doi: 10.25750/1995-4301-2026-1-177-188

Assessment of the hereditary component of fluoride ecotoxic load

© 2026. V. I. Starichenko ORCID: 0000-0001-9744-916X,
Institute of Plants and Animal Ecology UB RAS,
202, Vos'mogo Marta St., Yekaterinburg, Russia, 620144,
e-mail: starichenko@ipae.uran.ru

Fluoride is one of the most toxic and widely spread industrial pollutants of the environment. Identifying the hereditary component of fluoride accumulation plays a special role in environmental issues of fluoride pollution, as contributing to maintaining its level across generations. However, the factors of individual variability of fluoride accumulation in mammals, in particular, the hereditary component of accumulation variability, have not been identified. This is due to the methodological features of studying fluoride deposition parameters that are inaccessible in direct environmental observations. An experimental study on laboratory mice allows us to estimate the magnitude of the hereditary component of fluoride deposition. The fluoride accumulation was studied in the progeny of three strains of inbred mice (intrastrain correlation) against the background conditions and following chronic intake of the toxicant. The variant of the family analysis (intrafamily correlation) was also used. It is a classical approach to the hereditary variation of quantitative traits assessment. Fluoride entered the female mice body with food during the whole gestation period and up to the age of 1.5 month of the progeny. The assessment was performed with the control of the animal sex and litter size effect. Individual parameters of fluoride accumulation differed in certain experimental groups by 3–6.5 times. At the same time the specifics of fluoride accumulation was typical of the entire families. Combined hereditary component (intrastrain and intrafamily) of the fluoride accumulation was comparable with the hereditary correlation of morphological characteristics with known hereditary dependence of development ($R = 0.50–0.56$, $p < 0.0001$ and $R = 0.45–0.53–0.58$, $p < 0.0001$ respectively). Notably, the family component of the variability depending on the analysis option (the entire sample or just the experimental group) is comparable and exceeds the animal's strain effect by 2–3 times. For background fluoride level the hereditary dependence of its deposition is statistically insignificant. The results obtained can be extrapolated to field rodents.

Keywords: environmental toxicology, fluoride, bone tissue, inbred mouse strains, ANOVA, intraclass correlation coefficient, familial analysis.

УДК [615.916.1:546.16]+591.1+575.1

Оценка наследственной компоненты фторной экотоксической нагрузки

© 2026. В. И. Стариченко, д. б. н., в. н. с.,
Институт экологии растений и животных Уральского отделения РАН,
620144, Россия, г. Екатеринбург, ул. 8 Марта, д. 202,
e-mail: starichenko@ipae.uran.ru

Фтор – один из наиболее токсичных и широко распространённых промышленных загрязнителей природной среды. В экологических проблемах фторного загрязнения выявление наследственной компоненты его накопления играет особую роль как способствующей поддержанию его уровня в ряду поколений. Однако факторы индивидуальной изменчивости аккумуляции фтора у млекопитающих, в частности, наследственная составляющая вариабельности накопления, не выявлены. Это обусловлено методологическими особенностями исследования параметров депонирования фтора, недоступных для изучения в прямых экологических наблюдениях. Экспериментальное исследование на лабораторных мышах позволяет оценить величину наследственной компоненты депонирования фтора. Исследовали накопление фтора у потомства трёх линий инбредных мышей (внутрилинейная корреляция) в фоновых условиях и после хронического поступления токсиканта. Использовался также вариант семейного анализа (внутрисемейная корреляция). Это классический подход к оценке наследственной изменчивости количественных признаков. Самки получали фтор с пищей в течение всего периода беременности и до 1,5-мес. возраста потомства. При оценке учитывали эффект влияния пола животных и величины помёта в семье. Аккумуляция фтора у отдельных индивидов различалась в некоторых экспериментальных группах в 3–6,5 раз. В то же время особенности депонирования фтора оказались характерными для целых семейств. Общая наследственная составляющая (внутрилинейная и внутрисемейная) кумуляции фтора

сопоставима с наследственной корреляцией морфологических признаков, имеющих высокую наследственную детерминацию развития ($R = 0{,}50–0{,}56$, $p < 0{,}0001$ и $R = 0{,}45–0{,}53–0{,}58$, $p < 0{,}0001$ соответственно). В зависимости от варианта анализа (вся выборка или только опытная группа), семейная составляющая изменчивости сопоставима или значительно выше (в 2–3 раза) эффекта линейной принадлежности животных. Для фоновых уровней фтора наследственная обусловленность его депонирования статистически незначима. Полученные результаты могут быть экстраполированы на полевых грызунов.

Ключевые слова: экологическая токсикология, фтор, костная ткань, линейные мыши, дисперсионный анализ, коэффициент внутриклассовой корреляции, метод семейного анализа.

In the context of technogenic environmental pollution, the fluoride occupies one of the first places in terms of toxicity. It could be found in the emissions of the aluminum industry, ferrous industry, fertilizer production, ceramics and others. Damage by the fluoride and its compounds is viewed as general toxic one. However, its main acceptor is bone tissue where up to 90 % of the toxicant that enters the bloodstream accumulates irrespective of the routes of entry [1–5]. Fluoride incorporated in the skeleton retains in the bone tissue for a long time, thus becoming the permanent source of internal intoxication of the body.

A lot of publications are devoted to the study of the fluoride influence on the human and vertebrates animal body [1, 3, 6–11]. Their acute effects are manifested by respiratory failure, severe gastroenteritis, vomiting, diarrhea, shortness of breath, convulsions, ventricular tachycardia and other symptoms. Chronic effects lead to fluorosis, arthrosis-arthritis, skeletal and dental anomalies, and disruption of the immune and reproductive function [5].

Fluorides effects are studied in detail from the medical-hygienic and veterinary perspectives. It is known that for people exposed to the equal level the hazardous chemical, the effect may differ by two or three orders of magnitude [12].

For example, it was observed that the development and severity of the fluorosis in the aluminum production employees exposed to industrial intoxication almost similar in terms of intensity and time, differs considerably: in some individuals the health status remains unchanged for a long period of time, whereas others develop severe diseases [5, 13, 14].

In natural populations of small rodents inhabiting the areas adjacent to the ecotoxic enterprises some fluoride-resistant individuals could be found. Apparently, it is genetically preconditioned by the individual variability of fluoride sensitivity. Its range in wild populations is great. Population reactivity under chronic exposure to fluoride pollution is largely non-specific and is similar in manifestation to reactivity in radiation biocenoses, i. e. changes in reproduction, population size, and disrupted animal migration are observed. When exposed to number of generations, hereditary adaptation to a fluorotoxic environment develops [5]. Also, transgenerational epigenetic modification is induced in rodents of different species living in areas contaminated with various pollutants (including fluorides) [15].

The data on fluoride accumulation in the rodent skeleton [5, 10, 16] demonstrated that the scatter of its values could not be completely associated with the difference in consumed food and water rich in toxic elements and polluted air. For example, fluoride concentration in the skeleton of the house mouse (*Mus musculus*) in the vicinity of Tadzhik aluminum plant varies from 4700 to 16000 μg/g of the bone tissue [5].

In the works devoted to the ecology of mammals in a technogenically polluted natural environment with fluorine and fluorides, as well as industrial intoxication in humans, the indication of the individual variability of toxicant accumulation and its effect is limited only to the statement of its presence [5, 12–14].

The reason of such variability effect of the toxicant is on the one hand the individual sensitivity of the body to the effect of similar doses of the damaging factor, on the other hand – specific features of the toxicant kinetics (its distribution in organs and tissues and the period of time of its presence in the body). Genetic dependence of the sensitivity to the fluoride effects was shown in a lot of studies [8, 17, 18].

However, when studying the hereditary factors of the resistance, only body sensitivity to the fluoride effect is taken into account while the individual characteristics of the skeletal metabolism stay aside. And the mechanism of the development is not clear. Obviously, the development of the approaches to solve the task of individual prediction of the health status due to the damage by bone–seeking toxicants together with the evaluation of the body's sensitivity to the damaging factor requires due account of the individual metabolic activity of the skeleton.

Hereditary study of both the development of skeletal morphology and calcium metabolism is indirect evidence of the fact that the deposition of other bone-seeking substances in the skeleton also has hereditary component. It is logical to study the issue of hereditary determination of fluoride metabolism in an experiment, and to transfer the findings to natural populations of animals, humans and large farm animals.

In a laboratory experiment intrastrain comparisons are used to assess the hereditary component of variability. They proved themselves well in terms of qualitative parameters. However, for quantitative characteristics such assessments often give unsatisfactory results [19]. The classical approach to the assessing the hereditary component of the variability of quantitative parameters is the family analysis that does not require any genetic concept. Since all the individuals of the strain have the same genotype, family analysis could reveal possible epigenetic effects that alter the substance accumulation.

The present study is a continuation of investigating the hereditary (epigenetic) factors of bone–seeking substance accumulation (in particular ^{90}Sr) in the mammalian skeleton in mouse models [20, 21]. In addition to the interstrain comparison, we also used family analysis (the twin families' method).

The provision of the rationale of hereditary component of fluoride accumulation is within the scope of the toxicology and occupational pathology. The study of the heredity effect in the environmental problems of fluoride pollution, along with radionuclide contamination in nuclear accidents and carcinogenic petrochemical products, plays a special role. Epigenetic modification plays a significant role in the reactivity of rodents in impact territories, and their ability to adapt to a toxic environment is discussed. At the same time, experimental data should be representative enough to extrapolate to wild rodents, the most numerous order of mammals, and useful for studying other vertebrates from their natural habitat. Notably, some important aspects of the fluoride metabolism, for example, epigenetic inheritance, started to be discussed only recently. The issues of fluorosis also did not lose their importance [11, 14, 19, 22].

The aim of the study is to evaluate the hereditary (strain and family) component of the variability of fluoride deposition in bone tissue of the laboratory inbred mice under background and chronic intake.

Objects and methods of research

All experiments were carried out in accordance with Protocol No. 14 of the Bioethics Commission of the Institute of Plants and Animal Ecology UB RAS dated May 12, 2023.

In the study we used sexually mature laboratory CBA (from the Nursery "Rappolovo"), BALB/c (from the Nursery "Stolbovaya") and BC mice (the offspring of the second generation hybrids from the cross breeding of animal strains BALB/c – female and CBA – male, breeding during 10 years via closely related crossing and that have reached complete inbreeding), that were crossbred in the vivarium of the Institute of Plant and Animal Ecology of the Ural Branch of the Russian Academy of Science. Fluoride deposition in bone tissue was studied on the progeny of the mice of these strains.

Background levels of the fluoride accumulation were studied in the control group (vivarium ration); in the experimental group – after ingestion of an intoxicant (0.5 g/L sodium fluoride solution; 1 mL per mouse). The offspring received fluoride after birth – first with their mother's milk, then with food when they began to feed themselves (offspring age at the time of euthanasia was 1.5 months).

Since the birth of the offspring, the animals were kept by families (female and its litter). The ration of all the animals was enriched with in excess mineral feed of lump chalk and fresh greens to avoid calcium and vitamins deficit. After a month since birth females were separated from the offspring. The euthanasia of the offspring was performed by cervical vertebrae dislocation [23, 24]. After euthanasia the weight of the animal bodies, femur, and fluoride concentration in bone tissue were measured.

Fluoride in bone was measured with potentiometric method using fluoride-selective electrode after preliminary treatment of samples [25]. Fluoride concentration was expressed in $\mu g \cdot g^{-1}$ of dry bone. Family and strains of the animals were taken into account.

The subsequent dispersion analysis was based on the assumption of distribution normality of the analyzed parameters. Since the fluoride concentration both in the experimental and in the control groups had lognormal distribution, to follow the normality supposition logarithmic transformation was used. However, to simplify the expression we use the word combination "fluoride concentration", implying both the concentration itself and concentration logarithm. Body weight and femur weight have the distri-

bution that does not differ from the normal one.

Method of the family analysis has widely been used in research on heredity and presupposes the study of the manifestation of the traits in children, parents and close relatives. Its variant – method of twin families – is a combination of family and twin methods. The manifestation of the studied marker is compared in biologically related individuals irrespective of the fact whether the parents covariation is known – offspring or not [26]. The given methods belong to correlation studies.

Under the term "family" in this study we understand only the offspring of one female. It is connected with the fact that the comparison of data on fluoride accumulation in parents and children is incorrect due to the overwhelming contribution of age specific features to the value of deposition.

The progeny from 79 families was studied in the experiment (the number of animals $n = 582$): experimental group – 56 families ($n = 416$ animals); control group – 23 families ($n = 166$ animals). The number of the pups in families at birth varied in the range 2–20. But due to mothers' cannibalism by the end of the experiment three families had only one pup left. Experiment scheme is depicted in Figure 1.

Hereditary (strain and family) component of the changeability of fluoride accumulation in bone tissue was evaluated in comparison to the changeability of morphological characteristics (body weight and femur weight). Hereditary dependence of their development is known [19, 27–29].

Coefficient of intraclass correlation (R) that corresponds to the effect of the factors "strain" and "family" was used as an estimator. Assessment was performed with the control of the animal sex effect as well as the conditions of their development (different level of fluoride intake) and litter size in the family.

The term "random effects" in the context of dispersion analysis is used to designate factors which levels were not fixed beforehand, but were obtained from the sample during the experiment. Factors which levels are defined by the researcher are named fixed. It is assumed that the levels of a random factor are randomly selected from the general totality of all possible levels. In our case the heredity of the different strains and families within the strains cannot be completely known, that is we cannot study all the possible strains and families. Statistical analysis is based on a mixed model if some factors are assumed to be random, and some are fixed [30]. The assessment is performed only for the random factor.

Statistical conclusions (with 5 % significance level) were made based on a linear model with mixed effects where the factors "group", "sex" and covariate "litter size" were considered as fixed, and factor "family" as random. Factor "strain" was either fixed or random (depending on the particular assessment of the factors).

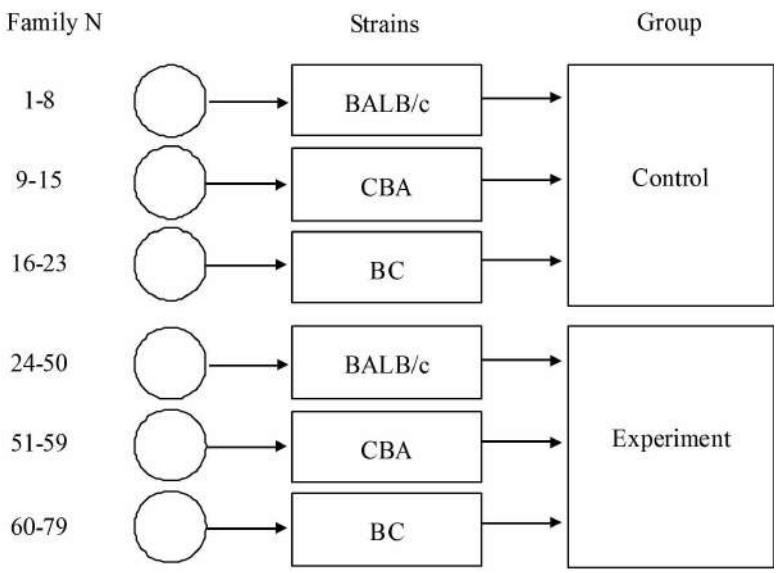

Fig. 1. The scheme of distribution of families in BALB/c, CBA and BC mice by experimental groups: control (background level of the fluoride), experiment (chronic fluoride intake). *Here and further in the figures and tables: CBA – mice from the Nursery "Rappolovo", BALB/c – mice from the Nursery "Stolbovaya", BC – the offspring of the second generation hybrids from the cross breeding of animal strains BALB/c (female) and CBA (male), the vivarium of the Institute of Plants and Animal Ecology UB RAS*

The assessment was carried out only for a random factor. Covariate (secondary independent variable) "litter size" was included into the analysis as it is known that for prolific mammals the number of the pups in the litter is one of the sources of the variability in the offspring weight indicator. That is why in experiments to assess the hereditary dependence of weight parameters, the number of offspring is artificially equalized or taken into account in statistical data processing.

The levels of the "family" factor are grouped within the "strain" fluoride level (Fig. 1). To get *F*-statistics for the studied effects in a mixed model of dispersion analysis the denominator synthesis was used [30, 31]. The coefficient of intraclass correlation (R) corresponding to the ratio of the dispersion component of the respective random factor to the complete dispersion, served as the estimator of the hereditary component of variability [31] (the percentage dispersion component is $R \cdot 100$ %).

The data were described with the mean and standard error and medians with quartiles. The significance of differences between the samples was assessed using Student's *t*-test and Newman-Keuls test. Statistical conclusions were made with 5 % significance ($p < 0.05$).

The data was analyzed using a licensed software package Microsoft Excel 2003 and Statistica 6.0 (StatSoft Inc.).

Results and discussion

The values of the weight characteristics and fluoride concentration in bone tissue of the experimental animals are given in Table 1.

Intergroup differences. A decrease in the body weight and femur weight was revealed in the animals from the experimental groups relative to the control (body weight 16.4 ± 0.1 g and 17.9 ± 0.2 g; femur weight: 0.0578 ± 0.0004 g and 0.0609 ± 0.0009 g, respectively). It could be either due to direct inhibitory effect of the fluoride in the period when they start to eat solid food or due to antenatal and postnatal effects of the fluoride that went through the placenta into the fetus blood during gestation period or was consumed with mother's milk [1, 2, 5, 32].

Intergroup differences in fluoride accumulation (control groups – 160 ± 2 μg/g, experimental groups – 2719 ± 45 μg/g) are associated with the different level of its receipt: control – background levels; experimental – chronic receipt.

Interstrain differences. When strains were compared in the control and experimental groups it turned out that the differences in the fluoride concentration between them are statistically insignificant (according to the Newman-Keuls Test), except for the CBA strain in the experimental group (Table 1). Considerable effect of the animals' strain on the fluoride accumulation was revealed only with the subsequent use of dispersion analysis.

Differences between males and females. The effect of the animal sex on weight values was not registered in all experimental groups (Table 1), although sex dimorphism of the weight in the majority of vertebrate species is well-known. The above effect (at the level of strains inside the group) on the fluoride accumulation was not observed. It is confirmed by the findings of many studies. In most of them the existence of sex-specific metabolism (of the fluoride and other bone-seeking elements) is not even discussed [2, 3, 32–34]

An exception is the pregnancy and lactation period when changes in the mineral metabolism are going on in the body of a female animal [35]. Another exception is the period of rapid growth when the skeleton weight is being formed. The rate of substance accumulation and its amount during this period differ in males and females because of the sex dimorphism in the skeleton size (body size) [36].

Individual differences. Individual values of fluoride deposition differ in some experimental groups in 3.0–6.5 times (variation coefficient of the fluoride concentration is – 23.5–36.5 %, of weight values – 9.9–16.2 %). Fluoride concentration in the bone tissue in some specimens in the control group varies from 90 to 268 μg/g and corresponds to the background level of this element content in the bone tissue of the mammals and humans – 50–450 μg/g [2, 5, 32, 37, 38]. In the experimental group fluoride accumulation is an order of magnitude higher – 980–6430 μg/g.

Notably, these specific features of fluoride accumulation typically relate to the entire families. The range of individual parameters of the deposition inside single families of the experimental groups is presented in Figure 2.

Dispersion analysis

Effect of the "strain" and "family" factor in the entire sample of animals. Results of the dispersion analysis of the effect of the "strain" and "family" factors are given in Table 2. Effect of the factors is statistically significant ($p < 0.0001$) for all the parameters. The contribution of the effect of the factor "strain" to the fluoride accumulation is 16.5 %, and to the development of the morpho-

Table 1

Weight characteristics and fluoride concentrations in bone tissue of the experimental and control animals (M ± m)

Group	Sex	*n*	Body weight, g	Femur weight, g	F, μg/g
Control	**BALB/c**				
	Males	17	21.0 ± 0.5	0.0729 ± 0.0023	161 ± 6
	Females	24	19.8 ± 0.3	0.0681 ± 0.0013	153 ± 7
	Group average	41 (8)*	20.3 ± 0.3 (15.0–25.7)**	0.0701 ± 0.0013 (0.0590–0.0895)	156 ± 5 (110–260)
	CBA				
	Males	17	17.6 ± 0.7	0.0573 ± 0.0027	161 ± 7
	Females	21	16.5 ± 0.6	0.0582 ± 0.0024	148 ± 6
	Group average	38 (7)	17.0 ± 0.4 (10.0–20.2)	0.0578 ± 0.0018 (0.0327–0.0775)	154 ± 5 (100–235)
	BC				
	Males	47	17.7 ± 0.4***	0.0587 ± 0.0018	168 ± 5
	Females	40	16.5 ± 0.3	0.0571 ± 0.0017	159 ± 5
	Group average	87 (8)	17.1 ± 0.3 (11.8–24.6)	0.0580 ± 0.0011 (0.0386–0.0902)	164 ± 4 (90–268)
Group average		166 (23)	17.9 ± 0.2 (10.0–25.7)	0.0609 ± 0.0009 (0.0327–0.0902)	160 ± 2 (90–268)
Experimental****	**BALB/c**				
	Males	81	17.9 ± 0.3***	0.0574 ± 0.0010	2773 ± 97
	Females	77	16.8 ± 0.3	0.0563 ± 0.0010	2603 ± 106
	Group average	158 (27)	17.4 ± 0.2 (8.6–25.1)	0.0569 ± 0.0007 (0.0347–0.0777)	2690 ± 72 (980–6430)
	CBA				
	Males	26	17.4 ± 0.2***	0.0594 ± 0.0010***	2957 ± 144
	Females	33	15.2 ± 0.2	0.0517 ± 0.0009	3141 ± 123
	Group average	59 (9)	16.2 ± 0.2 (10.0–19.1)	0.0551 ± 0.0008 (0.0405–0.0683)	3060 ± 94 (1720–5300)
	BC				
	Males	92	16.2 ± 0.3***	0.0598 ± 0.0010	2697 ± 92
	Females	107	15.3 ± 0.2	0.0588 ± 0.0009	2592 ± 100
	Group average	199 (20)	15.7 ± 0.2 (7.4–20.9)	0.0593 ± 0.0007 (0.0319–0.0803)	2640 ± 68 (1000–5140)
Group average		416 (56)	16.4 ± 0.1 (7.4–25.1)	0.0578 ± 0.0004 (0.0319–0.0803)	2719 ± 45 (980–6430)

*Note: * – number of families in the group, ** – min–max, *** – the differences between males and females are significant at P < 0.05 (Student's t-test), **** – fluoride receipts in antenatal and postnatal period.*

logical characteristics – 15–30 %, the "family" factor is 41.7 % and 46–48 %, respectively.

General hereditary component of the changeability (intrastrain plus intrafamily) in the entire sample of animals. The contribution of the factors "strain" and "family" to the total hereditary component of the changeability of the studied parameters is 52.9 % (13.5 and 39.4 %, respectively) for the body weight; 57.8 % (19.1 and 38.7 %, respectively) for the femur; 50.3 % (14.7 and 35.6 %, respectively) for the fluoride concentration (Table 3). It is clear that the changeability component dependent on the factor "strain" is 2–3 times less than the effect of the factor "family" both for the morphological and metabolic characteristics.

Hereditary component of variability (total intrastrain and intrafamily) for the control and experimental groups separately. Taking into account the discussion of the value of the total hereditary component of the changeability, it is of interest to perform analysis in the control and experimental groups separately. The data of Tables 4 and 5 are clearly demonstrated graphically in Figure 3.

The analysis of the control group has demonstrated that hereditary dependence of the fluoride accumulation for the background levels is not

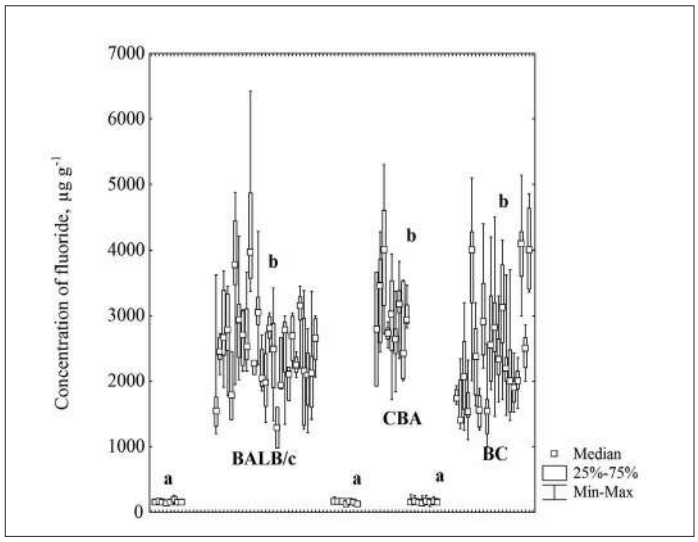

Fig. 2. Fluoride concentration (μg/g) in the bone tissue of the inbred mouse strains in certain families from the control (a) and experimental (b) groups (n = 582, number of families – 79)

Table 2

Coefficient of the intrastrain / intrafamily correlation of weight parameters and fluoride concentration in experimental animals (n = 582, 79 families)

Source of variance	Effect			Residue		F	$p\leq$	R
	Type	df	MS	df	MS			
Body weight (intrastrain correlation)								
Litter size	Fixed	1	996.62	2.11	63.89	15.60	0.0537	–
Group*	Fixed	1	370.75	19.56	5.82	63.73	0.0001	–
Sex	Fixed	1	220.54	436.13	4.19	52.62	0.0001	–
Strain	**Random**	2	105.64	576.00	4.04	26.13	0.0001	**0.155**
Body weight (intrafamily correlation)								
Litter size	Fixed	1	996.62	64.56	27.79	35.86	0.0001	–
Group	Fixed	1	370.75	69.14	20.21	18.34	0.0001	–
Sex	Fixed	1	207.90	273.76	3.87	53.69	0.0001	–
Strain	Fixed	2	111.96	73.25	16.52	6.78	0.0020	–
Family	**Random**	74	16.01	502.00	2.28	7.03	0.0001	**0.455**
Femur weight (intrastrain correlation)								
Litter size	Fixed	1	178693.9	2.07	57306.94	3.12	0.2153	–
Group	Fixed	1	222803.6	10.97	3925.78	56.75	0.0001	–
Sex	Fixed	1	9341.0	311.95	2431.54	3.84	0.0509	–
Strain	**Random**	2	95677.4	576.00	2294.63	41.70	0.0001	**0.229**
Femur weight (intrafamily correlation)								
Litter size	Fixed	1	631071.7	65.26	52363.93	12.05	0.0009	–
Group	Fixed	1	179172.7	69.51	37982.57	4.72	0.0333	–
Sex	Fixed	1	52083.5	258.11	6971.25	7.47	0.0067	–
Strain	Fixed	2	275632.6	73.31	30980.45	8.90	0.0003	–
Family	**Random**	74	30014.6	502.00	3944.81	7.61	0.0001	**0.478**
Fluoride concentration, ln (intrastrain correlation)								
Litter size	Fixed	1	1.23	2.10	1.35	0.91	0.4372	–
Group	Fixed	1	930.99	17.75	0.12	7939.53	0.0001	–
Sex	Fixed	1	0.35	418.86	0.08	4.18	0.0415	–
Strain	**Random**	2	2.24	576.00	0.08	28.12	0.0001	**0.165**
Fluoride concentration, ln (intrafamily correlation)								
Litter size	Fixed	1	1.23	63.29	0.51	2.42	0.1249	–
Group	Fixed	1	930.99	68.48	0.37	2513.03	0.0001	–
Sex	Fixed	1	0.34	301.69	0.08	4.49	0.0350	–
Strain	Fixed	2	2.24	73.15	0.30	7.36	0.0012	–
Family	**Random**	74	0.29	502.00	0.05	6.17	0.0001	**0.417**

*Note to Tables 2–5: df – number of degrees of freedom; MS – mean square deviation; * –experimental groups: control, fluoride receipts; bold font – the random factor being analyzed and the corresponding coefficient of the intrastrain/intrafamily correlation (R); a dash in a cell indicates that no assessment is performed for fixed factors.*

Table 3

General coefficient of the hereditary (intrastrain and intrafamily) correlation of weight parameters and fluoride concentration in experimental animals (n = 582, 79 families)

Source of variance	Effect			Residue		F	p ≤	R	ΣR
	Type	df	MS	df	MS				
Body weight									
Litter size	Fixed	1	996.62	3.32	80.29	12.41	0.0330	–	–
Group	Fixed	1	370.75	63.62	21.77	17.03	0.0001	–	–
Sex	Fixed	1	220.54	242.92	4.02	54.86	0.0001	–	–
Strain	**Random**	2	105.64	73.26	16.51	6.40	0.0028	**0.135**	–
Family	**Random**	74	16.01	502.00	2.28	7.03	0.0001	**0.394**	**0.529**
Femur weight									
Litter size	Fixed	1	631071.7	2.93	196182.9	3.22	0.1731	–	–
Group	Fixed	1	179172.7	56.25	42246.9	4.24	0.0441	–	–
Sex	Fixed	1	53158.2	201.62	7361.9	7.22	0.0078	–	–
Strain	**Random**	2	275095.2	73.32	30964.1	8.88	0.0004	**0.191**	–
Family	**Random**	74	30014.6	502.00	3944.8	7.61	0.0001	**0.387**	**0.578**
Fluoride concentrations, ln									
Litter size	Fixed	1	1.23	3.11	1.65	0.74	0.4495	–	–
Group	Fixed	1	930.99	60.23	0.40	2303.05	0.0001	–	–
Sex	Fixed	1	0.35	256.84	0.08	4.34	0.0382	–	–
Strain	**Random**	2	2.24	73.16	0.30	7.36	0.0012	**0.147**	–
Family	**Random**	74	0.29	502.00	0.05	6.17	0.0001	**0.356**	**0.503**

Table 4

Coefficient of the intrastrain and intrafamily correlation of weight parameters and fluoride concentration in the control group animals (n = 166, 23 families)

Source of variance	Effect			Residue		F	p ≤	R	ΣR
	Type	df	MS	df	MS				
Body weight									
Litter size	Fixed	1	336.72	2.84	98.14	3.43	0.1661	–	–
Sex	Fixed	1	50.20	34.79	3.73	13.46	0.0008	–	–
Strain	**Random**	2	110.97	18.89	14.49	7.66	0.0037	**0.382**	–
Family	**Random**	19	14.29	142.00	2.47	5.79	0.0000	**0.257**	**0.639**
Femur weight									
Litter size	Fixed	1	592832.7	3.25	152397.5	3.89	0.1359	–	–
Sex	Fixed	1	13392.2	47.63	6616.1	2.02	0.1613	–	–
Strain	**Random**	2	160684.2	18.91	29738.9	5.40	0.0139	**0.302**	–
Family	**Random**	19	29331.8	142.00	4503.3	6.51	0.0000	**0.314**	**0.616**
Fluoride concentrations (ln)									
Litter size	Fixed	1	0.02	5.97	0.064	0.35	0.5755	–	–
Sex	Fixed	1	0.18	157.62	0,03	5.20	0.0239	–	–
Strain	**Random**	2	0.04	18.62	0.05	0.70	0.5073	**0.000**	–
Family	**Random**	19	0.05	142.00	0.03	1.59	0.0666	**0.080**	**0.080**

significant while it exceeds 60 % for the weight parameters (Table 4, Fig. 3a).

Partly, the obtained results in the control group could be explained by the insignificance of the fluctuations in the background level of the fluoride content in one and the same medium (maternal body – at the antenatal stage, food stuffs and water – at the late stages of the onthogenesis). Because of this we do not detect (register) the effect of "strain" and "family" of an animal on the fluoride deposition.

However, one could not exclude a somewhat different mechanism for the formation of the fluoride background level in the bone. In this regard a comparative family study of the fluoride deposition in the control group at the stage of late

Table 5

Coefficient of the intrastrain and intrafamily correlation of weight parameters and fluoride concentration in experimental animals (n = 416, 56 families)

Source of variance	Effect			Residue		F	p ≤	R	$\sum R$
	Type	df	MS	df	MS				
Body weight									
Litter size	Fixed	1	799.97	7.87	32.27	24.79	0.0011	–	–
Sex	Fixed	1	168.31	156.51	4.24	39.72	0.0000	–	–
Strain	**Random**	2	28.47	51.19	16,59	1.72	0.1900	0.029	–
Family	**Random**	52	15.79	359.00	2.21	7.15	0.0000	**0.445**	**0.445**
Femur weight									
Litter size	Fixed	1	183167.6	2.94	153052.9	1.20	0.3554	–	–
Sex	Fixed	1	42541.0	59.98	8075.1	5.27	0.0252	–	–
Strain	**Random**	2	226023.0	51.17	27366.1	8.26	0.0008	**0.229**	–
Family	**Random**	52	26050.5	359.00	3734.5	6.98	0.0000	**0.348**	**0.577**
Fluoride concentrations, ln									
Litter size	Fixed	1	5.89	2.61	2.40	2.46	0.2283	–	–
Sex	Fixed	1	0.21	44.24	0.11	1.97	0.1675	–	–
Strain	**Random**	2	3.76	50.96	0.31	12.13	0.0000	**0.288**	–
Family	**Random**	52	0.30	359.00	0.05	5.56	0.0000	**0.275**	**0.563**

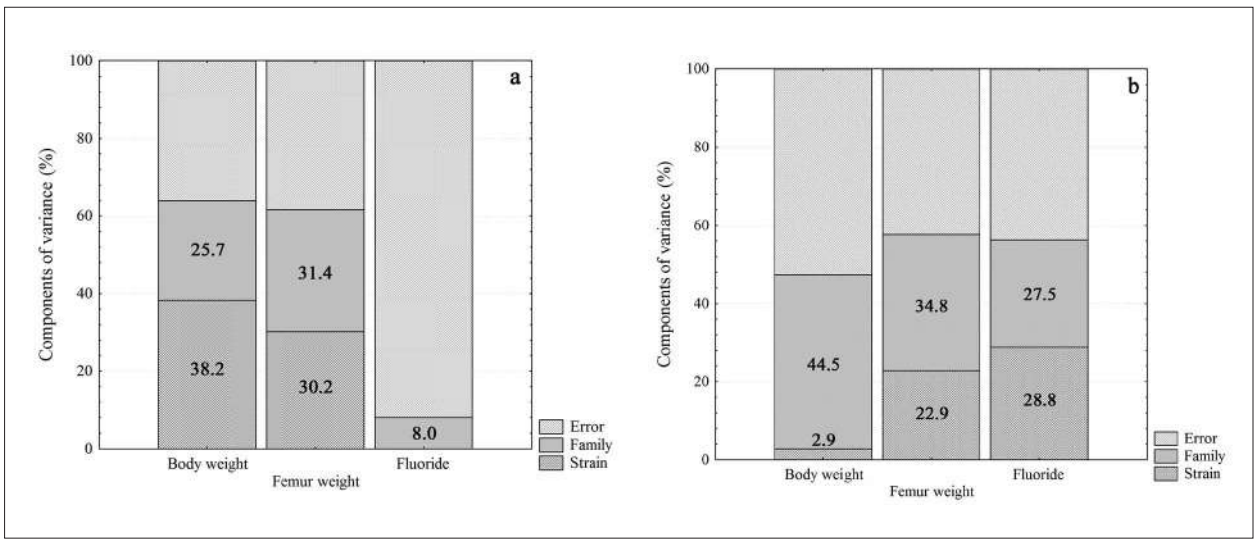

Fig. 3. Dispersion components (%) of the weight and fluoride concentration values, dependent on the strain and family of the animals in the control (a) and experimental (b) groups

antenatal and early postnatal development (until the transition to self-feeding) as well as finding the correlation between the level of the fluoride deposition in the maternal body and litter size will be of interest in the future.

The value of the hereditary dependence of the fluoride accumulation when the analysis performed only in the experimental group is 56.3 % (Table 5, Fig. 3b). The effect of the factor "strain" increases in 2 times relative to the combined sample against the background of some decrease in the effect of the factor "family" (Table 6). Total hereditary component of the dispersion is 56.3 and 50.3 %, respectively.

In the published papers there are few data on the hereditary dependence of the fluoride accumulation. For example, J.G. Carvalho et al. [39] studied the fluoride accumulation in the A/J and 129P3/J mice. Although the consumption of the fluoride by both of the strains was similar, its clearance in A/J mice was much higher. It led to much lower levels of the fluoride in the femur. 129P3/J strain retains more fluoride in the bone. In spite of this, 129P3/J is more resistant to the development of the teeth fluorosis [17].

Comparative analysis of the A/J, 129P3/J and SWR/J strains [8] showed both the presence of the inter-strain differences in its accumulation

Table 6

Coefficient of the intrastrain and intrafamily correlation of fluoride concentration in experimental animals with different variants of the analysis

Source of variance	Analyzed group		
	Control (n = 166)	Experimental (n = 416)	Control + Experimental (n = 578)
Strain	0.00 (p = 0.35)	0.308 (p < 0.001)	0.165 (p < 0.001)
Family	0.08 (p = 0.07)	0.386 (p < 0.001)	0.417 (p < 0.001)
Strain	0.00 (p = 0.51)	0.288 (p < 0.001)	0.147 (p < 0.001)
Family	0.08 (p = 0.07)	0.275 (p < 0.001)	0.356 (p < 0.001)
$\sum R$	0.08	0.563	0.503

in the femur and vertebra, and their absence (depending on the level of the fluoride receipts).

High intrafamily correlation (R = 0.50) was observed also for the ^{90}Sr deposition in CBA mice in the experiment with single administration of the ^{90}Sr [20]. The correlation value is little changed under the influence of the external factors modifying the growth processes in the skeleton [21].

The fact of the interfamily variability for the inbred animals, previously considered genetically homogeneous, can now be explained by proven non-isogeneity of strains, i.e. genetic variations within inbred mouse strains. Lead to this could be explained both by the residual heterogeneity of the individuals within the strains (spontaneous mutations, breeding errors, random crossing, etc.), and epigenetic variability [40–42].

Epigenetic character of the family component of the fluoride accumulation is related to the fact [43], that genetically homogeneous animals differ only in maternal food ration during pregnancy and lactation. There are no factors other than gestation and milk from different mothers that distinguish mice of the same litter from litters of the same strain kept under the same conditions that lead to different accumulations of fluoride and ^{90}Sr. The epigenetic nature of the inheritance of the deposition of these substances is also evidenced by literature data [44, 45].

The influence of the family is described in the studies of the ^{90}Sr accumulation in individual litters of beagle dogs [46]. The authors have noted that when puppies from one and the same litter have been taking ^{90}Sr with food for a long time, the curves of radionuclide accumulation are parallel. This situation is indirectly confirmed by a set of scientific papers on the food ration effect on the epigenetic labels [47, 48].

Family dependence of the ^{90}Sr accumulation was also found in animals from the natural habitat located in radioactively-contaminated territory of the East Ural Radioactive Trace – northern mole voles (*Ellobius talpinus* Pallas, 1770) – a specialized vole species living under the ground and characterized by family organization of the settlements and low ability to settle apart [49].

Thus, for the first time with the use of strain and family analysis of the litter of the three strains of the laboratory mice, the hereditary component of the variability (intrastrain and intrafamily, mainly epigenetic) of fluoride accumulation was evaluated. Hereditary dependence of fluoride accumulation was demonstrated. It is comparable to the variability in weight values that are most determined in vertebrates.

The obtained data could be both of fundamental and applied nature in particular for the ecology, health physics and toxicology. The finding of the mechanisms of individual specific features of the skeleton metabolism development will promote the improvement of the predictions of the accumulation and clearance of the bone–seeking pollutant. Quantitative data on the hereditary determination of fluoride metabolism obtained in the laboratory experiment will be extrapolated to natural animal populations living in contaminated areas.

Conclusions

1. The hereditary component of the variability (intrastrain and intrafamily correlation) of the fluoride accumulation in bones under background and chronic receipts of the toxicant (during the whole gestation period of female mice and up to the age of 1.5 month of the progeny) was assessed in the litter of three laboratory mice strains (BALB/c, CBA and BC).

2. In parallel the hereditary component of the variability of morphological characteristics (body weight and femur weight) with known hereditary dependence was evaluated. The assessment was performed with the control of animals' sex, conditions of the toxicant receipts as well as of the litter size in each family.

3. A significant hereditary (intrastrain and intrafamily) correlation of the fluoride accumulation in the bone tissue was found ($R = 0.503–0.563$, $p < 0.0001$), which was comparable to the correlation of the morphological characteristics ($R = 0.445–0.578$, $p < 0.0001$). It is indicative of the hereditary dependence of the fluoride accumulation.

4. Notably, the analysis of the total sample (control and experimental groups) has revealed that relative family component exceeds in 2–3 times the effect of animals' strain (correlation coefficient is 0.4–0.5 and 0.1–0.3 respectively, $p < 0.0001$) both for the fluoride concentration and morphological characteristics. The value of strain and family effect evens up in the analysis of the group that received increased amount of the fluoride. Under the background level of the fluoride receipts, its hereditary component is insignificant.

5. This study should be considered as materials for a biological basis for extrapolation to natural populations of vertebrates.

The author is grateful to I.A. Kshnyasev for his help in the statistical data processing and to N.M. Lyubashevsky – for fruitful discussion of the results.

This study was performed within the frameworks of state contract of the Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences (project No. 122021000077–6).

References

1. Faccini J.M. Fluoride and bone // Calcif. Tissue Res. 1969. V. 3. P. 1–16. doi: 10.1007/BF02058641

2. Knizhnikov V.A. Calcium and fluoride: radiation-hygienic aspects. Moskva: Atomizdat, 1975. 199 p. (in Russian).

3. Fluorine and fluorides. Environmental Health Criteria 36. Geneva: WHO, 1984. 100 p.

4. Boivin G., Meunier P.J. Fluoride and bone: Toxic effects and therapeutic role // Therapeutic uses of trace elements / Eds. J. Nève, P. Chappuis, M. Lamand. Boston, MA: Springer, 1996. P. 283–295. doi: 10.1007/978-1-4899-0167-5_49

5. Lyubashevskiy N.M., Tokar V.I., Shcherbakov S.V. Technogenic environmental pollution by fluoride (ecological and medical-social aspects). Yekaterinburg: Ural Branch of the RAS, 1996. 239 p. (in Russian).

6. Whitford G.M. The metabolism and toxicity of fluoride // Monogr. Oral Sci. 1996. V. 16. 156 p. doi: 10.1159/isbn.978-3-318-04022-7

7. Toxicological profile for fluorides, hydrogen fluoride, and fluorine. Atlanta, GA: Agency for Toxic Substances and Disease Registry (US), 2003. 404 p.

8. Mousny M., Banse X., Wise L., Everett E.T., Hancock R., Vieth R., Devogelaer J.P., Grynpas M.D. The genetic influence on bone susceptibility to fluoride // Bone. 2006. V. 39. No. 6. P. 1283–1289. doi: 10.1016/j.bone.2006.06.006

9. Yanin E.P. Fluoride in the environment: (prevalence, behavior, technogenic pollution) // Ecological expertise. 2007. No. 4. P. 2–98 (in Russian).

10. Gornostaeva E.A., Fuks S.L. The effect of fluorinated compounds on living organisms (review) // Theoretical and Applied Ecology. 2017. No. 1. P. 14–24 (in Russian). doi: 10.25750/1995-4301-2017-1-014-024

11. WHO. The public health impact of chemicals: knowns and unknowns WHO. International Programme on Chemical Safety. Geneva, 2016. 16 p.

12. Mogilenkova L.A., Rembovskiy V.R. Role of genetic polymorphism and differences in the detoxification of chemical substances in the human body // Hygiene & Sanitation. 2016. V. 95. No. 3. P. 255–262 (in Russian). doi: 10.18821/0016-9900-2016-95-3-255-262

13. Polzik E.V., Valova G.A., Zinger V.E., Sysoeva L.A. Some problems of biomedical prophylaxis occupational fluorosis // Occupational medicine and industrial ecology. 1995. V. 5. P. 44–47 (in Russian).

14. Lavryashina M.B., Ul'ianova M.V., Druzhinin V.G., Tolochko T.A. A study of the genetic basis of susceptibility to occupational fluorosis in aluminum industry workers of Siberia // Russ. J. Genet. 2003. V. 39. No. 7. P. 823–827.

15. Bol'shakov V.N., Vasil'ev A.G., Vasil'eva I.A., Gorodilova Yu.V., Kolcheva N.E., Lyubashevskii N.M., Chibiryak M.B. Technogenic morphological variation of the pygmy wood mouse (*Sylvaemus uralensis* Pall.) in the Urals // Russ. J. Ecol. 2012. V. 43. No. 6. P. 448–453. doi: 10.1134/S1067413612060033

16. Lyubashevsky N.M., Emelyanov A.M., Bakhtiyarova M.F., Juraev M.I., Kotomtsev V.V., Mokronosov A.A., Portnaya L.V., Chibiryak M.V., Starichenko V.I. Prospects for animal husbandry in the TADAZ area // Recommendations of the scientific and practical conference on reviewing the status of the results of the implementation of the "Target scientific and technical program of work to reduce emissions of pollutants into the environment by the Tajik Aluminum Plant to maximum permissible levels". Tursunzade, 1991. P. 84–90 (in Russian).

17. Everett E.T., McHenry M.A.K., Reynolds N., Eggertsson H., Sullivan J., Kantmann C., Martinez-Mier E.A., Warrick J.M., Stookey G.K. Dental fluorosis: variability among different inbred mouse strains // J. Dent. Res. 2002. V. 81. No. 11. P. 794–798. doi: 10.1177/0810794

18. Yan D., Willett T.L., Gu X.M., Martinez-Mier E.A., Sardone L., McShane L., Grynpas M., Everett E.T. Phenotypic variation of fluoride responses between inbred strains of mice // Cells Tissues Organs. 2011. V. 194. No. 2–4. P. 261–267. doi: 10.1159/000324224

19. Vogel F., Motulsky A.G. Human genetics: problems and approaches. Berlin, Heidelberg: Springer-Verlag, 2010. 981 p.

20. Starichenko V.I. Metabolism of osteotropic toxical substances: hereditary determination // Ecol. Genet. 2010. V. 8. No. 3. P. 27–37 (in Russian).

21. Starichenko V.I. Hereditary component of variation in ^{90}Sr deposition in inbred mice under exogenous conditions that affect bone formation // Appl. Radiat. Isot. 2018. V. 140. P. 126–132. doi: 10.1016/j.apradiso.2018.07.006

22. Everett E.T. Fluoride's effects on the formation of teeth and bones, and the influence of genetics // J. Dent. Res. 2011. V. 90. No. 5. P. 552–560. doi: 10.1177/0022034510384626

23. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the care and use of laboratory animals. Washington, D.C.: National Academies Press, 2011. 246 p.

24. Leary S., Underwood W., Anthony R., Cartner S., Corey D., Grandin T., Greenacre C., Gwaltney-Brant S., McCrackin M., Meyer R., Miller D., Shearer J., Yanong R., Golab G., Patterson-Kane E. AVMA guidelines for the euthanasia of animals: 2013 edition // J. Am. Vet. Med. Assoc. 2013. 102 p.

25. Khazemova L.A., Radovskaya T.L., Kruglova N.V., Kachalkova T.K. Fluorine assessment in plant material // Agrokhimiya. 1983. No. 6. P. 66–72 (in Russian).

26. Mather K., Jinks J.L. Introduction to biometrical genetics. London: Chapman and Hall Ltd., 1982. 238 p.

27. Falconer D.S., Mackay T.F.C. Introduction to quantitative genetics. Essex: Longman House, 1996. 448 p.

28. Beamer W.G., Donahue L.R., Rosen C.J., Baylink D.J. Genetic variability in adult bone density among inbred strains of mice // Bone. 1996. V. 18. No. 5. P. 397–403. doi: 10.1016/8756-3282(96)00047-6

29. Slemenda C.W., Turner C.H., Peacock M., Christian J.C., Sorbel J., Hui S.L., Johnston C.C. The genetics of proximal femur geometry, distribution of bone mass and bone mineral density // Osteoporos. Int. 1996. V. 6. No. 2. P. 178–182. doi: 10.1007/BF01623944

30. Scheffé H. The analysis of variance. New York: Wiley, 1959. 496 p.

31. Sokal R.R., Rohlf F.J. Biometry: the principles and practice of statistics in biological research. New York: W.H. Freeman & Co., 1995. 887 p.

32. Gabovich R.D. Fluoride and its hygienic value. Moscow: Medgiz, 1957. 250 p. (in Russian).

33. ICRP Publication 20. Alkaline earth metabolism in adult man. Oxford: Pergamon Press, 1973. 99 p.

34. Hefti A., Marthaler T.M. Bone fluoride concentrations after 16 years of drinking water fluoridation // Caries Res. 1981. V. 15. No. 1. P. 85–89. doi: 10.1159/000260504

35. Il'enko A.I., Krapivko T.P. Animal ecology in the radiation biogeocoenose. Moskva: Nauka, 1989. 224 p. (in Russian).

36. Shagina N.B., Tolstykh E.I., Degteva M.O. Improvements in the biokinetic model for strontium with allowance for age and gender differences in bone mineral metabolism // Radiat. Prot. Dosimetry. 2003. V. 105. No. 1-4. P. 619–622. doi: 10.1093/oxfordjournals.rpd.a006314

37. Fluoride concentrations in human bones // Nutr. Rev. 1959. V. 17. No. 5. P. 133–136. doi: 10.1111/j.1753-4887.1959.tb06433.x

38. Pharmacology of fluorides / Ed. F.A. Smith // Handbuch der experimentellen Pharmakologie. V. 20. Pt. 2. Berlin, Heidelberg, New York: Springer; Verlag, 1966. 452 p. doi: 10.1007/978-3-642-99973-4

39. Carvalho J.G., Leite A.L., Yan D., Everett E.T., Whitford I G.M., Buzalaf M.A.R. Influence of genetic background on fluoride metabolism in mice // J. Dent. Res. 2009. V. 88. No. 11. P. 1054–1058. doi: 10.1177/0022034509347249

40. Vasil'ev A.G. Epigenetic bases of phenetics: towards population meronomy. Ekaterinburg: Akademkniga, 2005. 640 p. (in Russian).

41. Casellas J. Inbred mouse strains and genetic stability: a review // Animal. 2011. V. 5. No. 1. P. 1–7. doi: 10.1017/S1751731110001667

42. Chebib J., Jackson B.C., López-Cortegano E., Tautz D., Keightley P.D. Inbred lab mice are not isogenic: genetic variation within inbred strains used to infer the mutation rate per nucleotide site // Heredity. 2021. V. 126. No. 1. P. 107–116. doi: 10.1038/s41437-020-00361-1

43. Lyubashevsky N.M., Starichenko V.I. Lactation and the phenomenon of biodiversity (epigenetics of the mineral metabolism of rodents) // Genetics of Populations: Progress and Perspectives: Proceedings of the International Conference commemorating the 80th birthday of Academician Y.P. Altukhov. Zvenigorod, April 17–21, 2017. Moskva, 2017. P. 152–154 (in Russian).

44. Kitsiou-Tzeli S., Tzetis M. Maternal epigenetics and fetal and neonatal growth // Curr. Opin. Endocrinol. Diabetes Obes. 2017. V. 24. No. 1. P. 43–46. doi: 10.1097/MED.0000000000000305

45. Ge Z.J., Sun Q.Y. Maternal epigenetic inheritance // Transgenerational Epigenetics. 2019. V. 13. P. 75–105. doi: 10.1016/B978-0-12-816363-4.00005-5

46. Goldman M., Della Rosa R.J. Studies of the dynamics of strontium metabolism under conditions of continual ingestion to maturity // Strontium metabolism: proceedings of an International symposium on some aspects of strontium metabolism / Eds. J.M.A. Lenihan, J.F. Loutit, J., G. Martin. London, New York: Academic Press, 1967. P. 181–194.

47. Jang H., Serra C. Nutrition, epigenetics, and diseases // Clin. Nutr. Res. 2014. V. 3. No. 1. P. 1–8. doi: 10.7762/cnr.2014.3.1.1

48. Murdoch B.M., Murdoch G.K., Greenwood S., McKay S. Nutritional influence on epigenetic marks and effect on livestock production // Front. Genet. 2016. V. 7. Article No. 182. doi: 10.3389/fgene.2016.00182

49. Starichenko V.I. Accumulation of ^{90}Sr in the bone tissue of northern mole voles in the head portion of the East Ural Radioactive Trace // Russ. J. Ecol. 2011. V. 42. No. 1. P. 64–70. doi: 10.1134/S1067413611010115