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HACETTEPE BULLETIN OF
NATURAL SCIENCES AND ENGINEERING

PUBLISHED BY THE
FACULTY OF SCIENCES OF HACETTEPE UNIVERSITY
Beytepe, Ankara, Turkey

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THE EFFECTS OF BHC AND HEPTACHLOR ON MICE

(BHC ve Heptaklorun Fareler Üzerine Etkileri)

M. Turan AKAY* , Ünal ALP*

S U M M A R Y

The low doses (50,100 and 200 ppm) of commercial BHC (Benzenehexachloride) and Heptachlor which are widely used in Turkey for pest control in agriculture were applied to the mice and their harmful effects were determined. They've both caused a decrease in body weight of mice and made histological damages in liver, kidney and spleen. We've we found toxic hepatitis characterized with granuloma, cellular degeneration due to the diminishing of the parenchymal cell-wall, the differences in cellular dimentions together with nucleus, vacuolar degeneration in liver , granuloma-like areas in kidney and increase in the amount of eosinophil leucocytes and erytrocytes in spleen. Due to the increase in drug-doses, the mice have showed tremor syndrome, difficulty in standing and walk and lastly anomalies in neurological behaviours.

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INTRODUCTION

Organochloride pesticides used for pest control in agriculture came up to men by spreading into the ecological surroundings in various ways and due to the more accumulation, cause some hazards in organisms. The chronic toxicity of technical BHC and its α, β and σ isomers retarded growth rate, and coused a decrease in body weight at 100 ppm or higher doses. It was found that the chief organ affected was liver, where as the kidney was affected to a lesser degree (FITZHUGH,1950). Hepatocarcinogenesis was observed in the mice treated with α, β,, σ or δ BHC, singly or in various combinations (ITO,1973). Commercial BHC and Heptachlor made similiar damages in liver of chicken embryos (KOLANKAYA, 1979). In this paper, light microscobic studies on liver, kidney, spleen, testis and ovary and anatomical studies in male and female albino mice treated with commercial BHC and Heptachlor have been discussed.

MATERIALS AND METHODS

In this study, 15-39 gr. weighed, 3,5 month old mice belonging to the Mus musculus L., O.ASA F_{15} strain were used and received from the lab. of experimental animals in Hacettepe University. Two females and one male mouse were housed in metalic individual cage in a room kept at 23°C and bedded under natural ligting conditions. At least, 15 mice were used for each dose of each pesticide. The relative humidity was 70 %. Pesticides were dissolved in aceton and after 20 minutes, 0,1 cc of this solution was added into the food weighed about 1.5 gr. While aceton froups were fed with the food containing only aceton, the control ate normal food. During the test, each mouse was given 4 capsule-

like food per day. The experiment went on ten weeks for groups receiving heptachlor and eight weeks for groups given BHC depending on the controls used in each drug. Mice dying during the experiment were excluded and instead of them new ones were used of the same weight and taken the same doses from the beginning of the test. All experimental animals were weighed hungrily in each week. Humidity, food and water supply were always controled. When the controls have given birth 8 or 10 weeks after the start of experiment, the test ended and then all experimental animals were killed by cervical dislocation process and all were autopsied and examined macroscopically for degeneration on organs. Liver, kidney, spleen, testis and ovary tissues were fixed in Bouin and Susa solutions for histological study. Fixed tissues were stained with hematoxylin and eosin stains. The piece taken above tissues blockaded in paraffin and cut by microtome in 7 microns.

R E S U L T S

I. THE EFFECT ON BODY WEIGHT AND FOOD CONSUMPTION:

Mice given commercial BHC Heptachlor within the experimental diets usually weighed less than control ones at the end of the experiment. Although the weight of all animals increased until the fourth week, after this time all mice receiving insecticides began to weaken with the decrease in food intake. There was no difference in food consumption and in body weight between the controls and those treated only with aceton. So, an increase in body weight of these animals was continuously seen until the experiment was over.

I. THE EFFECT ON REPRODUCTION

BHC and Heptachlor treated animals were failed to produce any new generation within 8 or 10 weeks though 15 of 20 mice of these litters in control group were eaten by their own mothers and the rest continued to live. (This action is seen normally). Only two fatty mice, one given 50, and the other given 100 ppm of BHC produced totally 13 litters and 8 of them were died after three days. Their mothers ate the rest. In aceton group, 7 mice of 10 produced 30 litters, 5 were eaten by their mothers and the rest lived continuously. There was a weight loss in new-born litters from BHC treated mice when copared with litters born from controls.

I. THE EFFECT ON NEUROLOGICAL BEHAVIOURS

There was not seen any important difference in neurological behaviours in the group of 50 ppm of BHC and Heptachlor treated mice from beginning to the end of the experiment. Male mice given 100 ppm of BHC and Heptachlor acted as control ones but half of the females had difficulty in standing and walk and leaned against the wall of the cage for support and could not right themselves if placed on the back. In addition to these, tremor of whole body including head, tail and extremities, self-mutilation and struggle with the other mice were also observed in many of these mice.

I. MORPHOLOGICAL FINDINGS

The colour of the liver, kidney and spleen of all mice treated with Heptachlor was seen darker red than controls and little visible dots were seen on the surface of the spleen in gross appearance. The fat tissue around all the organs especially liver, kidney, intestine

and spleen was found more than control ones. It was not found any difference in the ovaries and testes of mice given insecticides when compared with controls. The same results were also seen in BHC treated mice.

I. HISTOLOGICAL FINDINGS

I.a. Liver:

In groups that received commercial Heptachlor, we've found toxic hepatitis characterized with granuloma which was certainly caused by this insecticide in livers known to have an important role in metabolism. In this granulated liver tissue, the cells had their normal shape and their nuclei were more different than in normal cells. (FIGURE 1.3). Parenchymal cells of some areas of liver were slightly hypertrophic. Nuclear irregularities in cells in hypertrophic areas were rare and mononuclear. There were cells containing smaller and bigger nuclei than controls. (FIGURE 2). Cellular degeneration due to the fat storage of tissue, necrosis depending on the diminishing of the parenchymalcell-wall were observed. Beside these anomalies, there was an increase in the amount of kupffer cells and connective tissue cells such as fibroblasts around the hepatic veins, eosinophilic granulocytes in granuloma and reticular fibers causing fibrosis when compared the mice receiving commercial Heptachlor with control ones. It was seen that congestion was more than ever in higher concentration of the drug. The same results were obtained with the other insecticide BHC. The effects of insecticides on liver were in a lesser degree in doses of 50 and 100 ppm than in highes degree of dose 200 ppm of BHC and Heptachlor.The fameles were found more sensetive than males in all groups.

I-b. Spleen:

In spleen, it was found an increase in the quantity of erytrocytes and eosinophilic leucocytes that`s why it was seen that red pulp was widened. In mice receiving 200 ppm of commercial BHC and Heptachlor, the fibrosis was determined (FIGURE 4.5) No deleterious effect of aceton has been found both in liver and spleen.

I-c. Kidney:

The kidneys were the second organs affected by the insecticides but there were no many damages like in livers. The kidneys of the mice receiving 200 ppm of Heptachlor and BHC had granuloma including mononuclear cells, histiocytes and eosinophilic granulocytes. (FIGURE 6,7) These mononuclear cells were also found in fat tissue around the kidney.

The structure and the function of testis and ovary in all mice given all doses of both insecticides were essentially normal and no deleterious effect was determined.

D I S C U S S I O N

Previously, it was shown that BHC and its isomers kepon, heptachlor, aldrin, dieldrin and dieldrin group of compounds from chlorinated insecticides chronically retarded the growth rate, caused less body weight of the mice depending on the doses of drugs but on the contrary, caused an increase in liver weights, made negative effects on the food consumption and reproductive performance and made important damages especially in liver and kidney, respectively (MEHENDALE, 1977;,FITZHUGH, 1950. GAINES, 1960; HUBER, 1965; ITO, 1973_a, 1975_b; DenTONKELAAR, 1978). In our study, the mice given insecticides had less body weight than controls depending on the dose and as a result

anorexia arose by the effect of insecticides treated. Any new generation could not be received from insecticide treated mice although there could be found no pathological hazards in both ovaries and testes when examined microscopically. The pure gamma isomer of BHC broke down the testosteronemetabolism (FELLEGIOVA, 1977), DDT also made a negative effect on reproductive function of mice but its mechanism could not be known yet (WELCH,1969). By the way, it might be thought that the drugs used in test made an effect on hormonal mechanism. The metabolite of heptachlor and heptachlor are concentrated in butterfat of the milk of the cows (DAVIDOW, 1953). And it was reported that organochloride insecticides pass into the babies by way of placenta in human beings (YAMADA, 1976). That` s why we think that some newborn litters from BHC treated mice died. Chlorinated insecticides affect directly the central nervous system by inhibiting the acetylcholinesterase (BROOKS, 1973, 1974). Insecticides can accumulate in brain and cerebellum containing phospholipid in which these drugs can easily be stored so the animals showed anomalies in neurological behaviours such as struggle with each other and wound themselves. When treated in long period the carcinogenic characteristics of these insecticides are known and reported by many of the workers , previously (CABRAL, 1977, DAVIS, 1962). In our study, we` ve found toxic hepatitis characterized with granuloma in liver and granuloma like areas in kidney in 8 or 10 weeks. Vacuolar degeneration and congestion, anisocytosis, the differences in nuclei were also observed in liver. The similiar results were reported in chicken embryos (KOLANKAYA, 1975a, 1979b). These hazards were observed especially in females since they are more sensitive than males, and some weak males.

No deleterious effect of aceton in all tissues has been found since it evaporated before the mice were eating the food. Upon the finding represented here insecticides, apart from the usefulness in agriculture which lastsfor only a few days or weeks, have detrimental effects and keep this characteristic for a long time.

Ü Z E T

Tarımda zararlıları kontrol altında tutmak için Türkiye' de yaygın bir şekilde kullanılan ticari BHC ve heptaklorun 50,100 ve 200 ppm gibi düşük seviyedeki dozları farelere uygulandı ve zararlı etkileri tesbit edildi. İlaçların her ikisi de farelerin vücut ağırlığında düşmeye neden oldu, karaciğer, böbrek ve dalakta hücresel seviyede hasara yol açtı. Karaciğerde insektisit etkisiyle ortaya çıktığı saptanan granülomlarla karakterize toksik hepatit saptandı, parenkimal hücre duvarlarındaki erimeye bağlı olarak hücresel dejenerasyon, büyüklü küçüklü çekirdekler ve hücrelere ve ayrıca valuolar dejenerasyonu rastlandı. Böbrekte granülom benzeri bölgeler, dalakta ise eozinofil leukositlerin ve eritrositlerin sayısında artış görüldü. Bundan başka doza bağlı olarak hayvanlarda titreme sendromu, ayakta durmakta ve yürümede güçlük çekme gibi bazı anormal davranışlar gözlendi.

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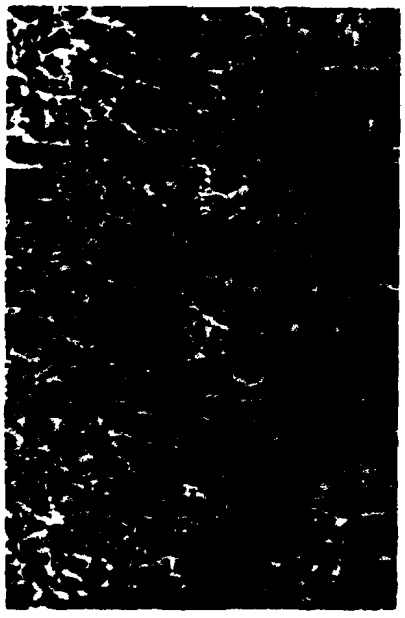

Figure 1:The liver of control mice magnification X 250

Figure 2:The liver of mice treated with 50 ppm of Heptachlor and BHC. magnification X250

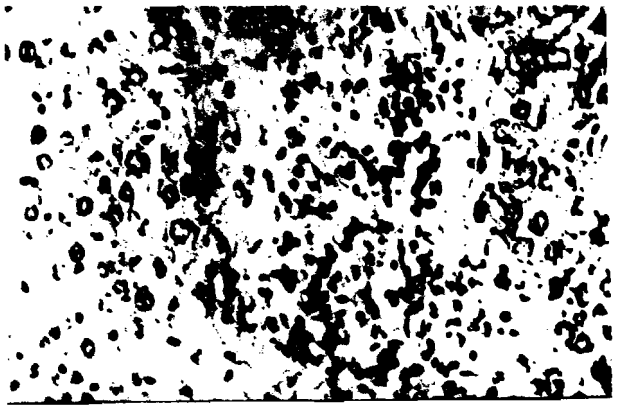

Figure 3: The granuloma in liver of mice given 200 ppm of Heptachlor and BHC magnification X 250

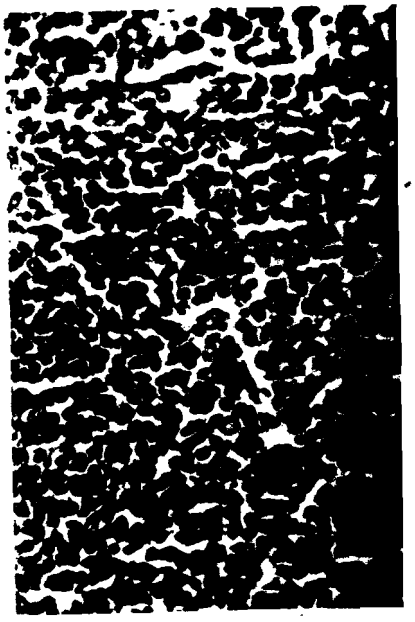

Figure 4: The spleen of control groups magnification X 400

Figure 5: The fibrosis in spleen of mice given 200 ppm of BHC and Heptachlor magnification X400

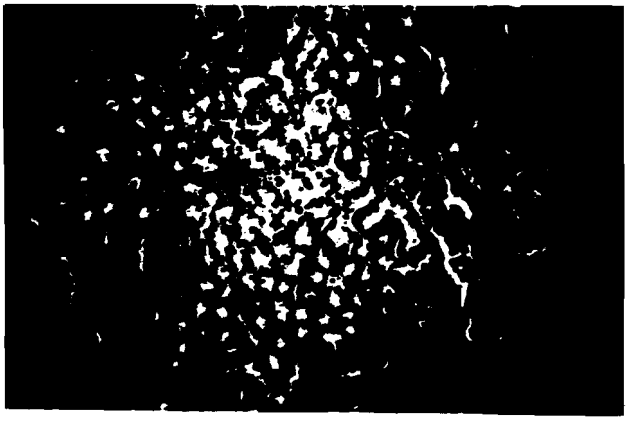

Figure 6: The kidney of control groups magnification X100

Figure 7: The kidney of mice receiving 200 ppm of Heptachlor and BHC magnification X 100

EFFECT OF L-ASCORBIC ACID ON GROWTH OF POLIOVIRUS IN HELA CELLS

(L-askorbik asidin Poliovirusun HeLa Hücrelerinde Üremesine Etkisi)

Çervin Çırakoğlu*

SUMMARY

Effect of various concentrations of L-ascorbic acid on growth of poliovirus in HeLa cells was investigated.

L-ascorbic acid at concentration 120 mg/ml was found to inhibit poliovirus growth totally.

The mechanism of this antiviral effect of L-ascorbic acid was also investigated and it was found that L-ascorbic acid inhibited poliovirus growth by inhibiting adsorption of virus to the cell.

INTRODUCTION

Antiviral effect of guanidine; 2,6 diaminopurine; pactamycin and ammonia was established for poliovirus (CARP, 1964; MUNYON, 1964; STEELE and BLACK, 1967; TABER, et. al., 1971; WARD, 1978). These chemicals were found toxic for cells.

L-ascorbic acid is described in medical literature as virtually non-toxic (PAULING, 1970a). Also it was indicated that phagocytes must have certain concentrations of ascorbic acid in them to be functional (PAULING, 1970b). Above function of it gave the idea to study it's antiviral effect for poliovirus.

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MATERIALS AND METHODS

I. CELLS AND MEDIA

HeLa cells were used for virus growth in this experiment. HeLa cell culture was obtained from IRSC, Villejuif, France and grown as monolayers at 37°C in 200 ml glass culture flasks in Eagle's minimal essential medium (MEM) supplemented with 10% foetal bovine serum and antibiotics.

II. VIRUS GROWTH AND TITRATION METHOD

Brunhilde strain of poliovirus $Type_1$ was obtained from N.I.H., Bethesda, Maryland, U.S.A. HeLa cells were infected with poliovirus at M.O.I. of 10 per cell and incubated for 15 hours at 37°C. Poliovirus multiplies in seven hours, but then remain inside the host cells for very long periods of time, eight to ten hours on the average and are then released in a burst (HOWES, 1959). Therefore we incubated the infected cells for 15 hours.

TCD_{50} (Tissue Culture $Dose_{50}$) method was used since it was established more sensitive and economic than plaque method for virus titration (SCOTTI, 1977). Hank's BSS (Basal Salt Solution) was used for dilution medium. 0.1 ml was planted from each dilution to three culture tubes and cytopathic effect (CPE) was observed under microscope.

III. L-ASCORBIC ACID SOLUTION

L-ascorbic acid was obtained from Sigma Chemical Co. St.Louis, Missouri, U.S.A. 6 gr of L-ascorbic acid was dissolved in 20 ml of distilled water in a capped bottle. It was sterilized through millipore filter after it was dissolved completely. Fresh L-ascorbic acid was prepared for each experiment.

RESULTS

I. Growth of Poliovirus in HeLa cells at various concentration of L-ascorbic acid

HeLa cell monolayers were infected with poliovirus at M.O.I. of

10 per cell. Titer of poliovirus increased from 10^7 TCD_{50}/0.1 ml to 10^{11} TCD_{50}/0.1 ml in 15 hours after infection. Thus 4 log units (10^4) increase in virus titer in a control culture. Growth of poliovirus at various L-ascorbic acid concentrations is shown in Table 1.

Table 1. Growth of poliovirus concentrations of L-ascorbic acid containing media.

Infected HeLa Cell cultures	L-ascorbic acid (mg/ml)	Beginning virus titer	Virus titer 15 h after infection	Viral yield
Control	-	10^7	10^{11}	10^4
1	10	10^7	10^{10}	10^3
2	25	10^7	10^{10}	10^3
3	50	10^7	10^9	10^2
4	85	10^7	10^8	10
5	120	10^7	10^7	-

II. Adsorption of Poliovirus to HeLa cells at 37°C

Two $25cm^2$ plastic flasks were infected with poliovirus at M.O.I. of 10 per cell and put at 37°C. Titer of unadsorbed viruses were determined by taking 0.1 ml amount from each culture after 5, 15, 30 and 60 minutes of incubation. Adsorption of poliovirus to HeLa cells is shown in Table 2.

Table 2. Adsorption of poliovirus to HeLa cells at 37°C.

Time (minutes)	% of unadsorbed virus
5	85
15	50
30	30
60	5

It was established that 85 mg/ml L-ascorbic acid inhibited poliovirus growth 3 log units and 120 mg/ml of it inhibited their growth totally. So it was concluded that presence of 85 mg/ml L-ascorbic acid in the medium was responsible to inhibit 50% of adsorption of poliovirus to HeLa cells, where as 99% adsorption was inhibited by 120 mg/ml (Fig 1).

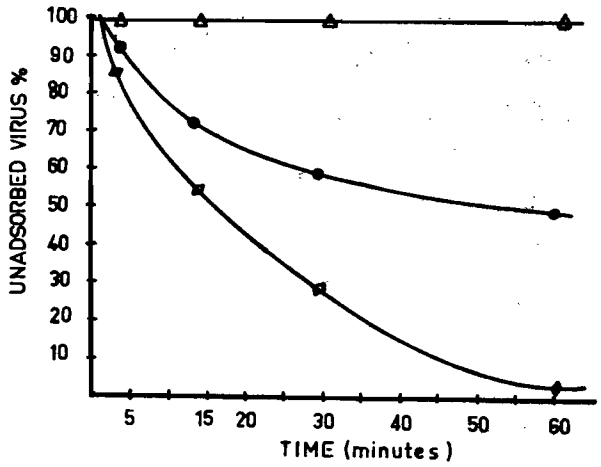

Fig 1. Adsorption of poliovirus to HeLa cells at 37°C in control culture and L-ascorbic acid containing media.
Symbols : □, control; o, 85 mg/ml L-ascorbic acid containing medium; ▲, 120 mg/ml L-ascorbic acid containing medium.

DISCUSSION

Effect of various L-ascorbic acid concentrations on growth of poliovirus was investigated in this study. It was reported that L-ascorbic acid did not effect the growth of herpes simplex, adenovirus and influenza virus (WALKER, et.al. 1967). But SCHWERDT and SCHWERDT (1975) reported that L-ascorbic acid inhibited rhinovirus growth.

In our present study it is established that -Lascorbic acid inhibits poliovirus growth in HeLa cells. It has been also found that L-ascorbic acid inhibits poliovirus growth by inhibiting

adsorption of virus to the cell.

It can be proposed that strong reducing agent L-ascorbic acid reduces the functional groups on the cell receptors that play role in adsorption of virus.

Before infection takes the virus particle is lying free on a mucous membrane or in the tissue space of the target organ. In such aposition free lying virus particle is completely inert, since multiplication can only take place after penetration to the cell has occured. In this free lying position the virus can be neutralized by circulating antibody (BAUER, 1977).

L-ascorbic acid inhibited adsorption of poliovirus to the cell. So free virus can be neutralized by circulating antibody under invivo conditions.

ÖZET

L-askorbik asitin çeşitli konsantrasyonlarının poliovirusun HeLa hücrelerinde üremesine etkisi araştırıldı. 120 mg/ml L-askorbik asit poliovirus üremesini tamamen inhibe etti. L-askorbik asitin antiviral etkisinin mekanizması da araştırıldı ve L-askorbik asitin, virusun hücreye adsorbe olmasını inhibe ederek poliovirus üremesini inhibe ettiği bulundu.

ACKNOWLEDGEMENT

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REVISION OF CHONDROSTOMA SPECIES OF TURKEY

(Türkiye Chondrostoma Tür'lerinin Revizyonu)

Mustafa Kuru*

SUMMARY

Twentyfive systematical important meristic and morphometric characters of four Chondrostoma species of Turkey were examined for 447 specimens. T-tests were used to compare characters. Also, the Coefficient of Difference formula was used to determine the subspecies status. These values were considered not sufficient to distinct the Chondrostoma of Turkey into four species, as previously thought or even subspecies.

INTRODUCTION

Up till now, four Chondrostoma species, Ch. nasus (LINNAEUS,1758), Ch. regium (HECKEL,1843),Ch. cyri KESSLER, 1877 and Ch. colchicum KESSLER, 1899, were found in Turkey. The distribution of these species (map 1) was given by LADIGES (1966). No Chondrostoma specimens were catched in the areas until 1966, which marked with question marks in this map. Gerede stream is very sufficient for Chondrostoma living. During this study we tried several times and in different ways, but no Chondrostoma specimens were catched as it was previously. From the streams between Muğla and Antalya where no Chondrostoma specimens were catched before, was identified as Ch. nasus by BALIK (1979). The number of pharyngeal teeth are used in the keys to distinct species from each other (HECKEL, 1843-46 ; SLASTENENKO, 1955-56; BERG, 1949 ; LADIGES, 1966; KURU, 1971; BALIK, 1975). But, as seen in table 1., it is imposible to distinct these species from each other using the pharyngeal teeth and some other characters.

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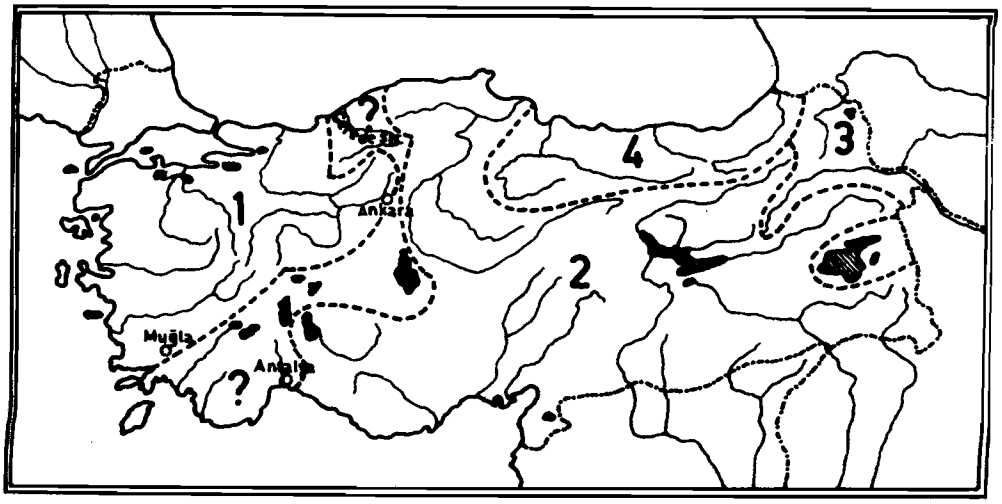

Map 1. The distribution of Chondrostoma species in Turkey. 1)Ch.nasus, 2)Ch.regium, 3)Ch.cyri, 4)Ch.colchicum, ?)No Chondrostoma specimens were catched (From LADIGES,1966).

In this work, 447 specimens of four Chondrostoma species from different streams and lakes of Turkey,were examined and compared for 25 meristic, morphometric and other characters. A re-examination is given here to resolve the systematical status of the species.

Table 1. Some important systematical characters of Chondrostoma species according to previous studies.

Species	Total numb. of D. ray	Total numb. of A. ray	Pharyn. teet	Lat. Line
Ch. nasus and ssp.	(11) 12-13 (14)	(12) 13-14 (15)	6-6 (7-6,6-5)	(50) 57-65
Ch. regium	11-12 (13)	12-14	(6)7-6,6-7	60-73
Ch. cyri	10-11 (12)	(11)12-13	5-6(6-6)	54-62
Ch.colchicum	11-12	12-13	6-5	57-64

MATERIALS AND METHODS

The specimens which were studied in this work, were obtained from the following people and localities (Ch. nasus, from region 1, Ch. regium, from region 2, Ch. cyri, from region 3 and Ch. colchicum, from region 4, in map 1).

Ch. nasus: Demirköprü dam-Manisa 1970, 2* (Leg.T.ongan); Bakır stream-Bergama 13. IX. 1971, 4, Gümüldür stream-İzmir 28.VI.1972, 5, Göksu (Gediz river)-Muradiye 30.VI.1972, 6, Gölhisar lake-Ulupınar 29.X.1976, 3 (Leg. S. Balık); Hamam stream-Kızılcahamam 9.VII.1978, 18, Seydisuyu-Yıldızören 15.VII.1978, 5, Kokar stream-Kütahya 22.VII. 1978, 8, Kokar stream-Çayırhan 22.VII.1978, 6, Koca stream-Beypazarı 30.VII.1978, 5, Seydisuyu-Seyitgazi 15.V.1979, 12, Sakarya river-Polatlı 22.VII.1979, 7, Mudurnu stream-Hasanbey 28.VII.1979, 6, Göksu-Osmaneli 12.VIII. 1979, 8, Kanal stream-Camidüzü 27.VIII.1979,4, Aladağ stream-Çayırhan 28.VIII.1979, 6, Ova stream-Yenikent 2.V.1980, 8 (Leg.F.Erk'akan).

Ch.regium: Beyşehir lake-Konya 1972, 4(Leg.T.Ongan); Murat river-Ahır village (Elazığ) 6.V.1971, 3, Cullap stream-Urfa 16.IX.1971, 4, Akziyaret stream-Kızıltepe 18.IX.1971, 5, Karasu-Tercan 20.IX.1971, 5, Karasu-Kandilli 25.IX.1971, 4, Habur river-Ceylanpınar 16.X.1971, 5, Batman stream-Batman 19.X.1971, 4, Murat river-Tutak 17.VI.1973, 4, Tohma stream-Malatya 5.V.1974, 2, Çağcağ stream-Nusaybin 9.V.1974, 5, Pisyar stream-Kozluk, 11.V. 1974, 3, Dicle river-Hasankeyf 19.VI.1974, 2, Başur stream-Baykan 20.VI.1974, 6, Avuski stream-Beşiri 20.VI. 1974, 6, Karasu-Muş 21.VI.1974, 8, Fırat river-Keban 14. VII.1974, 3, Dicle river-Samanyolu village (Batman) 12. VII.1974, 6, Hacıkamil stream-Siverek 16.VII.1974, 8, Ambar stream-Diyarbakır 17.VII.1974, 6, Dicle river-Diyarbakır 17.VII.1974, 4, Pisyar stream-Kozluk, 18.VII. 1974, 4, Karasu-Erzincan 11.VIII.1974, 3(Leg.M.Kuru); Beyşehir lake Konya 20.VI.1979, 5(Leg.Ü.Erdemli); Beyşehir lake-Konya 2.X.1980, 6 (Leg. Ü. Erdem).

Ch. cyri: Aras river-Horasan 18.VII.1970, 4, Aras river-Köprüköy 18.VII.1970, 13, Çıldır lake-Çıldır 28. VII.1971, 7, Kars stream-Kars 29.VII.1971, 12, Aras river-Hasankale 16.IX.1971, 5, Ölçek stream-Ardahan 27.VII. 1974, 7, Damal stream-Damal 28.VII.1974, 9, Akçalar stream-Arpaçay 29.VII.1974, 10, Kura river-Ardahan

*Numbers following dates,indicate number of specimens.

29.VII.1974, 12, Aras river-Karakurt 6.VIII.1974, 9, Kura river-kotanlı village (Ardahan) 7.VIII.1974, 14, Çiftekőprü stream-Ardahan 20.VIII.1974, 3, (Leg.M.Kuru).

Ch. colchicum: Tortum stream-Tortum 25.IV.1971, 2, Çoruh river-Artvin 1.V.1971, 8, Çoruh river-Borçka 1.V. 1971, 5, Çoruh river-Yusufeli 6.VI.1971, 7, Çoruh river-İspir 19.VII.1971, 7, Kelkit stream-Reşadiye 31.VIII.1971, 3, Kelkit stream-Şebinkarahisar 1.IX.1971, 12, Masat stream-Bayburt 6.VIII.1974, 11, Kelkit stream-Niksar 8. VIII.1974, 5, Çekerek stream-Göynücek 9,VIII.1974, 9, Yeşilırmak-Tokat 10.VIII.1974, 14,Almus dam-Tokat 10.VIII. 1974,6, Yeşilırmak-Pazar, 11.VIII.1974,21,(Leg. M. KURU).

Counts and measurements were made on 113 adult specimens of Ch. nasus, 115 Ch. regium, 109 Ch. cyri and 110 Ch. colchicum. The range in total length for Ch. nasus was 79-180 mm., Ch. regium 104-270 mm., Ch. cyri 113-256 mm. and Ch. colchicum 107-199 mm. All dorsal, anal, ventral, pectoral and caudal rays were recorded. Scales were counted in lateral series from the one touching the postero-dorsal corner of the operculum to the last large scales on the caudal peduncle (L.line)*. The number of scales around the narrowest part of the caudal peduncle (SACP)* and the number around the body just anterior to the ventral fins (SAB)*, left and right pharyngeal teeth (LPh.T. and RPh.T)* and gill rakers on the outer side of the first gill arch (GR)* were counted.

The following measurement were taken with a milimetric ruler. Total length (TL)* : Distance from tip of snout to the end of longest lobe of caudal fin. Head length (HL)*: Distance from tip of snout to the end of gill cover bone (operculum). Head depth (HD)*: Measured at occiput, i.e., above where the first vertebra attached to cranium. Body depth (BD)*: Maximum depth of body exclusive of fins. Caudal peduncle depth (CPD)*: Minimum depth of this region. Eye width (EW)*:Longitudinal diameter of eye. Snout length (SL)*: Distance from tip of snout to anterior margin of eye. Interorbital distance (ID)*: Bony distance between orbits. Dorsal fin height (DH)*: Length of its longest ray. Anal fin height (AH)*: Length of its longest ray. Pectoral fin length (PL)*: Distance from anterior edge to tip. Ventral fin length (VL)*: Distance from anterior to tip. Mouth width (MW)*: Distance between corners of mouth. Ventral fin-Vent distance (V-A)*: Distance between ventral fin base and anal aperture. Pecto-Ventral distance (P-V)*: Distance between

*Notations in tables.

pectoral and ventral fin bases.

Values for head length, head depth, body depth, caudal peduncle depth, ventral fin-vent distance and pecto-ventral distance were entered as a proportion of total length. Eye width, snout length, interorbital distance, dorsal fin height, anal fin height, pectoral fin length, ventral fint length, head depth and mouth width were entered as a proportion of head length. The values are discussed in the results.

RESULTS AND DISCUSSION

Meristic and certain other characters which were examined, are summarized in tables 2 and 3. For comparison of the characters of species, t-tests were used and no significant differences between the characters of four Chondrostoma species were found (Table 4). For this reason, the coefficient of difference ($CD = \frac{Ma-Mb}{SD_a+SD_b}$)* formula was used to test the subspecies status. Again all of the calculated values were nonsignificant ($CD < 1.28$ for all characters of species).

These factors are considered not sufficient to distinguish these four species from one other and according to the priority rule Ch. regium, Ch. cyri and Ch. colchicum can be accepted as synonyms of Ch. nasus (L.,1758).

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CD = Coefficient of difference, M_a = Mean of character of species a , M_b = Mean of character of species b, SD_a = Standart deviation of character of species a, SD_b = Standart deviation of character of species b.

Table 2. Comparison of some meristic characters for Ch. nasus, Ch.regium, Ch.cyri and Ch. colchicum. (S.D. Standart deviation, n=number of specimens).

Characters	Ch.nasus (n=113)			Ch.regium (n=115)		
	Range	Mean	S.D.	Range	Mean	S.D.
D.rays	10-13	11.63	±3.36	11-13	11.82	±2.07
A.rays	9-14	11.78	±4.41	10-15	11.75	±2.57
V.rays	8-12	9.74	±2.95	9-12	9.03	±2.36
P.rays	10-19	13.78	±3.05	11-19	13.71	±4.51
C.rays	15-18	16.66	±2.45	15-20	16.68	±3.68
L.line	44-68	58.36	±9.53	57-73	62.77	±7.91
SAB	27-47	37.49	±9.97	32-49	39.36	±5.21
SACP	14-21	17.29	±4.45	14-19	16.89	±3.82
LPh.T	5-6	5.83	±0.91	5-7	6.38	±1.73
RPh.T	5-7	5.86	±1.64	5-7	6.05	±1.33
G.rakers	15-32	24.57	±7.06	18-36	27.92	±7.70

Characters	Ch.cyri (n=109)			Ch.colchicum(n=110)		
	Range	Mean	S.D.	Range	Mean	S.D.
D.rays	10-12	10.84	±1.08	9-12	10.79	±3.53
A.rays	10-11	10.84	±0.83	9-12	11.17	±0.54
V.rays	9-10	9.48	±2.66	8-11	9.13	±1.54
P.rays	9-15	12.32	±4.19	10-16	13.49	±4.48
C.rays	14-19	16.58	±4.39	14-20	16.27	±4.02
L.line	52-62	56.25	±4.90	50-64	57.48	±6.96
SAB	29-40	32.86	±3.50	29-40	34.79	±5.77
SACP	13-18	15.92	±2.45	13-19	15.74	±3.90
LPh.T	5-6	5.61	±0.92	5-6	5.86	±0.84
RPh.T	5-6	5.09	±0.63	5-6	5.37	±1.48
G.rakers	17-32	22.20	±6.39	19-33	23.28	±3.79

Table 3. Comparison of morphometric characters for Ch. nasus, Ch.regium, Ch.cyri and Ch.colchicum . (S.D. Standart deviation, n=number of specimens).

Characters	Ch.nasus (n=113)			Ch.regium (n=115)		
	Range	Mean	S.D.	Range	Mean	S.D.
TL/BD	4.03- 5.90	4.90	±2.29	4.31- 7.60	5.71	±1.67
TL/HL	4.00- 5.90	5.12	±2.60	4.71- 7.89	6.16	±2.61
TL/HD	5.57- 8.13	7.12	±2.32	6.59- 9.64	7.95	±2.08
TL/CPD	8.21-14.63	11.30	±3.68	10.39-18.14	13.41	±3.98
TL/V-A	4.17- 8.12	5.98	±2.17	4.26- 7.76	5.77	±2.35
TL/P-V	3.33- 5.17	3.87	±2.03	3.22- 5.43	4.26	±1.27
HL/HD	1.21- 1.80	1.40	±0.39	0.95- 1.65	1.29	±0.47
HL/EW	3.29- 5.83	4.54	±2.30	3.00- 5.60	4.14	±2.13
HL/SL	3.13- 6.60	4.39	±3.30	3.14- 6.80	4.68	±2.65
HL/DH	0.91- 1.38	1.14	±0.45	0.86- 1.45	1.06	±0.53
HL/AH	0.93- 1.88	1.42	±0.56	1.12- 1.85	1.46	±0.50
HL/VL	0.93- 1.85	1.45	±0.59	1.06- 1.75	1.40	±0.43
HL/ID	2.20- 3.50	2.66	±1.10	1.73- 3.43	2.62	±1.50
HL/MW	2.90- 5.00	3.79	±2.61	2.57- 5.50	3.77	±1.80
HL/PL	1.00- 1.61	1.28	±0.33	0.95- 1.50	1.19	±0.38

Characters	Ch.cyri(n=109)			Ch.colchicum(n=110)		
	Range	Mean	S.D.	Range	Mean	S.D.
TL/BD	4.82- 6.65	5.68	±0.98	4.38- 7.08	5.34	±1.10
TL/HL	5.67- 8.95	7.29	±1.05	5.59- 8.96	6.91	±1.84
TL/HD	7.79-12.80	9.37	±3.60	6.30- 9.42	7.96	±1.87
TL/CPD	9.94-15.40	12.21	±1.48	10.69-14.50	12.34	±3.71
TL/V-A	5.43- 8.33	6.90	±1.43	5.46- 8.82	6.71	±1.44
TL/P-V	3.38- 5.14	4.18	±0.94	3.05- 5.19	4.16	±1.20
HL/HD	0.93- 1.91	1.29	±0.27	0.68- 1.57	1.16	±0.31
HL/EW	3.20- 5.25	3.90	±1.84	2.83- 5.50	3.36	±1.38
HL/SL	3.14- 5.25	4.26	±1.45	3.17- 5.33	3.88	±1.55
HL/DH	0.78- 1.08	0.90	±0.27	0.62- 1.21	0.91	±0.31
HL/AH	0.95- 1.27	1.14	±0.78	0.74- 1.47	1.11	±0.29
HL/VL	0.84- 1.40	1.13	±0.23	0.77- 1.36	1.10	±0.45
HL/ID	1.82- 3.00	2.26	±1.81	1.70- 2.75	2.18	±0.77
HL/MW	2.56- 4.20	3.21	±1.97	2.11- 3.67	2.86	±1.39
HL/PL	0.78- 1.24	1.01	±0.18	0.68- 1.33	0.97	±0.34

Table 4. Comparison of some meristic and morphometric characters for Chondrostoma species (t=calculated t value, p=probability between characters of species, 1=Ch.regium 2=Ch.nasus, 3=Ch.cyri, 4=Ch. colchicum).

Varieties	Gill rakers		Left Ph. teeth		Right Ph. teeth		Scal.around C.Peduncle	
	t	p>	t	p>	t	p>	t	p>
1→2	0.32	0.50	0.27	0.50	0.09	0.80	-0.07	0.80
1→3	0.55	0.50	0.37	0.50	0.60	0.50	0.20	0.80
1→4	0.49	0.50	0.24	0.80	0.34	0.50	0.21	0.80
2→3	0.25	0.80	0.17	0.80	0.42	0.50	0.26	0.50
2→4	0.15	0.80	-0.02	0.80	0.22	0.80	0.26	0.50
3→4	-0.14	0.80	-0.20	0.80	-0.18	0.80	0.04	0.80

Varieties	Scal.around body		L. line		Branch.rays of C. fin		Branch.rays of P. fin	
	t	p>	t	p>	t	p>	t	p>
1→2	0.17	0.80	0.36	0.50	0.50	0.80	-0.01	0.80
1→3	0.98	0.20	0.66	0.50	0.02	0.80	0.22	0.80
1→4	0.58	0.50	0.48	0.50	0.07	0.80	0.03	0.80
2→3	0.42	0.50	0.19	0.80	0.02	0.80	0.29	0.50
2→4	0.22	0.80	0.07	0.80	0.09	0.80	0.05	0.80
3→4	-0.29	0.50	-0.15	0.80	0.05	0.80	-0.19	0.80

Varieties	Total rays of V. fin		Total rays of A. fin		Total rays of D. fin		TL/BD	
	t	p>	t	p>	t	p>	t	p>
1→2	-0.19	0.80	-0.01	0.80	0.05	0.80	0.20	0.50
1→3	-0.13	0.80	0.31	0.50	0.39	0.50	0.02	0.80
1→4	-0.03	0.80	0.19	0.80	0.27	0.50	0.17	0.80
2→3	0.07	0.80	0.20	0.80	0.22	0.80	-0.30	0.50
2→4	0.17	0.80	0.13	0.80	0.17	0.80	-0.16	0.80
3→4	0.11	0.80	-0.33	0.50	0.01	0.80	0.23	0.80

Varieties	TL/HL		TL/HD		TL/CPD		TL/V-A	
	t	p>	t	p>	t	p>	t	p>
1→2	0.28	0.50	0.27	0.50	0.39	0.50	-0.07	0.80
1→3	-0.37	0.50	-0.36	0.50	0.26	0.50	-0.39	0.50
1→4	-0.22	0.80	-0.01	0.80	0.19	0.80	-0.31	0.50
2→3	-0.75	0.20	-0.53	0.50	-0.22	0.80	-0.35	0.50
2→4	-0.55	0.50	-0.28	0.50	-0.20	0.80	-0.27	0.50
3→4	0.18	0.80	0.34	0.50	-0.03	0.80	0.09	0.80

Varieties	TL/P-V		HL/DH		HL/ID		HL/AH	
	t	p>	t	p>	t	p>	t	p >
1→2	0.17	0.80	-0.11	0.80	-0.02	0.80	0.05	0.80
1→3	0.05	0.80	0.25	0.80	0.15	0.80	0.36	0.50
1→4	0.06	0.80	0.22	0.80	0.24	0.80	0.55	0.50
2→3	-0.14	0.80	0.45	0.50	0.19	0.80	0.30	0.50
2→4	-0.12	0.80	0.41	0.50	0.35	0.50	0.47	0.50
3→4	0.01	0.80	-0.02	0.80	0.04	0.80	0.04	0.80

Varieties	HL/PL		HL/VL		HL/MW		HL/EW	
	t	p>	t	p>	t	p>	t	p>
1→2	-0.18	0.80	-0.07	0.80	-0.01	0.80	-0.13	0.80
1→3	0.39	0.50	0.51	0.50	0.21	0.80	0.08	0.80
1→4	0.42	0.50	0.48	0.50	0.38	0.50	0.28	0.50
2→3	0.70	0.20	0.49	0.50	0.17	0.80	0.21	0.80
2→4	0.65	0.50	0.46	0.50	0.30	0.50	0.42	0.50
3→4	0.11	0.80	0.46	0.80	0.14	0.80	0.23	0.80

Varieties	HL/SL		HL/HD	
	t	p>	t	p>
1→2	0.07	0.80	-0.18	0.80
1→3	0.13	0.80	0.00	0.80
1→4	0.24	0.80	0.21	0.80
2→3	0.03	0.80	0.23	0.80
2→4	0.13	0.80	0.47	0.50
3→4	0.18	0.80	0.32	0.50

ÖZET

Türkiye'de bulunan dört Chondrostoma tür'ünün 447 örneği, 25 önemli sistematik özellik bakımından incelenmiş ve birbirleriyle karşılaştırılmıştır. Özelliklerin önem farkını kontrol için t-testi ve farklılık katsayısı bağıntısından yararlanılmıştır. Elde edilen verilere göre, daha önce düşünüldüğü gibi Türkiye Chondrostoma örneklerinin dört ayrı tür hatta alttür olarak kabul edilemeyeceği saptanmıştır.

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