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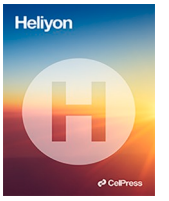

Research article

Cytome analysis (micronuclei and nuclear anomalies) in bioindication of environmental pollution in animals with nuclear erythrocytes

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ABSTRACT

Assessment of cytogenetic homeostasis of indicator animals is of great importance in ecological monitoring. The simplest method of its study is micronucleus analysis. Animals with nuclear erythrocytes are often used as indicator animals. In addition to the micronuclei usually recorded, a wide range of cytological nuclear and cellular abnormalities (cytomic analysis) is encountered when assessing the spontaneous level and under the influence of anthropogenic factors. Spontaneous frequency of cytogenetic disorders in 36 species of fish, amphibians, reptiles and birds was studied. Ecological monitoring of territories of Kazakhstan with different types of pollution (radiation, petrochemical, pesticide, heavy metals, due to rocket and space activities) was carried out with the help of separate species of animals. The results of the study include comparative descriptions, schematics and microphotographs clearly demonstrating a wide range of cytological anomalies of nuclear erythrocytes of animals of different classes. The greatest spectrum of nuclear anomalies in the studied animals was registered at petrochemical and pesticide contamination of territories. Depending on the tasks and climatic-geographical conditions, all investigated species can be used as bioindicators. *Testudo horsfieldii* is an exception for desert regions due to high spontaneous micronuclei level in this species. A review of the names of the main nuclear anomalies is carried out and variants of its ordering are proposed.

1. Introduction

Environmental pollution is the reason for the increase in the mutation rate and the volume of genetic load in human and animal populations. For environmental monitoring of unfavourable regions, modern ecology is increasingly focused on natural diagnostic tools using bioindicator species. Methods of bio-testing and bioindication provide an opportunity to get a quick answer to the complex biological response of an ecosystem or population, about the degree of influence of various anthropogenic factors on it.

Ecotoxicological analysis of natural animal populations can determine both summative assessment of toxic effects of chemical and physical factors on populations of free-living organisms and study of biosystems' responses to toxicants at the population, organismal

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and genetic levels (Schuijt et al., 2021; Pérez-Iglesias et al., 2023). Cytogenetic characteristics of peripheral blood of amphibians, fish, reptiles and birds living near the pollution indication sites are used as biomarkers to detect mutagenic impact of anthropogenic factors on living organisms [1–3].

The main problem with using wild animals as test subjects is the lack of data on species-specific patterns of genome instability and methods for its assessment. There are many different methods for assessing the level of genome instability, but the use of most of them is limited either by their low informativeness or by considerable difficulties, especially in the field. Cytogenetic homeostasis, expressed in the maintenance of the karyotype, is one of the indicators of organismal state. In many cases, it is easiest to characterise it using the micronucleus test, which consists in counting the frequency of cells with micronuclei (Favio et al., 2015; [4–6]) and other cytological abnormalities of blood cells. Therefore, its informativeness, simplicity and accessibility have ensured its popularity in studies of this kind.

The connection between micronuclei and exposure to environmental factors was first established in experiments with root meristematic cells exposed to ionising radiation [7]. Micronuclei induced by chemical exposure were described in Ehrlich tumour cells treated with colchicine [8]. Thus, even the first studies showed that multiple factors are responsible for nuclear abnormalities.

The use of the micronucleus test in the study of the effects of mutagenic and carcinogenic drugs on the organism is due to the relative simplicity and stability of the results [9]. The advantages of the micronucleus test include rapidity, independence from the karyotype of the species under study, often containing a large number of small, poorly distinguishable chromosomes, reliability, stability, and the fact that testing can be performed in tissues with low mitotic activity. Of great importance is the possibility to conduct lifetime, non-invasive screening to determine the dynamics of changes in this indicator over time.

The presence of micronuclei in cells can be considered a universal indicator of contamination [1,10], which can be used to quickly and accurately determine the clastogenicity of compounds and substances needed in household, industry, agriculture and medicine. This allows improving the quality of environmental monitoring in various man-made accidents, environmental disasters involving powerful releases of pollutants into the environment.

In recent years, the so-called micronucleus cytome analysis, a method for assessing nuclear disruption in cells, has been used to

Fig. 1. Scheme of location of the study areas. 1,2,3 - areas of petrochemical pollution in the Kazakhstan part of the Caspian Sea region (Atyrau, Bautino, Aktau). Atyrau, Bautino, Aktau); 4 - the area of fall of the 1st stage of Soyuz-2 launch vehicles (Kostanay region); 5 - the area of fall of the 1st stage of Proton-M launch vehicles (Karaganda region); 6 - the area of emergency fall of Dnepr launch vehicle; 7 - Balkhash region (heavy metal pollution); 8 - areas of local pesticide pollution; 9 - the area of local radiation pollution; 10 - control region (areas near Lake Alakol).

evaluate genome instability. As a rule, it is used on binuclear peripheral blood lymphocytes with cytokinetic blockade [11,12] and on human buccal epitheliocytes in medical studies [13,14]. But also in animals with nuclear erythrocytes, along with micronuclei, a rather wide range of morphological types of nuclear and cellular abnormalities also occur, and they can provide additional information on ecotoxicological effects. However, their classification, information on their full spectrum and genesis are few. The inconsistency of names of the registered anomalies in nuclear erythrocytes of animals creates additional difficulties.

Therefore, the aim of the present work included: studying the spontaneous indices of several of species of non-mammalian vertebrate species (fish, amphibians, reptiles and birds) that can be used as potential bioindicators; using individual species to assess different types of anthropogenic factors; accumulating and analyzing the maximum spectrum of abnormalities found in nuclear erythrocytes in different classes of animals; analyzing the names of nuclear abnormalities used by different authors and making suggestions for their ordering.

2. Experimental section

2.1. Materials and methods

To obtain primary ecological-faunistic materials and collect biomaterial samples, expeditionary work was carried out in the spring-summer period (April–September) in the Caspian, Ile-Balkhash, and South Kazakhstan regions of Kazakhstan. The general assessment of species diversity, distribution and living conditions of the studied animals was based on our own observations and publications on these vertebrate groups (fish of Kazakhstan, herpetofauna, birds) [15–17].

2.2. Objects of research

This study was approved by the Local Ethical Commission of Kazakh-Russian Medical University N⁰52 by September 05, 2017 and of the Institute of Human and Animal Physiology N⁰3 by 28.03. 2022 (Almaty, Kazakhstan).

The objects of study were natural populations – 19 species of fish of families *Cyprinidae* (13), *Gobiidae* (1), *Esocidae* (1), *Percidae* (1), *Salmonidae* (2), *Labridae* (1); amphibians (lake frog (*Pelophylax ridibundus*), toad); reptiles (representatives of the genus *Eremias*, Central Asian turtle); birds (*Columba livia*) living in ecologically favorable conditions and near places with established features of habitat pollution by xenobiotics (pesticides, rocket fuel, oil products, heavy metals and radionuclides). The species names of the animals studied are presented in the respective tables in the Results section.

2.3. Characteristics of the surveyed territories

The survey areas are presented in Fig. 1.

Petrochemical pollution. Atyrau region (Atyrau city (1)) of the Republic of Kazakhstan is located to the north-east of the Caspian Sea. The oblast is rich in various mineral resources and is the oldest oil and gas producing region of Kazakhstan. Mangistau region (Bautino (2) and Aktau (3)) is washed by the Caspian Sea from the west. It produces 25 % of Kazakhstan's oil and the main focus of the region is the oil industry, which accounts for 70.6 % of the total industrial output of the region. The cities of Aktau and Bautino are seaports [18].

2.3.1. Impact of rocket and space activities

The drop area of the first stage (side blocks) of the Soyuz-2 launch vehicle is located in Dzhangeldinsky district of Kostanay region (4). All stages used T-1 fuel (aviation kerosene) as propellant and liquid oxygen as oxidizer.

The area of the Proton rocket first stage fall is located in the territory of Ulytau district of Karaganda region (5) and occupies a total area of 160 thousand hectares. For 40 years, the area has been under the influence of numerous crashes of the first stage of the Proton launch vehicle. All rocket stages use unsymmetrical dimethylhydrazine (NDMH or "heptyl") as propellant components.

The area of the Dnepr launch vehicle accident (6). The accident occurred on July 27, 2006 in the northern part of the Kyzylkum desert 130 km south of the Baikonur Cosmodrome (coordinates of the center of impact: 44°34′24.7 ″N, 62°58′13.9 ″E). An explosion of rocket fuel components (heptyl and amyl) occurred at the launch site. As a result of the explosion, the so-called "Big Sinkhole" was formed with a diameter of 50 m, depth of 15 m and a berm height of 2.5 m.

Heavy metal pollution. Balkhash region (7) has large deposits of polymetallic ores, coal, construction materials, which are actively developed; its pollution is also related to the operation of copper smelting and zinc smelting plants, which cause uncontrolled pollution. Pollution of surface waters of Lake Balkhash is noted for copper (10.0 MAC), zinc (1.1 MAC), nickel (5.93 MAC), magnesium (7.98–8.6 MAC) and others [19].

Pesticide pollution (8). The survey sites included former warehouses of obsolete stocks of unutilised pesticides, adjacent territories and water bodies of settlements of Kerbulak and Talgar districts (Kyzylkairat, Amangeldy, Enbekshi, Belbulak, Beskainar). In Taukaraturuk settlement of Almaty oblast there are no warehouses of unutilised pesticides, but pesticides were used on former agricultural lands more than 20 years ago.

Radiation contamination. At the end of the 80's at the Suluchekinskoye uranium deposit there was experimental uranium mining by in-situ borehole leaching, which was soon completely stopped, the boreholes were liquidated and reclamation was carried out. However, in the contour of the self-injecting well, located within the boundaries of the seismic station "Kalkan" (9) (State Research and Production Enterprise "Altyn-Emel"), the EDR values were established in the range (0.462–4.158 μSv/h), which noticeably exceeds the

permissible values (EDR - 0.094–0.200 μSv/h).

Control region. (10) The Alakol region includes Lake Alakol and its surroundings. It is a saline, drainless lake located on the border of Almaty and East Kazakhstan oblasts. There is a protected biosphere Alakol Reserve on the entire lake area.

2.4. Cytogenetic studies of animals

Blood sampling and preparation of smears were carried out in the field in accordance with the proposed recommendations [20,21]. Peripheral blood samples were taken from the studied animals to perform laboratory hematological studies. The method of blood collection depended on the species and size of the animal. In fish, blood was taken from the heart, gill vein, tail artery, by cutting off the tail. In reptiles by cutting off the claw phalanx of the finger of the hind limb or the tip of the tail. In large amphibians by cutting off the claw phalanx of a finger of the hind limb, in small amphibians, from the heart or after decapitation. In birds, blood was collected from underwing vessels using aseptic solution. If possible, blood was collected from the animals under study without removing them from the environment.

Sample processing was performed in laboratory conditions. Peripheral blood smears were fixed in 96 % ethyl alcohol for 30 min, dried and stained with 4 % Romanowsky-Giemsa solution for 20 min.

The frequency of micronuclei and cytological abnormalities was recorded in normochromic erythrocytes of peripheral blood on a "Zeiss AxioLab A.1" microscope under oil immersion and magnification 16x100. During cytogenetic examination all erythrocyte structure abnormalities differing from normal morphology were recorded [22]. 10–20 thousand erythrocytes were examined from each examined individual. Photodocumentation was made of the most characteristic and interesting abnormalities of peripheral blood erythrocytes at x100 magnification.

2.5. Statistical analysis

For statistical analysis, the arithmetic mean and its deviation (M±SE%) were calculated. The reliability of the mean difference was assessed on the basis of Student's t-test. The statistical significance threshold of $p \leq 0.01$ was applied. Statistical and correlation analyses of data were performed using Excel (Microsoft Corporation, Redmond, Washington, DC, USA). Data were tested using Pearson correlation, Spearman correlation.

3. Results and discussion

3.1. Spontaneous level of micronuclei and nuclear abnormalities in peripheral blood erythrocytes of the studied animals

The main method of environmental monitoring is the comparative analysis of control and tested animal populations. The complexity of comparative analyses lies in the fact that different animal species have different spontaneous frequency of cytogenetic abnormalities [23,24] and sensitivity to various anthropogenic factors. Moreover, the frequency of cytogenetic abnormalities in fish such as *Cyprinus carpio* depends on habitat temperature [25]. And Tomazelli et al. (2022) showed that the frequency of micronuclei and nuclear abnormalities was greater in omnivorous birds. This demonstrates that the physiological and ecological characteristics of each species may affect the result of biomonitoring differently.

To identify the spontaneous level and mutagenic influence on living organisms as a result of exposure to unfavourable environmental situation, cytogenetic characteristics of blood of the most common animal species in Kazakhstan were used as biomarkers.

The results of the study of micronuclei levels and various nuclear abnormalities in control populations of different animal species with nuclear erythrocytes (fish, amphibians, reptiles, birds) are presented in Table 1.

In different fish species, even within the same family, the level of micronuclei differed 2–3 times. In representatives of the largest family *Cyprinidae*, the average frequency of cells with micronuclei was 0.032 ± 0.006 % and varied from 0.015 to 0.06 %. The discrepancies in control indicators of micronuclei frequency in erythrocytes of fish across different literature sources can vary by orders of magnitude, even when considering studies on the same species. Many researchers have shown that the background level of micronuclei in fish is 0.5–1‰ [20,26]. The proportion of cells containing micronuclei in a sample of silver carp inhabiting the Tom River averaged 0.057 ± 0.030 %. The similar indicator of crucian carp inhabiting Lake Azhendarovo was 0.13 ± 0.07 ‰ [27]. At the same time, other authors demonstrate micronucleus frequency rates of 0.25 ± 0.03 % in Ukrainian scaly carp [28] or 18.12 ± 0.15 ‰ in pikeperch [29] and 2.91 ± 0.15 % in bream from the Volga-Caspian Canal [30].

The spectrum of cytological abnormalities in cytomic analysis included erythrocytes with micronuclei; binuclear erythrocytes; amitosis; erythrocytes with cytoplasmic abnormalities in the form of "tail", invagination of cytoplasm, invagination of nuclear envelope, and others. Of about 20 fish species studied, the highest frequency of cytological abnormalities was registered in the Amur bitterling and Caspian goby. The frequency of erythrocytes with micronuclei for a particular species does not depend on the level of cytological abnormalities. The results of cytogenetic analysis of *Rhodeus sericeus* are the best illustration of this. The frequency of micronuclei in representatives of this species is at the average level (0.02 %). But the frequency of nuclear abnormalities in them is very high with formation of all kinds of combinations of binuclearity with invagination of nuclear envelope. Probably, this is a species trait not related to the influence of any factors.

Pelophylax ridibundus was studied in two control regions. It is shown that the frequency of micronuclei in erythrocytes of both populations of lake frogs practically does not differ. In ponds with flowing water from artesian sources it is 0.19 ± 0.010 %, in Alakol region - 0.20 ± 0.007 %. At the same time, it should be noted some level of heterogeneity of cytogenetic indices in the studied groups of

Table 1
Spontaneous level of cytogenetic abnormalities in erythrocytes of animals with nuclear erythrocytes captured in control territories.

Animal species	Number of animals	Total cells	MN, %	Amitosis, %	Poikilo-cytosis, %	Nuclear invagination, %	Binucleated, %
Fish							
Pseudorasbora parva	6	60000	0.027 ± 0.006	0.006 ± 0.003	0	0	0.002 ± 0.002
Diptychus dybowskii	5	25000	0.024 ± 0.01	0	0	0.004 ± 0.004	0
Rutilus caspicus	5	50000	0.04 ± 0.010	0.014 ± 0.005	0.010 ± 0.005	0.023 ± 0.005	0.008 ± 0.004
Cyprinus carpio	10	100000	0.06 ± 0.02	0.04 ± 0.01	0	0.06 ± 0.02	0
Cyprinus	10	100 000	0.02 ± 0.01	0.01 ± 0.002	0	0.2 ± 0.03	0
Aspius aspius	4	40000	0.027 ± 0.008	0.035 ± 0.009	0.003 ± 0.002	0.01 ± 0.005	0.01 ± 0.005
Rutilus rutilus	8	80000	0.015 ± 0.005	0.003 ± 0.002	0.001 ± 0.001	0.003 ± 0.002	0.002 ± 0.002
Carassius carassius	8	80000	0.025± 0.005	0.015± 0.004	0	0.022± 0.005	0.017± 0.004
Blicca bjoerkna	8	80000	0.051 ± 0.008	0.005 ± 0.002	0.005 ± 0.002	0.006 ±0 .002	0.001 ± 0.001
Scardinius erythrophtha-lmus	10	100000	0.024± 0.005	0.008± 0.003	0	0	0
Abramis brama	5	50000	0.06 ± 0.01	0.02 ± 0.006	0.02 ± 0.006	0.02 ± 0.006	0
Pomatoschistus microps	10	100000	0.02 ± 0.004	0	0	0	0
Rhodeus sp.	5	50000	0.02 ± 0.006	0	0	2.2 ± 0.06	2.5 ± 0.07
Gobius macro-phthalmus	11	110000	0.036± 0.007	0.012± 0.004	0.025± 0.006	0.046± 0.007	0
Esox lucius	3	30000	0.01 ± 0.005	0	0	0	0
Perca schrenkii	18	180000	0.03 ± 0.004	0.011 ± 0.002	0	0.007 ± 0.002	0.006 ± 0.001
Salmo trutta	10	100000	0.02 ± 0.01	0.10 ± 0.03	0	0.03 ± 0.01	0
Salvelinus alpinus	4	40000	0.05 ± 0.01	0.02 ± 0.007	0.06 ± 0.01	0.06 ± 0.01	0
Anampses meleagrides	15	150000	0.042± 0.005	0.008± 0.002	0.05± 0.006	0.03± 0.004	0.003± 0.001
Amfibians							
Bufotes perrini	4	50000	0.13 ± 0.005	0.006 ± 0.003	0.005 ± 0.003	0.04 ± 0.01	0.01 ± 0.007
Pelophylax ridibundus	38	760 000	0.2± 0.070*	0.006 ± 0.001	0.06 ± 0.01	0.04 ± 0.02	0.04 ± 0.02
Reptiles							
Eremias velox	15	750000	0.023 ± 0.011	0.02 ± 0.008	0.003 ± 0.003	0.08 ± 0.019	0.007 ± 0.004
Eremias lineolata	5	25000	0.025 ± 0.014	0	0.003 ± 0.002	0.1 ± 0.02	0.01 ± 0.006
Eremias intermedia	4	40000	0.03 ± 0.008	0.03 ± 0.008	0	0.1 ± 0.016	0
Trapelus sanguinolentus	4	40000	0.32 ± 0.027	0.003 ± 0.002	0.14 ± 0.016	0.47 ± 0.034	0.02 ± 0.001
Testudo horsfieldii	8	80000	10.85 ± 0,03	0	0.14 ± 0.013	3.05 ± 0.03	0
Birds							
Melanocorypha calandra	4	40000	0.10 ± 0.016	0.001 ± 0.001	0	0.075 ± 0.013	0
Motacilla alba	4	60000	0.025 ± 0.008	0.04 ± 0.01	0.025 ± 0.008		0
Motacilla flava	4	60000	0.015 ± 0.005	0		0.02 ± 0.008	0
Oenanthe isabellina	8	90000	0.06 ± 0.01	0	0.002 ± 0.002	0.03 ± 0.08	0
Charadrius leschenaultii	4	30000	0.07 ± 0.016	0.02 ± 0.008	0	0.02 ± 0.008	0.003 ± 0.008
Acridotheres tristis	5	50000	0.03 ± 0.008	0.005 ± 0.003	0.01 ± 0.004	0.005 ± 0.003	0.01 ± 0.004
Columba livia	10	100000	0.027 ± 0,005	0.013 ± 0.004	0.006 ± 0.002	0.08 ± 0.009	0.017 ± 0.005
Passer ammodendri	6	30000	0.06 ± 0.02	0.05 ± 0.014	0	0	0
Phylloscopus collybita	4	40000	0.02 ± 0.006	0.001 ± 0.001	0.002 ± 0.002	0	0
Curruca nana	4	40000	0.017 ± 0.006	0	0	0.03 ± 0.008	0

frogs. According to the literature data in lake frogs, control indices vary from 0.05 to 0.1 % [3,31] to 0.27 ± 0.03 % [32]. The spectrum of cytological abnormalities was represented by single cells with cytoplasmic abnormalities in the form of a "tail" and invagination of the nuclear envelope.

The level of micronuclei in the background area of Eremias lizards was 0.023–0.03 %. In control populations of bisexual *D. raddei* and parthenogenetic *D. armeniaca* lizards, the level of micronuclei in erythrocytes ranged between 0.06 ± 0.04 and 0.20 ± 0.13 ‰ (Simonyan et al., 2018). The spontaneous frequency of micronuclei determined by *Tupinambis merianae* was 0.95 ± 0.27 ‰ [33]. *Trapelus sanguinolentus* showed a relatively high level of both micronuclei (0.32 ± 0.027 %) and nuclear anomalies - various forms of invagination of the nucleus (0.47 ± 0.034 %). The Central Asian tortoise (*Testudo horsfieldii*) shows a very high level of micronuclei and invagination of the nucleus (Table 1). At the same time, demonstrating rather high heterogeneity of the studied individuals by these parameters. In addition, we excluded from the analysis several individuals (visually healthy), in which the micronuclei frequency was 30–40 %. This is probably an individual peculiarity of the species. At such levels of cytogenetic abnormalities in background areas, this species is not recommended as a bioindicator species.

Analysis of several species of natural bird populations revealed a range of variation in micronucleus frequency of 0.015–0.1 %. According to literature data, micronucleus frequency in different bird species varies from 0 to 0.8 ‰ [34], which is consistent with our data. However, due to the vast habitat area and mobility of birds, they are not often used for monitoring studies.

Table 2 presents a comparative analysis of the spontaneous level of cytogenetic disorders in the control areas of the main bioindicator species used by different authors for monitoring studies. Even within the same species (e.g. *Pelophylax ridibundus*), the spontaneous level differs by 2–3 times among different authors. These differences probably depend on the region, the degree of "cleanliness" of the control area, and the researcher himself. Therefore, comparative analysis of the results obtained by different authors should be carried out with a certain degree of skepticism.

3.2. Study of micronuclei and nuclear anomalies in bioindicator species in areas with different types of pollution

Fish. Fish are considered the most appropriate target for screening mutagenic and carcinogenic chemical compounds in water because they metabolise, accumulate chemicals in water and react to toxic compounds in a similar way to higher vertebrates. The use of fish as aquatic bioindicators is for the following reasons: fish have a long life cycle, can be used for marine and freshwater genotoxicity analyses, aquaculture assessment and *in situ* in laboratory conditions with different periods of exposure to toxicants [40]. Fish inhabiting natural water bodies are widely used as bioindicators of cumulative exposure to aquatic pollutants [2,41]. The frequency of micronuclei (MN) in fish is a sensitive biomarker for detecting genotoxic damage induced by petrochemicals, heavy metals and pesticides [42–46]. Fish exposed to pesticide, petrochemical and radiation impact were studied. The results of the study of micronuclei level and various nuclear anomalies in fish living in water bodies with different types of pollution are presented in Table 3.

Cytogenetic analysis of fish caught near the storage sites of old, unutilised and banned pesticides revealed a statistically significantly increased ($p \leq 0.001$) frequency of erythrocytes with micronuclei and nuclear cell abnormalities compared to the control. A high level of cytological abnormalities was detected not only in ichthyofauna caught near the warehouse with unutilised pesticides

Table 2
Comparative analysis of the spontaneous level of cytogenetic disorders in the main bioindicator species in the control territories according to the literature data.

Animal species	MN, %	Amitosis, %	Poikilocyto-sis, %	Nuclear invagination, %	Binucleated, %
Fish *(Cyprinidae)*					
Catla catla [35]	0.8 ± 0.29				
Catla catla [36]	0.89 ± 0.06	0.10 ± 0.01	0.10 ± 0.04	N- 0.15 ± 0.10	0.35 ± 0.01
Cyprinus carpio [25]	0.29 ± 0.05			0.45 ± 0.09	0.20 ± 0.09
Carassius gibelio [26]	0.93 ± 0.23				
Labeo rohita [36]	0.50 ± 0.18	0.55 ± 0.00	0.60 ± 0.17	N- 0.35 ± 0.10	0.15 ± 0.00
Cirrhina mrigala [36]	0.24 ± 0.12	0.82 ± 0.02	0.10 ± 0.01	N-0.82 ± 0.04	0.24 ± 0.02
Ctenopharyngodon Idella [36]	1.97 ± 0.39	0.20 ± 0.03	0.49 ± 0.11	N- 0.30 ± 0.02	0.10 ± 0.01
Abramis brama [26]	0.75 ± 0.48				
Amphibians					
Bufotes variabilis (Özgül, 2020) [32]	0.21 ± 0.16	1.94 ± 1.20		N- 2.45 ± 0.70 K-1.12 ± 0.71	0.05 ± 0.07
*Pelophylax ridibundus (*Cördük, 2018) [37]	0.16 ± 0.06	0.61 ± 0.22		N-0.96 ± 0.36 K-0.064 ± 0.01	
Pelophylax ridibundus [38]	0.07 ± 0.1	0.09 ± 0.1		N-0.03 ± 0.0 K-0.05 ± 0.0	0.01 ± 0.0
Pelophylax ridibundus (Özgül, 2020) [32]	0.27 ± 0.21	2.03 ± 0.86		N- 1.49 ± 0.76 K-0.76 ± 0.41	0.21 ± 0.60
Pelophylax Ridibundus [3]	0.07 ± 0.01			N- 0.05 ± 0.01 K-0.02 ± 0.01	
Birds					
Columbina picui [39]	0.87 ± 0.19			N-1.77 ± 0.84	0.35 ± 0.18
Zenaida auriculate [39]	0.40			N- 2.50	0.10

N - Notched; K - Kidney shaped; L - Lobed.

Table 3
Results of cytogenetic analysis of erythrocytes of fish caught in water bodies with different types of pollution.

Place of capture	Animal species	Number of animals	Total cells	MN, %	Amitosis, %	Poikilo-cytosis, %	Nuclear invagina-tion, %	Binuclea-ted, %
Pesticide contamination								
Water bodies in the vicinity of pesticide warehouses	*Pseudorasbora parva*	45	376000	0.17± 0.010[a]	0.027± 0.003[a]	0.006± 0.001	0.007± 0.001	0.003± 0.001
Control		6	60000	0.027 ± 0.006	0.006± 0.003	0	0	0.002± 0.002
Water bodies near agricultural plantations	*Diptychus dybowskii*	37	364 000	0.13± 0.006[a]	0.001± 0.001	0.006± 0.001	0.01± 0.002[a]	0
Control		5	25000	0.024 ± 0.009	0	0	0.004± 0.004	0
Radiation contamination								
Water body near a mothballed uranium mine	*Anampses meleagri-des*	10	200000	0.076 ± 0.006[a]	0.034± 0.006[a]	0	0	0.019± 0.003*
Control		15	150000	0.042 ± 0.005	0.008± 0.002	0.05± 0.006	0.03± 0.004	0.003± 0.001
Water body near a mothballed uranium mine	*Pseudorasbora parva*	8	160000	0.053 ± 0.006[a]	0.03± 0.004[a]	0	0	0.023± 0.004*
Control		6	60000	0.027 ± 0.006	0.006± 0.003	0	0	0.002± 0.002
Petrochemical contamination								
Atyrau	*Scardinius erythroph thalmus*	13	130000	0.048 ± 0.004[a]	0.0124± 0.002[a]	0.0005± 0.0005	0.017± 0.003	0.007± 0.002
Control		10	100000	0.024 ± 0.005	0.008± 0.003	0	0	0
Aktau	*Rutilus rutilus*	10	10000	0.076 ± 0.010[a]	0.012± 0.004[a]	0.013± 0.004[a]	0.018± 0.004[a]	0.002± 0.001
Control		8	80000	0.015 ± 0.005	0.003± 0.002	0.001± 0.001	0.003± 0.002	0.002± 0.002
Bautino Bay	*Gobius macroph-thalmus*	11	110000	0.071 ± 0.008[a]	0.021± 0.005	0.049± 0.005[a]	0.067± 0.008	0.012± 0.003
Control		4	40000	0.036 ± 0.007	0.012± 0.004	0.025± 0.006	0.046± 0.007	0
Bautino Bay	*Rutilus caspicus*	10	100000	0.196 ± 0.014[a]	0.071± 0.008[a]	0.021± 0.005	0.025± 0.005	0.067± 0.008
Control		5	50000	0.04± 0.010	0.014± 0.005	0.010± 0.005	0.023± 0.005	0.008± 0.004

[a] $p \leq 0{,}01$ between biomonitoring cytogenetic analysis data and relevant control data.

(0.17 ± 0.01 %), but also in the water body (0.13 ± 0.006 %), where there are no pesticide warehouses nearby, but pesticides were used on agricultural lands in the past (more than 20 years ago) [47].

Currently, control of radioactive contamination of the surface layer of the atmosphere on the territory of Almaty region is carried out by specialised units of RSE "Kazgidromet". According to their data in Almaty region average values of radiation gamma background of the surface layer of the atmosphere are within 0.12–0.14 μSv/h, which does not exceed the natural background. However, the relatively favorable radioecological condition in general in Almaty region does not exclude the probability of local radioactive contamination. A number of studies show that in areas where uranium mining operations were carried out within the boundaries of the Iliysk uranium ore province, local radioactive contamination may manifest itself over time for various reasons of natural and anthropogenic origin [48–50]. Such a situation occurred at the site of exploration, short-term operation, conservation and liquidation of the pilot mine at the Sulucheki uranium deposit. In the reservoir, in the contour of the self-pouring well, EDR values in the range of 0.462–4.158 μS/h were established, which noticeably exceeds the permissible values.

The micronucleus test results of fish from this reservoir revealed an increased ($p \leq 0.01$) frequency of erythrocytes with micronuclei

and other cytological abnormalities compared to fish from control populations. In this case, the nuclear and cytoplasmic abnormalities detected are the result of exposure to gamma radiation inducing gene- and cytotoxicity, similar to the findings of [35] and [49]. Meanwhile, a study of two fish species revealed an interspecific difference in the frequency of cytogenetic abnormalities in spotted thicklip loach and stone moroko.

In erythrocytes of fish caught in the Caspian region, where the main polluting factors are petrochemical compounds and heavy metals, the frequency of micronuclei and cytological abnormalities also statistically significantly ($p \leq 0.01$) differs from the control values.

In fish exposed to different ecotoxicological impact, not only the increased frequency of cytological disorders compared to the control, but also a wider range of them was revealed. Changes in erythrocyte morphology were revealed in *Rutilus caspicus*; different representatives had erythrocytes with a more rounded shape, light-coloured nucleus and cytoplasm, while others, on the contrary, had more elongated cells and dark cytoplasm (the method, staining time and dye concentration were identical). It is difficult to say what these differences are related to at this stage, perhaps it is due to a physiological condition or some disease. Some specimens of *Rutilus caspicus* showed vacuolisation of erythrocyte cytoplasm (up to 3 %) and invagination of nuclei (up to 1 %). In one specimen of this species, up to 20 % of erythrocytes at different stages of degradation were recorded, while in most of the analysed fish this indicator did not exceed 0.5–1%. The presence of these disorders with a certain statistical frequency can be considered as evidence of manifestation of chronic toxicosis of fish organism.

According to the frequency of occurrence of degenerative disorders in the analysis of erythrocytes of peripheral blood of fish, their greatest number was recorded in petrochemical pollution. Thus, representatives of ichthyofauna react sensitively to different types of pollution, but different cytogenetic sensitivity is observed for different species, even within the same family.

Amphibians. Amphibians also fulfil all the requirements for species used for bioindication. Such characteristics as the biphasic life cycle of these animals, in which they are exposed to both aquatic and terrestrial environments, make them universal bioindicators (Gregorio et al. 2019). At the same time, they reflect the local habitat condition, as they have no pronounced tendency to migrate. Their semi-permeable skin and physiological adaptation to live in very specific microhabitats allow amphibians to be successfully used as bioindicator species in monitoring environmental pollution and the degree of (geno)toxicity of anthropogenic factors [51–56]. We used one of the main representatives, the lake frog (*Pelophylax ridibundus*), to carry out bioindication. It is a widespread amphibian species that has distinct and convenient traits for investigation, is highly sensitive to pollutants and mutagens [38]. Lake frogs for cytogenetic analysis were captured in pesticide contaminated areas, in areas where 1st stages of launch vehicles fell during launching of spacecraft with different types of fuel, in areas contaminated with petroleum products and heavy metals (Table 4).

In lake frogs caught in all regions with different types of pollution there is a significantly increased frequency of erythrocytes with micronuclei compared to control regions ($p \leq 0.01$). The spectrum of cytological disorders under the influence of genotoxicants included - binuclear erythrocytes; amitosis; erythrocytes with various disorders of cytoplasm, invagination of cytoplasm; invagination of nuclear envelope, nuclear-free cells, and vacuolysis.

Table 4
Results of cytogenetic analyses of *Pelophylax ridibundus* caught near sites with different types of pollution.

Place of capture	Total cells	MN, %	Nucleus-free cells, %	Amitosis, %	Poikilocytosis, %	Nuclear invagination, %	Binucleated, %	Vacuolisation, %
Pesticide contamination								
Water bodies in the vicinity of pesticide warehouses	180000	0.47± 0.016[a]	0.01± 0.005	0.08± 0.007	0.19± 0.010	0.08± 0.007	0.03± 0.004	0.028± 0.004
Water bodies near agricultural plantations	300000	0.32± 0.014[a]	0.003± 0.003	0.005± 0.002	0.073± 0.007	0.017± 0.007	0	0
Heavy metals								
Balkhash region	106915	0.71± 0.01	0.024± 0.005	0.015± 0.004	0.022± 0.004	0.016± 0.004	0.06± 0.007	0.03± 0.005
Petrochemical contamination								
Atyrau	110 000	0.59± 0.02[a]	0.03± 0.005	0.07± 0.008	0.2± 0.013	0.1± 0.01	0.05± 0.007	0.03± 0.005
Kulsary	100000	0.39± 0.02[a]	0.02± 0.004	0.04± 0.005	0.1± 0.01	0.15± 0.011	0.02± 0.004	0.02± 0.004
Rocket and space activities								
Soyuz-2 LV 1st stage fall area, Kostanay region	80000	0.29± 0.06	0.025± 0.005	0.29± 0.06	0.17± 0.01	0.1 ± 0.01	0.1± 0.01	0
Proton LV 1st stage fall area, Karaganda region	4000	0.35± 0.09	0	0.1± 0.05	0.1± 0.05	0.15 ± 0.06	0	0
Control regions								
Clean ponds with running water	200000	0.19± 0.010	0.01± 0.002	0.005 ± 0.001	0.05± 0.01	0.05 ± 0.01	0	0
Alakol region	540 000	0.2± 0.070[a]	0.01± 0.001	0.008 ± 0.001	0.07± 0.01	0.03 ± 0.002	0.04± 0.002	0

[a] $p \leq 0.01$ between biomonitoring cytogenetic analysis data and control data.

Reptiles. Reptiles are also sensitive bioindicators of environmental pollution [57]. This is particularly relevant when analysing the impact of anthropogenic factors in desert and semi-desert regions, where the choice of indicator animals is very limited [58]. Depending on the need to assess a particular habitat, different reptile species are used as bioindicators, including snakes, crocodiles [59], turtles [60], and various species of lizards. They are particularly sensitive to heavy metal and pesticide pollution.

The species *Eremias velox* from the genus *Eremias* were used as indicator animals in the analysis of the consequences of rocket-space activity on the territory of Kazakhstan (the area of the fall of side stages of Soyuz-2 LV and the accident of RS-20 Dnieper LV). Cytogenetic indices characterising the genetic status of herpetofauna representatives were determined for them (Table 5).

The frequency of micronuclei in erythrocytes of examined lizards in the area of the Dnieper LV crash exceeds the control values almost 12 times ($p \leq 0.01$) and there is a significant ($p \leq 0.01$) excess of the frequency of cytological abnormalities. In the area where the side stages of the Soyuz-2 LV crashed, the frequency of micronuclei is increased 1.5 times compared to the control and does not have such serious environmental consequences as at the site of the Dnieper LV crash.

Birds. Birds are also quite sensitive bioindicators of the impact of various environmental factors. And, despite some difficulties in their use, the assessment of genotoxicity of the environment with the evaluation of cytogenetic characteristics of bird erythrocytes finds its application in some studies [61,39]. Synanthropic species that are ecologically related to human settlement are often used to assess the quality of the human environment. The frequency of micronuclei in different bird species varies from 0 to 0.8 ‰ [34]. As an indicator species, a convenient target is the rock dove (*Columba livia*), a widespread polytypic species whose natural range covers a large part of Eurasia and North Africa. The attachment of rock pigeons to their nesting sites creates conditions for the long-term impact of environmental factors on these animals and makes them a suitable object for biomonitoring of the ecology of their habitats (del Hoyo, Collar, 2014).

Capture and cytogenetic analysis of erythrocytes of *Columba livia*, nests of which are located near the sites of disposal of unutilised pesticides revealed a significant (0.05 ± 0.07 %) ($p \leq 0.05$) excess of micronuclei frequency compared to the control (0.027 ± 0.005 %) and a slight increase in cytological disorders. During cytogenetic analysis of erythrocytes, the following disorders were predominantly recorded: binuclear erythrocytes; displacement of the nucleus; erythrocytes with cytoplasm disorders in the form of "tail", invagination of the nuclear envelope.

3.3. Analysis of nuclear cellular abnormalities in the examined animals

The results obtained by studying micronuclei in erythrocytes of peripheral blood of bioindicators (fish, amphibians, birds) are quite correlated with the level of detected pollutants. Thus, the total concentration of POP-pesticides in water and soil samples taken from former pesticide storage areas was 8.9 mg/kg and exceeded MAC 60 times. In the vicinity of former agricultural lands, where pesticides have not been used for more than 20 years, the total concentration of POPs was 1.7 mg/kg, and the concentration of individual POP-pesticides in soil exceeded the MAC 17 times. It should be noted that the ratio of DDT and its derivatives allows to approximately estimating the time of appearance and decomposition of residual concentrations of pesticides contained in soils. Thus, the ratio (DDE + DDD)/DDT> 1 indicate the "old" use of DDT and its active transformation by microorganisms.

From five points of Ile-Balkhash region (Balkhash town, Kapshagay town, Priozersk town, Bakanas settlement and Chunja settlement) (Table 2 presents data of average frequency of cytogenetic disorders) the highest frequency of cells with micronuclei in erythrocytes of frogs' blood was found in Balkhash town and Kapshagay town (0.84 ± 0.02 and 1.09 ± 0.02 %, respectively). These results are quite natural, based on the level of pollutants emitted by the smelting plant in Balkhash city and SDPP in Kapshagai city. In the other settlements there is also a significantly increased frequency of erythrocytes with micronuclei (0.45–0.67 %). The main pollutants in Ile-Balkhash region are heavy metals. In water up to 24 MPC of cobalt, in silt - nickel (1.7 MPC), chromium (1.7–7.5 MPC), cadmium (1.4–1.6 MPC), cobalt (1.6–1.9 MPC). These data reflect the general level of pollution in the Ile-Balkhash basin.

To assess the sensitivity of the studied bioindicators, a comparative analysis of the micronucleus test data and the results of genotoxicity assessment of water samples taken in the places of animal capture in model experiments were carried out. The correlation dependence (+0.88) of the content of cells with micronuclei in erythrocytes of frog blood and the frequency of chromosomal aberrations in peripheral blood lymphocytes of humans at *in vitro* exposure to the studied water samples was revealed.

Table 5
Results of cytogenetic analysis of *Eremias velox* exposed to the effects of results of space activity.

Variant	Total cells	MN, %	Nuclear invagination, %	Amitosis, %	Poikilocytosis, %	Vacuolization, %	Binucleated, %
Accident area of LV Dnieper	120000	0.275 ± 0.01[a]	0.175 ± 0.01	0.05± 0.006[a]	0.20± 0.01	0.0875± 0.008	
Soyuz-2 rocket booster stage 1 drop region	60000	0.037 ± 0.008[b]	0.086 ± 0.01	0.02± 0.005	0.007± 0.003		0.003 ± 0.002
Control	30000	0.023 ± 0.009	0.08 ± 0.019	0.02± 0.008	0.003± 0.003		0.007 ± 0.004

[a] $p \leq 0.01$ between biomonitoring cytogenetic analysis data and control data.
[b] $p \leq 0.05$ between biomonitoring cytogenetic analysis data and control data.

The level of cytogenetic disorders in indicator animals (fish and lake frogs) caught in the Caspian region (Atyrau city, Aktau city, Bautino Bay) reflects the pollution of this region by alkanes, oil products (2–6 MAC) and heavy metals (cadmium, lead, nickel - ≥2 MAC) determined in water and bottom sediment samples.

The impact of rocket and space activities is also reflected in the cytogenetic status of indicator animals (amphibians, reptiles). The least impact is caused by the development and jettisoning of rocket stages, including the minimum impact of the Soyuz-2 booster stage

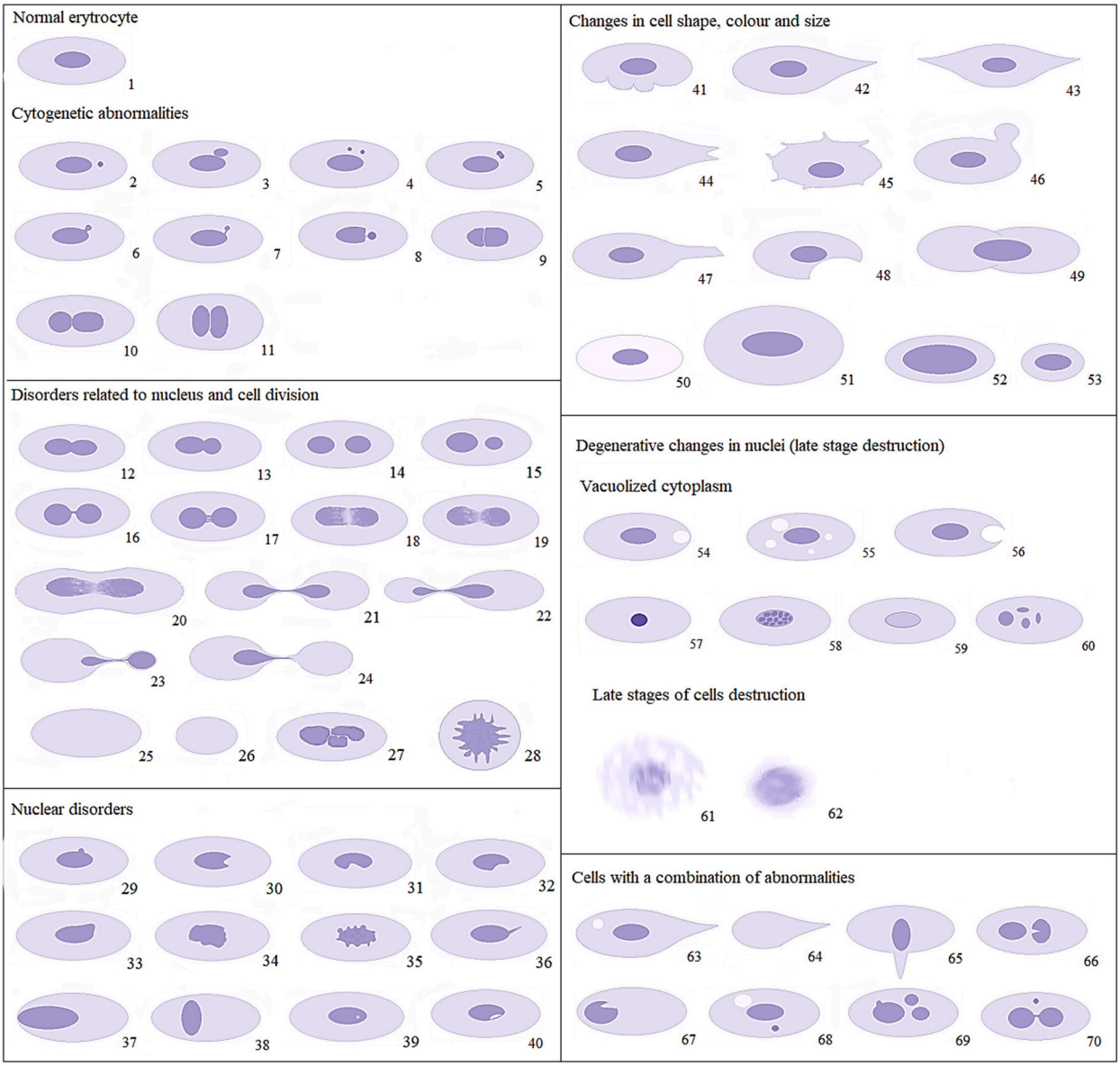

Fig. 2. Schematic representation of erythrocytes with micronuclei and nuclear abnormalities. 1 - normal erythrocyte. **Cytogenetic disorders:** 2 - erythrocyte with micronucleus; 3 - erythrocyte with large micronucleus adjacent to the main nucleus; 4 - erythrocyte with two micronuclei; 5 - unformed nuclear material; 6, 7 - micronuclei connected by chromatid bridge; 8–11 - protrusions. **Disorders related to nucleus and cell division:** 12, 13 - lobed nuclei; 14, 15 - binucleated erythrocytes; 16, 17 - binucleated erythrocytes connected by one or more nucleoprotein bridges; 18–24 - amitosis; 25, 26 - nuclear-free erythrocytes; 27 - fragmentation of the nucleus into no more than 3–4 structures, but not apoptosis; 28 - cell in the bloodstream at the stage of mitosis. **Nuclear disorders:** 29 - nuclear buds; 30–34 - invagination of nuclear envelope; 35 - bubbling nucleus; 36 - nucleus with nucleoprotein tail; 37, 38 - displacement of nuclei - the nucleus is located not in the center, but displaced to the edge of the cell, sometimes in contact with the envelope (walled nucleus); 39 - nuclear vacuole; 40 - perinuclear vacuole. **Changes in shape, colour and size of cells:** 41–49 - poikilocytosis; 50 - hypochromia; 51 - macrocyte; 52 - enlargement of the nucleus; 53 - microcyte. **Degenerative changes:** 54–56 - vacuolised cytoplasm; 57 - karyopicnosis; 58 - karyorrhexis (chromatin condensation); 59 - karyolysis; 60 - apoptosis; 61, 62 - destruction of nuclei and cells. 63–70 - **cells with a combination of disorders**. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 6
Overview of terminology used for nuclear abnormalities in animal nuclear erythrocytes.

Appearance	Name variants	Our offer
	**blebbed nucleus** [36,37,41,54,67–69]; **nuclear bud** [39,70–74] **asymmetrical constricted nucleus** (if the protrusion is large) [69] **con brote** (with sprout) [60]	**Nuclear bud**
	**Nucleoplasmic bridges** [39,72] **nuclear bud on filament** [73] **nuclear bud** [75]	**Nuclear bud on filament**
	**Nuclear tails** [39,72] **nuclear bud** [31]	**Nuclear (nucleoplasmic) tail**
	**kidney-shaped** [31,68,69,73] **kidney nucleus** [76] **notched nuclei** (31,72, 37,75]) **Reniform** [25,67,74,77] **with an even deeper cut – Lobed** [67] **hook shape nucleus** [68] **Notched nuclei** [31,36,39,41,54,69,71,76] **nuclear bud** [25] **Lobed nucleus** [75,77]	**Nuclear invagination**
		
	**Lobed nucleus** [31,41,71,74] **deshaped nuclei** [36] **Multilobulated** [69] **Segmented** [67]	
		
	**Lobed nucleus** [37,70]; **“eight” shape nucleus** [31]; **Symmetrical constricted nucleus** [69] **Eightshaped** [77] **Binucleated cell** [74]	**Lobed nucleus**
		
	**dumbble shaped nuclei** [36] **Binucleus** [31,39,71,76,72] **blebbed nucleus** [78] **Eight-shaped nuclei** [73] **notch** [71] **Amitosis** [66]	**Amitosis**
		
	**nucleoplasmatic bridge** [31,74] **Amitosis** [66]	
	**blebbed nucleus** [37]	**Blebbed nucleus**

with T-1 fuel (aviation paraffin), and somewhat greater impact by the development of Proton M stages with fuel - unsymmetrical dimethylhydrazine (UDMH or "heptyl"). The Dnieper LV accident in 2006 caused the greatest damage not only to the surrounding landscape, but also to indicator animals (herpetofauna). This is indicated not only by the frequency of micronuclei, but also by the number of cytological abnormalities. Similarly, the results of Cordük et al. (2018) showed that the amount of heavy metals contained in the stations was closely related to the percentage of total nuclear abnormalities and each type of abnormality in *P.ridibundus* erythrocytes.

The nuclear erythrocytes of vertebrates (birds, reptiles, amphibians and fish) are normally oval or rounded (in some fish species) with a centrally located oval or rounded nucleus that stains burgundy-violet when stained with Giemsa dye.

The size of erythrocytes is specific not only for each class, but also for the animal species (supplementary material, Figs. 1 and 2). The largest erythrocytes are in salamanders, and among them amphiuma, which has giant erythrocytes - up to 70 μm in length, the lake frog 22–24.5 μm, and the toad 27 μm. The erythrocytes of reptiles are smaller than those of most amphibians - 15–21 μm. For example, in crocodile - 20.6 μm, in lizards of the genus *Eremias* - 14.5–16 μm. Erythrocytes of fish and birds are smaller than those of reptiles and vary considerably. For example, in fish, from 9.5 × 7.5 μm in pikeperch to 19.0 × 11.5 μm in common crucian carp. The size of erythrocytes of birds varies within 9–16 μm. For example, in chickens they average 11-12 x 6–8 μm, in the common pigeon 13-15 x 7–8 μm. They are usually more elongated, with a similarly elongated nucleus that is not always smooth (slightly wavy).

The main emphasis in ecological monitoring is, of course, on the frequency of micronuclei. They arise in the process of cell divisions from: lagging acentric fragments resulting from structural disorders of chromosomes (clastogenic effect); whole chromosomes lagging behind in anaphase, which did not enter the daughter nuclei after the completion of mitosis telophase, due to the fact that they were not properly attached to the division spindle during the segregation process in anaphase (aneugenic effect). Micronuclei can also result from damage to the division spindle or centromere, from pathological mitosis, or from the first stage of nucleus fragmentation (karyorrhexis) during apoptotic cell death. It has been found that micronuclei can also be formed from fragmented chromosomal material representing remnants of the nucleoplasmic bridge destroyed in telophase [62,63]. There is strong experimental evidence in favour of each of these mechanisms. A nuclear envelope is formed around such chromosomal fragments and lagging chromosomes, and they become morphologically similar to nuclei, only smaller in size [64].

Nuclear budding, in which the nucleus has an outgrowth into the cytoplasmic space, can also be considered as another mechanism of micronucleus formation and as an independent process. In this process, the nucleus first forms a lobe, which then separates and forms a micronucleus - this process is well illustrated by micronuclei with irregular edges, pressed against the main nucleus. It has been suggested that the non-mitotic formation of micronuclei is a pathway of ejection of genetically defective chromatin [65].

Additional information about pathological processes occurring in response to genotoxic factors can be obtained by analysing the structure of cells that differ from the normal morphology characteristic of a given cell type and animal species. Nuclear anomalies may indicate not only degenerative processes in the cells in which they are observed, but also the fact of previous chromosomal aberrations as a result of which they were formed. Therefore, the use of cytomic analysis, a method of assessing nuclear and cellular abnormalities in vertebrate nuclear erythrocytes, increases its informativeness and importance in environmental monitoring. Nuclear abnormalities include nuclear budding, various types of invagination of the nuclear envelope, formation of nucleoplasmic bridges, protrusions of various kinds, binucleated erythrocytes, amitosis, "tail cells" and other often interrelated disorders [66].

The relative ease of detection of nuclear and cellular anomalies in animal nuclear erythrocytes and the great variety of their morphological forms led to the use of different names for the same or similar anomalies by different authors, or, on the contrary, different names are used for the same anomalies. Table 6 summarises the patterns of the most common anomalies and the names used by different authors to refer to them. This terminological ambiguity creates difficulties for comparative analyses of spontaneous and induced nuclear and cellular anomalies arising from different factors. We have taken the liberty of suggesting the use of some of the most common and some new names. Let us focus on the proposed new names.

The most frequent anomalies are jagged nuclei, nuclei with a dent, kidney-shaped nuclei, or not et al. regular in shape. But all of them are probably minimal and/or maximal manifestations of one process - invagination of the nuclear envelope. The subjective perception of different researchers regarding these anomalies varies, which is presented in the table in the form of mixing the names of these anomalies. In addition, none of the researchers perform comparative numerical changes for these anomalies separately. Therefore, it is advisable to summarise the frequencies of these anomalies with the definition of their name as invagination of the nuclear envelope or deformation of the nucleus.

The next group of anomalies, characterised by a diversity of opinions, includes cells with closely located nuclei, sometimes connected by nucleoplasmic bridges, nuclei connected by a wide or narrow strand of chromatin material (with or without involvement of the cytoplasm), and binuclear cells. But again, the subjective visualisation of different authors introduces certain discrepancies and many authors mix the manifestations of this process, taking into account as binuclear cells.

Closely located nuclei (possibly not of the same shape and size), sometimes merging at some distance, nuclei connected by nucleoplasm bridges - the term protrusion is used for similar types of abnormalities in the analysis of buccal epitheliocytes [79,80]. Broken egg type protrusion appears as a large micronucleus connected by a bridge of nucleoplasm. A protrusion of the "tongue" type is a similar disorder with 2–3 bridges of nucleoplasm [81]. Protrusions similar to micronuclei may be formed by chromosome fragments or lagged whole chromosomes in the case of division spindle disruption, the nuclear envelope around which is connected to the main nucleus envelope. There is also a suggestion that nuclear protrusions may be formed by budding of interphase nuclei. According to Nikiforov et al. [82], such formations are a consequence of closely spaced micronuclei, unbroken bridges and translocated chromosomes with abnormally long arms. In many cases, they may also result from disturbed nuclear division or alterations in the cytoskeleton of the cell. It has been shown experimentally that this leads to changes in the volume and shape of the nucleus, including the formation of protrusions [83].

Nuclei with nucleoplasmic tails are probably the result of the loss of micronuclei connected to the main nucleus by a nucleoplasmic bridge or broken bridges formed by aberrant chromosomes with abnormal behaviour in the anaphase of division. In this regard, protrusions, nuclei with tails similar to micronuclei should be attributed to cytogenetic disorders.

Nuclei bound by a wide or narrow tether of chromatin material are the result of amitosis, a simple division of the cell nucleus in two in the interphase state without a division spindle. The nucleus and the cell, pulling over, takes a dumbbell-like shape, the hereditary material is distributed not always evenly, but randomly. In this case, as a rule, a chromatin bridge of varying length and width between the dividing parts of the nucleus is observed. Nucleus division can also occur without cytoplasmic tugging, in which case the absence of cytokinesis leads to the formation of binuclear cells. The phenomenon of amitosis was first described by the German biologist Robert Remak in 1841, and the term was proposed by the histologist Walter Flemming in 1882. It is characteristic that under different physiological states of the organism, various forms of amitosis occur, which have their own species specificity. In this regard, various variants of the described anomaly are proposed to be called amitosis. The formation of other cellular anomalies - schistocytes and nuclear microcytes - is also associated with the phenomenon of amitosis. The formation of nuclear and nuclear-free microcytes is usually the result of completed amitosis. A nuclear microcyte is the result of relatively regular amitosis, while a schistocyte is the result of irregular amitosis. It occurs when cytokinesis is disrupted, with the nucleus moving to one of the poles of the cell from which the microcyte buds off, leaving part of the cytoplasm as a nucleusless cell. Cytological evidence for these phenomena is presented in the supplementary material, in the gallery of microphotographs. There is probably another mechanism for schistocyte formation as well, since nuclear-free erythrocytes of normal, full size have been recorded in the common pigeon.

Lobed nuclei (eight-shaped nuclei) with symmetrical or non-symmetrical parts of the nucleus. They have a symmetrical constriction perpendicular to the long axis of the nucleus as if tugging the nucleus without disturbing its integrity or significantly increasing its length. In the description of nuclear abnormalities in buccal epithelium, this anomaly is referred to as “circular notched nuclei”. The anomaly is formed during incomplete mitosis as a result of damage to the division spindle. This is probably the pathway (initial stage) of binuclear cell formation, so these anomalies should be considered separately.

As mentioned above, many authors interpret in one way or another connected nuclei in a cell as binuclear cells. Thus, many authors consider cells at the early (not completed) stage of amitosis, when the nuclear envelope is not formed in the place of their separation, nuclei that are close to each other and/or connected by chromatin strands (bridges) having a drop-shaped shape to be binuclear cells. It is suggested that cells with separately (at a distance) lying nuclei of round-oval shape with a clearly formed shell of these nuclei should be considered as binuclear cells.

Amitosis, lobed nuclei, and binuclear cells are indicators of proliferation. Perinuclear vacuole results from invagination of the nuclear envelope, with the formation of a rounded zone of discoloured cytoplasm and karyoplasm in the perinuclear space of stained cells. This disorder is considered a characteristic sign of cell necrosis, and is referred to the phenomena of early destruction of the nucleus. Vacuolisation of the nucleus - formation of rounded unstained cavities in the nucleus as a result of chromatin lysis is also referred to the signs of early nucleus destruction. Chromatin condensation is accompanied by the formation of clumps and strands, between which there remain cavities filled with karyoplasm.

On the basis of all the above, it is proposed to distinguish the following groups of disorders and their constituent abnormalities characteristic of animals with nuclear erythrocytes.

3.3.1. Cytogenetic disorders

- Micronuclei, one or more, of varying size and shape;
- Branching micronuclei (micronuclei connected by a chromatin bridge);
- Protrusions (adjacent nuclei of different shapes, nuclei connected by 1–3 chromatid bridges);
- Nuclei with a nucleoplasmic tail;
- A cell in the bloodstream at the stage of mitosis.

3.3.2. Cytological disorders

According to the nature and degree of manifestation of cytological disorders in nuclear erythrocytes, they are conditionally divided into 4 groups.

1. Nuclear disruptions
   - Deformation of the nucleus (invagination of the nuclear envelope). The nucleus has an irregular shape while maintaining normal dimensions. There is an internal or external invagination of the nuclear envelope of different shape and size, with many nuclear buds;
   - Walled nucleus. The nucleus is not located in the centre, as in a normal cell, but is displaced to the edge of the cytoplasm, sometimes in contact with the membrane;
   - Perinuclear vacuole - characteristic of the initial stages of nucleus destruction;
   - Vacuolised nucleus.
2. Disorders related to nucleus and cell division
   - Lobed nucleus, possibly an initial or incomplete stage of amitosis or binuclear cell formation;
   - Amitosis - simple division of the cell and nucleus without a division spindle, when, by tugging, it assumes a dumbbell-like shape;
   - Binuclear cells - there are two nuclei inside a normal-sized cell. The size and shape of the nuclei do not correspond to the norm, the chromatin structure is not abnormal;

- Fragmentation of the nucleus (but not apoptosis);
- Cells with a combination of abnormalities - red blood cells with 2–3 types of different abnormalities.

3. Changes in the shape, colour and size of cells
   - Poikilocytosis. Change in the shape of red blood cells. Pathological erythrocytes with outgrowths in the form of "tails" (drop-shaped cells), bulges, "bitten", etc. are observed. Their presence indicates a decrease in the elasticity of the cell membrane, characteristic of inhibition of erythropoiesis;
   - Hypochromia, the discoloured cytoplasm of a cell, indicates a lack of hemoglobin content in the cell;
   - Anisocytosis. A red blood cell with a reduced/increased (microcyte/macrocyte) size of the cell itself or its nucleus. Their formation is possible in the presence of various pollutants in the environment;
   - Nuclear-free erythrocyte (schistocyte).
4. Degenerative changes in cells and nuclei (late stages of nuclear destruction)
   - Vacuolised cytoplasm. There may be one to several vacuoles of different sizes in an erythrocyte. Sometimes there is a rupture of the plasma membrane of the cell by the vacuole. Associated with clearing the cell of harmful extracellular toxins. Characteristic of exposure to toxic factors or at the initial stages of the development of the pathological process in the body;
   - Karyopicnosis - Thickening of the chromatin of the nucleus, which becomes dark and structureless;
   - Karyorrhexis - disintegration of nuclear chromatin into separate structures. Intermediate stage of necrobiosis - between karyopycnosis and karyolysis;
   - Karyolysis is the loss of chromatin staining ability and its dissolution;
   - Apoptosis is the fragmentation of the nucleus;
   - Lysis is a late stage of cell destruction. The process that consists of the disintegration of the cell. The nucleus loses its structure, cytoplasm is often absent.

The specificity and severity of certain types of nuclear anomalies are characteristic of a certain class and species of animal. That said, in animals such as amphibians that have a biphasic life cycle, sensitivity may differ in the same species depending on life stage (tadpoles and adults) (Rana catesbeiana) [84]. In different classes of animals cytopathological changes of erythrocyte structure occur with different frequency and have a characteristic spectrum. According to the experience of our current and previous studies we can say that the greatest spectrum and frequency of cytological abnormalities in animals with nuclear erythrocytes is characteristic of fish - birds - reptiles in descending order, least of all in amphibians. In fish, with the greatest spectrum of abnormalities, most often occur disorders associated with invagination of the nuclear envelope, division of the nucleus - amitosis, binuclear cells, nucleusless cells, violations of the size and shape of cells, especially in the form of "tails" (droplet-shaped cells). In amphibians, erythrocytes with outgrowths in the form of "tails", initial stages of amitosis and nucleusless microcytes are the most common. Of the nuclear anomalies, invaginated, lobed, and nuclear kidney nuclei occur in descending order [3]. In the common toad, displacement of nuclei in cells is common. In fish and amphibians, there are differences in amitotic cell formation. Fish are characterised by a single long thin thrust between two nuclei, while amphibians are characterised by a thick, short thrust consisting of multiple thrusts of nuclear material. At vacuolysis of cytoplasm in fish contains from one to several small vacuoles, in lake frogs and toads there are very large vacuoles sometimes even with rupture of the cell membrane. Reptiles are characterised by poikilocytosis, bud nuclei and invagination of the nuclear envelope. In synanthropic bird species (*Columba livia* and *Acridotheres tristis*), micronuclei on the pedicle, segmentation of the nucleus, displacement of the nucleus, and invagination of the nuclear envelope are common. Amitosis and poikilocytosis are rare. In field species of birds, only invagination of the nuclear envelope was recorded among nuclear anomalies. At the same time, the frequencies of registered cytological disorders and micronuclei are not connected with each other by definite ratios and can be both the result of anthropogenic factors and species or individual peculiarity of the studied animal.

Publications on the results of testing various factors based on nuclear anomalies in animal nuclear erythrocytes usually do not contain detailed information and microphotographs of rare anomalies. However, this is of some interest and broadens the spectrum of detectable anomalies. To some extent we decided to fill this gap and in the supplementary material we present microphotographs of different variants of frequently occurring and rare nuclear and cellular anomalies. Their schematic representation is presented in Fig. 2.

4. Conclusion

The conducted study of spontaneous frequency of cytogenetic disorders in 36 species of animals with nuclear erythrocytes demonstrated different levels of abnormalities and specificity for each species and class of animals. Under certain conditions, all studied species can be used as bioindicators, depending on the objectives and climatogeographical characteristics of the areas. Benthic fish species, e.g. Gobius macro-phthalmus, are better suited to analyze bottom sediments, lizards in desert regions, etc. An exception for desert regions is Testudo horsfieldii, due to the high spontaneous micronucleus levels in this species. The study of individual representatives of fish, amphibians, reptiles and birds during ecological monitoring revealed the specificity of nuclear anomalies characteristic of different classes of animals. The greatest spectrum and frequency of cytological abnormalities in animals with nuclear erythrocytes are characteristic of fish - reptiles - birds and amphibians in descending order. The analysis of animals captured in the territories with different types of pollution revealed the greatest spectrum of arising nuclear abnormalities in petrochemical and pesticide pollution. A comparative description is made, schemes and microphotographs are presented, clearly demonstrating a wide range of cytologic anomalies of nuclear erythrocytes of animals of different classes. A review of the names of the main nuclear anomalies is carried out and variants of its ordering are proposed.

Taking into account not only micronuclei but also a wide range of cytological abnormalities provides additional information about

the processes occurring in response to environmental stressors and/or mutagens. In many cases, these changes accompany compensatory processes occurring in tissues, for example, during functional overload, starvation, poisoning, or denervation. In addition, nuclear and cytoplasmic abnormalities in erythrocytes of peripheral blood of the studied animals indicate the development of degenerative processes in the organism caused by the complex influence of exogenous and endogenous factors. The level of these abnormalities can serve as an indicator of the state of the organism of the studied animals and their adaptive capabilities.

CRediT authorship contribution statement

Oksana Cherednichenko: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Igor Magda:** Writing – review & editing, Validation, Methodology, Data curation. **Serikbay Nuraliyev:** Methodology, Funding acquisition, Formal analysis. **Anastassiya Pilyugina:** Visualization, Methodology, Data curation. **Dinara Azizbekova:** Writing – review & editing, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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