68]. Despite the variations in LD<sub>50</sub> levels according to types of animals, 2,4-D was accepted as moderately toxic for animals [69]. World Health Organization informed that teratogenic and embryo toxic dosage of 2,4-D in mammals and birds was 10 ppm whereas its lethal dosage varied between 100 and 300 ppm [70]. Some animals, for example dogs, has more sensitivity against 2,4-D than rats and humans. It was informed that malignant tumors were formed in dogs which were in contact with grass exposed to 2,4-D [69]. LD<sub>50</sub> values were found as 375 mg/kg for mouse, 666 mg/kg for rats, 800 mg/kg for rabbits and 541 mg/kg body weight for chicks [71].

World Health Organization informed that teratogenic effect dosage of 2,4-D in fish was 1 ppm [70]. In United States of America, 0.01 ppm-1.00 ppm 2,4-D was

determined in the fish and mussels living in the water close the areas where 2,4-D was used [72]. In another study, 2,4-D was reported in surface water if 2,4-D was used in close areas [73]. In the study which was carried out with *Lepomis macrochirus* -bluegill- and *Mola mola*, LD<sub>50</sub> dosage was 263 mg/L. In a study which was carried out with commercial rainbow trout, on the other hand, LD<sub>50</sub> dosage was found as 377 mg/L. As a result of 2-month observation for oyster and clams, the value was found as 3.8 ppm [74]. LD<sub>50</sub> dosage which was given for 2,4-D isooctylester in fish was determined as 62-153 mg/L for rainbow trout and 5-68 mg/L for blue fish [75]. In a study carried out in America, the residuals of this herbicide were found in mushrooms and plants in the areas where 2,4-D was applied. Moreover, fish and crustaceans were exposed to 2,4-D by using aquatic herbicides [70]. In a research of Fairchild *et al.* [76], it was determined that using 2,4-D in order to get under control undesired grass which had aquatic and terrestrial origin posed a risk in terms of surviving of rainbow trout, *Salvelinus confluentus* and other types of trouts. In another study in Hong Kong, it was stated that 200 mg/l and more dosages of 2,4-D ceased the photosynthesis of *Scenedesmus quadricauda* which were of green algae and development of chlorophyll-a by inhibiting its synthesis [77].

In a study performed with *Poecilia reticulata*, acute toxic effect of 2,4-D and its effect on behavior variations were investigated. In the experiments, the movements such as deceleration, grouping, going down and up slowly in aquarium and clustering in regions where air was in excess were observed in fish. 96-hour LC<sub>50</sub> value for *Poecilia Reticulata* fish in this experiment was determined as 30 ppm. It was observed that 50% of fish died at the end of 96 hours in the aquarium where this dosage was applied. At high dosages, on the other hand, irregular swimming, balance disorder, sudden mobilizations were observed in fish [78]. In the experiments of Yalcinkaya [20] which were performed with *Poecilia reticulata* type fish, upside-down and vertical swimming, sudden jumping motion, balance disorder, grouping on the surface to take air and color fading were observed in fish which were exposed to low, moderate and high dosages of 2,4-D. Parallel to increase in herbicide concentrations, neuronal loss was determined in vertebra. Intracellular edema and deformations in Nissl granules depending on the increase in dosage were observed. Moreover, pyknotic cells were observed from place to place. Gliosis in continuing histologic results was among other histopathologic symptoms.

Uyanıkgil *et al.* [79] investigated the effect of 2,4-D acid on medulla spinalis of poecilia reticulata type fish and as a result, it was determined that 2,4-D acid was quite neurotoxic for poecilia reticulata. In the study of Gül *et al.* [80], they searched for LC<sub>50</sub> value of *Capoeta tinca* (*Capoeta capoeta umbla*, HECKEL, 1843) and it was observed that at 25 ppm, no fish died, at 50 ppm 3 fish, at 75 ppm 4 fish, at 80 ppm 6 fish and 100-125 and 150 ppm all fish died. As a result, LC<sub>50</sub> value of 2,4-D in *Capoeta tinca* was found as 82.2759 g.

Pathologic process of 2,4-D material on *Tinca tinca* members was followed by taking its 96-hour acute dosage into consideration. The fish were investigated after 1-2-5-8-12 day intoxication periods. The symptoms showed that there was reduction and deformation in kidney tissue and variations occurred in the cells forming excretory system [81].

In a toxicologic study carried out in Finland, toxic dosages of 2,4-dichlorophenoxyacetic acid and 2-methyl-4-chlorophenoxyacetic acid (MCPA) were applied on rats, mice, guinea pigs, Syrian hamsters, rabbits and chickens and it was determined that 2,4-D and MCPA caused damages in blood vessels of brain on rats and on mice, respectively. Moreover, 2,4-D material caused a restriction in motive power of circulation of blood serum in the spinal cord region of mice [82]. Ferri *et al.* [83] investigated the possible effects of 2,4-D acid on the brains of new-born rats and as a result of the research, 2,4-D acid caused a variation at the level of reactive oxygen in the brain region of new-born rats and affected enzymatic activities in their defense mechanisms. As a result of experiments performed on rats with 2,4-D, subacute intoxication was observed in rats, their body weights decreased, variations were observed in their organ weights and some histopathological findings were determined in their livers [84-86]. As a result of the study of Sulik *et al.* [87], variations were determined in the kidney ducts of rats which were fed by sodium salt of 2,4-D acid. In the study of Ar *et al.* [88], it was shown that the presence of dimethylamine salt of 2,4- dichlorophenoxyacetic acid in human milk caused impairments in odontogenesis of young rats. In the study of Fukuyama *et al.* [89], it was determined that 2,4-D acid was an allergen for respiration.

Elio [90] treated chicken embryo with 2,4-D before incubation and then injected 0, 0.5, 1.2 and 4 mg 2,4-D acid into the film of egg shell. Pure 2,4-D and 2,4-D isooctylester having 37% commercial formula were used throughout the tests. As a result, brother chromatid variations at low dosages (P < 0.05) were observed after 4 days in the group to which 4 mg 2,4-D acid was applied.

During long-term exposure to 2,4-D, brother chromatid variations were observed increasingly. It was also observed that 2,4-D caused degenerative variations in ovary tissue of rats and the fertility of animals decreased with dosages above 50 mg/kg/day [91, 92]. Özdas *et al.* [93] indicated that 2,4-D herbicide caused histopathological damage in testis tissue of rats.

In a study carried out with 2,4-D, it was determined that continuous application of 2,4-D to mice, guinea pigs and rabbits caused anorexia, weight loss, vomiting, scleroderma, depression, a general angriness and muscle weakness [94, 20].

## RESULTS AND SUGGESTIONS

This study was carried out in order to examine the ecotoxic risk factors of 2,4-D acid in terms of environment and human health in the light of literature. The ecotoxic researches performed on human beings showed that 2,4-D intake caused variations in some biochemical parameters of living things, soft tissue sarcomas were observed, the rate of becoming cancerous in organs such as stomach, pancreas, lung, skin and urinary bladder increased, the number of sperms in males decreased and the risk of becoming prostate cancer also increased. Moreover, it was also found that it caused an increase in central nervous system, circulatory/respiration, urogenital or muscle and skeleton abnormalities.

The findings of animal experiments carried out on fish and aquatic organisms with 2,4-D showed that balance loss and vertical swimming, sudden jumping movement, grouping on the surface to take air and deformations in their color were observed in fish. Moreover, neuronal loss in spinal cord, deformations in nissl granules, teratogenic and toxic effects were observed in fish depending on the increase in herbicide concentration and acute or chronic exposure to this pesticide put the life of aquatic organisms in danger. As a result of experiments carried out on rats with 2,4-D, subacute intoxication was observed in rats, their body weights decreased, variations were observed in their organ weights and some histopathological findings were determined in their livers and kidneys as well as 2,4-D caused degenerative variations in ovary tissue.

2,4-Dichlorophenoxyacetic acid (2,4-D) is the most commonly used phenoxy herbicide in agriculture and gardening and it was determined that these pesticides have toxic effects on birds and useful pests and affect aquatic life, little invertebrates, fish, frogs, reptiles and algae negatively. Since 2,4-D free acid cannot dissolve in water and cannot be absorbed by soil particles well, it can pass to the groundwater through leakage [95].

The toxic effect of 2,4-D acid and the pesticides including it on fish is higher than its environmental effect on other aquatic living things. For example, LC<sub>50</sub> concentration range for salmon trouts is between 1.0 and 100 mg/L and mostly causes sudden deaths. In a study, when catfish was exposed to 10 mg/L dosage, approximately 10% of all population died within 48 hours [96, 25]. (When 110 mg/L dosage was applied to ocean sunfish (*Mola mola*), some variations in their swimming motions were observed. However, any effect wasn't observed in crabs when 2,4-D acid derivative pesticide was applied for 85 days to some young crabs. When brown shrimps were exposed to 2 mg/L dosage for 48 hours, an increase in their death rate was observed [24, 25].

As this is the case in other pesticides, it's a known fact that 2,4-D acid mixes into the air, soil and water and has toxic effects directly or indirectly to the environment and directly to the ecosystem. It was shown with studies that 2,4-D has toxic effect especially on freshwater fish, algae and other aquatic organisms as well as sea organisms at low levels. According to this, it's possible to say that the usage of 2,4-D as a herbicide is extremely harmful for the ecosystem. For this reason, it's very important to raise the awareness of public in terms of ecologic risk factors that may occur depending on unaware usage of 2,4-D acid. It seems as it'll be an obligation to bring new arrangements about its usage in our country.

**Conflict of Interest:** The authors declare that there are no conflicts of interest.

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